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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.

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Perbedaan Efektivitas Penggunaan Metformin-Insulin *versus* Metformin-Vildagliptin Terhadap Profil Glikemik Pasien DMT2 di Instalasi Rawat Jalan RSISA Periode 2022

Comparison in Effect between Metformin-Insulin and Metformin-Vildagliptin on Glycemic Profile in Outpatients with Type 2 Diabetes Mellitus at Sultan Agung Islamic Hospital in 2022

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Abstrak

Diabetes Mellitus (DM) merupakan penyakit tidak menular yang ditandai dengan gangguan kondisi metabolisme dengan gejala umum yaitu peningkatan kadar glukosa darah (hiperglikemia). Jumlah kasus DM semakin meningkat dari tahun ke tahun. Tata laksana *terapi* pasien DM tipe 2 dapat dilakukan melalui 4 pilar, yaitu edukasi, pola makan, aktivitas fisik, dan terapi farmakologi. Terapi farmakologi obat antidiabetik terbagi atas injeksi insulin dan oral. Tujuan penelitian ini adalah untuk mengetahui perbandingan efektivititas penggunaan obat metformin-insulin *versus* metformin-vildagliptin pada pasien DM tipe 2 di RS Islam Sultan Agung Semarang. Penelitian ini termasuk jenis penelitian observasional. Pengambilan data secara *retrospektif*. Data dilakukan analisis menggunakan SPSS dengan uji parametrik yaitu uji *independent* t-test dan uji non parametrik menggunakan *Mann-Whitney test* untuk mengetahui perbandingan efektivitas 2 kelompok obat. Hasil penelitian analisis perbandingan efektivitas penggunaan Metformin-insulin *versus* metformin-vildagliptin terhadap profil glikemik diperoleh nilai signifikansi profil glikemik HbA1c 0,494 p(>0,05) dan GDS 0,638 p(>0,05). Kesimpulan yang dapat diambil dari penelitian ini menunjukkan bahwa tidak terdapat perbedaan yang signifikan terhadap profil glikemik pada kelompok metformin-insulin maupun metformin-vidagliptin.

Kata kunci: Diabetes Mellitus tipe 2; Efektivitas; Insulin; Metformin; Vildagliptin

Abstract

Diabetes Mellitus (DM) is a non-communicable disease characterized by metabolic disorders with common symptoms, namely increased blood glucose levels (hyperglycemia). The number of DM cases is increasing from year to year. Type 2 DM therapy can be managed through 4 pillars: education, diet, physical activity, and pharmacological therapy. Pharmacological therapy of antidiabetic drugs is divided into insulin injections and oral administration. This study aimed to compare the effectiveness of metformin-insulin versus metformin-vildagliptin in type 2 DM patients at Sultan Agung Islamic Hospital Semarang. This research includes observational research—retrospective data collection. Data were analyzed using SPSS with a parametric test, namely the independent t-test and the non-parametric test using the Mann-

Whitney test to compare the effectiveness of the two drug groups. The results of the comparative analysis of the effectiveness of using metformin-insulin versus metformin-vildagliptin on the glycemic profile obtained a significance value for the glycemic profile of HbA1c 0.494 p (> 0.05) and GDS 0.638 p (> 0.05). The conclusions that can be drawn from this study indicate that there is no significant difference in the use of each drug group on the glycemic profile.

Keywords: *Diabetes Mellitus type 2; Effectiveness; Insulin; Metformin; Vildagliptin*

1. PENDAHULUAN

Diabetes Mellitus (DM) merupakan penyakit tidak menular yang ditandai dengan gangguan kondisi pada metabolisme dengan gejala umum yaitu peningkatan kadar glukosa darah atau dikenal dengan hiperglikemia (Sa'dyah *et al*., 2021). Diabetes Mellitus dapat timbul disebabkan adanya kondisi abnormal pada sekresi insulin oleh pankreas, kerja insulin atau keduanya (Purnamasari, 2014). Pasien diabetes mellitus beresiko mengalami komplikasi apabila kadar glukosa darah tidak ditangani dengan baik. Adanya komplikasi dapat mempengaruhi kondisi kesehatan pasien (Anshari *et al*., 2023)

Diabetes Mellitus tipe 2 merupakan kasus DM yang banyak terjadi. WHO memperkirakan jumlah peningkatan pasien yang menderita Diabetes Mellitus (DM) tipe 2 di Indonesia terhitung dari 8,4 juta orang yang terjadi pada tahun 2000 menjadi 21,3 juta orang yang diperkirakan akan terjadi di tahun 2030. Berdasarkan perkiraan dari *International Diabetes Federation* (IDF) pada tahun 2019 – 2030 terdapat peningkatan jumlah penderita diabetes dari 10,7 juta orang menjadi 13,7 juta orang pada tahun 2030. Keseluruhan jumlah kasus karena DM yang terus meningkat dan tidak tertanganinya pasien DM dengan baik, maka hal ini dapat menimbulkan komplikasi (PERKENI, 2021).

Pengelolaan terapi DM tipe 2 yang diterbitkan oleh Perkumpulan Endokrinologi Indonesia (PERKENI) dapat melalui 4 pilar, yaitu edukasi atau pengetahuan, pengaturan makanan, pengaturan kegiatan fisik, dan terapi farmakologi. Terapi farmakologi obat antidiabetik terbagi atas injeksi insulin dan oral. Terapi obat antidiabetik oral yang sudah digunakan di Indonesia antara lain, metformin, SU (sulfonilurea), glinid, TZD (thiazolidindione), penghambat glikosidase alfa, penghambat DPP4, dan penghambat SGLT 2 (Sihotang et al., 2018).

Pemberian kombinasi obat antidiabetik oral dengan insulin dapat diberikan pada pasien DM tipe 2 apabila pasien belum menunjukkan perbaikan dalam melakukan pengaturan pola hidup (PERKENI, 2021). Vildagliptin dapat digunakan sebagai monoterapi atau terapi tambahan dengan obat lain, seperti metformin. Pengobatan pada pasien DM tipe 2 seringkali diberikan metformin sebagai pilihan pertama terapi. Kombinasi penggunaan Metformin-vildagliptin menghasilkan pengurangan HbA1c yaitu 0,7%. (Kristin, 2016). Efektivitas terapi obat antidiabetik dilihat berdasarkan ketercapaian gula darah pasien sesuai. Pemilihan obat antidiabetik berdasarkan keuntungan, kerugian, dan ketersediaan obat (Arini dan Kurnianta, 2019). Penggunaan vildagliptin yang ditambahkan pada algoritma terapi DM tipe 2 merupakan

terapi baru yang belum banyak digunakan, karena keterbatasan informasi mengenai efektivitas terapi obat tersebut, maka untuk itu perlu adanya informasi terkait penggunaan terapi tersebut. Pemberian terapi kombinasi metformin-insulin yang telah dilakukan oleh (Madelina *et al*., 2018) menunjukkan bahwa kombinasi terapi metformin-insulin banyak digunakan. Metformin berperan dalam meningkatkan sensitivitas terhadap insulin sehingga insulin dapat bekerja lebih baik. Dalam penelitian (Fitriyani *et al*., 2021) kombinasi metformin-insulin menunjukkan penurunan HbA1c yang lebih baik serta memberikan penambahan berat badan yang lebih kecil dibandingkan dengan kombinasi oral lainnya. Kedua penelitian tersebut belum dijelaskan efektivitas kombinasi metformin-vildagliptin terhadap profil glikemik pasien DM tipe 2. Pengendalian glikemik pada pasien DM tipe 2 dengan menggunakan pengobatan terapi obat antidiabetik sangat penting karena dapat digunakan untuk mendukung dalam menegakkan diagnosis serta memanajemen terapi yang diberikan. Penelitian ini memiliki tujuan yaitu untuk mengetahui perbedaan efektivitas penggunaan metformin-insulin dan metformin-vildagliptin.

2. METODE

2.1. Subyek penelitian

Subyek penelitian ini adalah pasien DM tipe 2 yang mendapatkan terapi metformin-insulin dan metformin-vildagliptin dengan sampel sebanyak 34 orang. Penentuan ukuran sampel penelitian ini menggunakan Persamaan 1. Berdasarkan perhitungan tersebut, sampel minimal yang dibutuhkan sebanyak 34 orang.

$$n = \left[\frac{(Z\alpha+Z\beta)S}{X1-X2}\right]^2 \; n = \left[\frac{(1{,}64+1{,}28)6}{40-37}\right]^2 \; n = \; 5{,}84^{\,2} \; n = 34{,}11$$

Persamaan 1. Perhitungan besar sampel penelitian analitis kategorik-numerk berpasangan. Keterangan: Deviat baku alfa ($Z\alpha$), Deviat baku beta ($Z\beta$), Simpang baku dari selisih nilai antarkelompok (S), Selisih sminimal rerata yang dianggap bermakna (X_1-X_2) (Dahlan, 2013).

2.2. Teknik pengambilan sampel

Teknik pengambilan sampel dalam penelitian menggunakan teknik observasional dengan melihat data rekam medis pasien DM tipe 2 di RS Islam Sultan Agung Semarang dari bulan Januari-Oktober 2022 yang berisi identitas pasien meliputi nama, usia, jenis kelamin, berat badan, hasil pemeriksaan glukosa pasien, serta terapi yang diberikan. Penelitian yang dilakukan termasuk jenis penelitian kuantatif. Rancangan penelitian bersifat deskriptif analitik, pengambilan data secara *cohort* retrospektif. Kriteria inklusi penelitian ini diantaranya: usia dewasa akhir – lansia (36 – >65 tahun), pasien DM tipe 2 yang mendapat terapi metformin-insulin tunggal dan metformin-vildagliptin, pasien DM tipe 2 dengan HbA1c >6%. Selain itu kriteria eksklusi penelitian ini yaitu pasien dengan komplikasi ginjal, pasien yang sedang hamil dan pasien dengan data rekam medis yang tidak lengkap.

2.3. Analisis hasil

Analisis hasil penelitian menggunakan software *Statistical Product and Service Solution* (SPSS), pada awal analisis dilakukan uji normalitas (*shapiro-wilk*) dan uji homogenitas. Data dinyatakan terdistribusi normal dan homogen (p> 0,05) kemudian diteruskan dengan uji parametrik, apabila hasilnya data tidak terdistribusi normal dilanjutkan menggunakan uji non-parametrik. Analisis data perbandingan dua kelompok menggunakan uji t-*test* untuk hasil data yang terdistribusi normal dan *mann whitney* untuk hasil data yang tidak terdistribusi normal. Penelitian yang dilakukan (Kristin, 2017) pemberian terapi kombinasi metformin-vildagliptin menghasilkan pengurangan HbA1c 0,7% sehingga terapi tersebut efektif dalam menurunkan kadar HbA1c, sedangkan dalam pemberian terapi metformin-insulin memberikan penurunan HbA1c sebanyak 0,2% (Arini dan Dwipayana, 2020). Penelitian Oktaviani *et al* (2022) menjelaskan, suatu terapi menunjukkan hasil efektif apabila terapi yang diberikan mampu menurunkan atau memberikan perbaikan profil glikemik dan terapi menunjukkan hasil yang tidak efektif apabila terapi yang diberikan belum memberikan penurunan atau perbaikan profil glikemik.

3. HASIL DAN PEMBAHASAN

3.1. Distribusi pasien

Distribusi subyek pasien Diabetes Mellitus Tipe 2 mencangkup gambaran karakteristik usia, jenis kelamin, IMT dan jaminan pengobatan.

3.1.1. Distribusi karakteristik pasien Diabetes Mellitus Tipe 2

Distribusi karakteristik subyek pasien Diabetes Mellitus (Tabel 1.) didapatkan hasil sebagian besar pasien Diabetes Mellitus tipe 2 berusia >45 tahun sebanyak 58 pasien (85,30%), penelitian ini sejalan dengan penelitian yang dilakukan oleh Yanti *et al* (2020). Hasil Riset Kesehatan Dasar tahun 2018, pada usia tua, lebih dari 50 tahun, terjadi resistensi insulin yang menyebabkan adanya penurunan sensitivitas insulin sehingga menyebabkan peningkatan kadar glukosa. Selain itu, disebabkan menurunnya kemampuan jaringan otot dan lemak untuk meningkatkan glukosa dan menyebabkan kadar glukosa darah terus meningkat. Kadar glukosa darah berkaitan dengan bertambahnya usia, karena toleransi glukosa yang berkurang dan dikaitkan dengan penurunan sensitivitas sel perifer terhadap insulin akibatnya bisa mempengaruhi kadar glukosa dalam darah. Seseorang akan mengalami penurunan fungsi sel beta pankreas dan mengalami penurunan pada fungsi fisiologis pada usia 40 tahun. Penurunan ini dapat menyebabkan berkurangnya sintesis protein, penurunan massa tubuh dan massa tulang serta peningkatan persentase lemak tubuh (Yanti *et al*., 2020).

Pasien yang terlibat dalam penelitian ini didominasi oleh pasien dengan jenis kelamin perempuan sebanyak 43 orang (63,24%). Penelitian ini didukung oleh penelitian yang dilakukan Yanti *et al*., (2020) terkait faktor yang berkontribusi terhadap glukosa darah. Pada penelitian Yanti *et al.,* (2020) pasien DM tipe 2 dengan jenis kelamin laki-laki sejumlah 27

orang (40,9%) sedangkan pasien dengan jenis kelamin perempuan sejumlah 39 orang (59,1%). Pasien perempuan mengalami siklus bulanan (sindrom pramenstruasi) dan pascamenopause yang menyebabkan distribusi lemak dalam tubuh lebih mudah tertimbun akibat proses hormonal tersebut dan beresiko mengalami peningkatan kadar glukosa, selain itu juga sensitivitas insulin yang menurun dapat menyebabkan kadar glukosa meningkat (Yanti *et al*., 2020). IMT pasien DM tipe 2 paling tinggi pada rentang 25-29,9 sebanyak 46 pasien (67,65%). Hasil penelitian yang didapatkan ini didukung oleh penelitian yang pernah dilakukan oleh Adnan *et al*., (2013) yang menunjukkan adanya timbunan lemak yang tinggi dapat mengakibatkan tingginya asam lemak bebas dan merangsang oksidasi lemak sehingga berefek terhadap fungsi penggunaan glukosa di dalam otot. Pengobatan yang dijalankan oleh pasien Diabetes Mellitus membutuhkan terapi yang bersifat jangka panjang, sehingga diperlukan biaya yang tidak sedikit dalam melakukan pengobatan. JKN (Jaminan Kesehatan Nasional) banyak dimanfaatkan oleh pasien, hal ini dikarenakan dapat memberikan kepastian jaminan perlindungan serta kesejahteraan sosial bagi setiap individu agar mendapatkan kehidupan yang layak (Putri *et al*., 2019).

Tabel 1. Distribusi karakteristik pasien Diabetes Melllitus Tipe 2 di Instalasi Rawat Jalan RS Islam Sultan Agung Semarang.

Karakteristik Pasien	Jenis Terapi	
	Metformin-Insulin (n=34)	Metformin - Vildagliptin (n=34)
Usia		
<45 tahun (n=10)	4 (11,76%)	6 (17,65%)
>45 tahun (n=58)	30 (88,24%)	28 (82,35%)
Jenis Kelamin		
Laki-laki (n=25)	10 (29,41%)	15 (44,12%)
Perempuan (n=43)	24 (70,59%)	19 (55,88%)
IMT		
18,5 - 22,9 (n=14)	8 (23,53%)	6 (17,65%)
23 - 24,9 (n=2)	0	2 (5,88%)
25 - 29,9 (n=46)	22 (64,71%)	24 (70,58%)
>30 (n=6)	4 (11,76%)	2 (5,89%)
Jaminan Pengobatan		
PBI (n=15)	10 (29,41%)	5 (14,71%)
Non PBI (n=53)	24 (70,59%)	29 (85,29%)

3.1.2. Distribusi berdasarkan penggunaan obat antidiabetik

Penggunaan obat antidiabetik kombinasi metformin-insulin pada terapi Diabetes Mellitus tipe 2 (Tabel 2.) dapat diuraikan pasien dengan kombinasi metformin-insulin Glulisin (Apidra) sebanyak (11,76%); metformin-insulin Lispro (Humalog) sebanyak (17,65%); metformin-insulin Aspart (Novorapid) sebanyak (35,30%); metformin-degludec (Ryzodeg) sebanyak (29,41%); metformin-insulin Novomix sebanyak (5,88%). Sehingga dari hasil tersebut

diperoleh pasien DM tipe 2 yang mendapat terapi paling banyak yaitu terapi kombinasi metformin-insulin Aspart (Novorapid). Penggunaan *short* dan *long acting* yang digunakan pada penelitian ini disesuaikan dengan kondisi pasien. Kondisi pasien dengan nilai GDS yang sangat tinggi perlu diberikan terapi kombinasi insulin *rapid acting* yang bekerja menurunkan gula darah dengan segera dan mengontrol gula darah saat makan, dan insulin *long acting* unyuk menjaga kondisi insulin basal pada saat malam hari agar gula darah tetap dalam kondisi stabil (Atika *et al*., 2016).

Selanjutnya, penggunaan obat antidiabetik kombinasi metformin-vildagliptin (Tabel 2.) sebanyak 3 pasien (100%). Hasil penelitian yang diperoleh dalam penelitian ini didukung oleh penelitian Wahyuni *et al.,* (2012) bahwa penggunaan terapi insulin kerja cepat/pendek (*rapid acting*) lebih banyak digunakan dibandingkan insulin kerja panjang. Hal tersebut dikarenakan keuntungan dari penggunaan insulin kerja cepat dalam memperbaiki nilai HbA1c dibandingkan dengan insulin kerja panjang. Selain itu, jenis insulin *rapid acting* memungkinkan pemindahan insulin di saat makan karena kerjanya yang cepat serta insulin jenis ini dapat diberikan sebelum makan tanpa mengganggu kontrol glukosa (Inayah *et al.*, 2016).

Tabel 2. Distribusi penggunaan obat antidiabetik Pasien Diabetes Mellitus Tipe 2 di Instalasi Rawat Jalan RS Islam Sultan Agung Semarang.

Jenis Terapi	N	Persentase (%)
Metformin- Insulin (N=34)		
Metformin-Insulin Glulisine	4	11,76
Metformin-Insulin Lispro	6	17,65
Metformin-Insulin Aspart	12	35,30
Metformin-Insulin Degludec	10	29,41
Metformin-Insulin Novomix	2	5,88
Metformin-Vildagliptin (N=34)		
Metformin-Vildagliptin	34	100

3.2. Perbandingan efektivitas terapi

3.2.1. Efektivitas metformin-insulin versus metformin-vildagliptin terhadap HbA1c

Hasil analisis efektivitas terapi kombinasi obat antidiabetik yang diperoleh pada parameter HbA1c (Tabel 3.) dari 34 pasien yang mendapatkan terapi metformin-insulin terdapat 10 pasien yang mengalami penurunan (efektif) kadar HbA1c dengan rata-rata penurunan 1,06 sedangkan pasien yang mendapatkan terapi metformin-vildagliptin dari 34 pasien terdapat 18 pasien yang mengalami penurunan (efektif) HbA1c rata-rata penurunan 0,21 dan p 0,494 (>0,05). Hasil analisis dengan menggunakan uji *t-independent* memberikan hasil tidak ada perbedaan yang signifikan dalam pemberian 2 kelompok obat terhadap HbA1c. Hasil penelitian ini didukung oleh penelitian yang dilakukan Wuryandari *et al*., (2021) bahwa penggunaan terapi kombinasi harus memiliki mekanisme kerja yang seimbang dan saling menguntungkan. Peran metformin pada penggunaan terapi metformin-insulin yaitu

meningkatkan kepekaan insulin serta peran insulin sebagai insulin endogen untuk mengontrol insulin yang dihasilkan oleh sel b pankreas sehingga kadar glukosa bisa dikendalikan. Selain itu, penggunaan terapi metformin-vildagliptin juga memiliki efek yang sama menguntungkan yaitu metformin dengan meningkatkan kepekaan insulin serta vildagliptin berperan menghambat enzim *dipeptidyl peptidase* sehingga dapat menaikkan sekresi insulin dan menekan sekresi glukagon (Hardianto, 2020).

Tabel 3. Efektivitas metformin-insulin *versus* metformin-vildagliptin terhadap HbA1c pasien Diabetes Mellitus Tipe 2 di Instalasi Rawat Jalan RS Islam Sultan Agung Semarang.

Jenis Obat	Efektif	Tidak Efektif	p *value*
Metformin-Insulin (n=34)	10	24	0,494
Metformin-Vildagliptin (n=34)	18	16	

3.2.2. Efektivitas metformin-insulin *versus* metformin-vildagliptin terhadap GDS

Hasil analisis efektivitas terapi kombinasi obat antidiabetik pada parameter GDS dengan jumlah sampel 34 pasien (Tabel 4) dari 34 pasien yang mendapatkan terapi metformin-insulin terdapat 10 pasien yang mengalami penurunan (efektif) kadar GDS dengan rata-rata penurunan 23,35. Pasien yang mendapatkan terapi metformin-vildagliptin dari 34 pasien terdapat 18 pasien yang mengalami penurunan (efektif) GDS dengan rata-rata penurunan 15,97 dan p 0,638 (>0,05). Kedua hasil tersebut menunjukkan tidak ada perbedaan yang signifikan terhadap GDS.

Tabel 4. Efektivitas metformin-insulin *versus* metformin-vildagliptin terhadap GDS pasien Diabetes Mellitus Tipe 2 di Instalasi Rawat Jalan RS Islam Sultan Agung Semarang.

Jenis Obat	Efektif	Tidak Efektif	p *value*
Metformin-Insulin (n=34)	15	19	0,638
Metformin-Vildagliptin (n=34)	18	16	

Penelitian ini didukung oleh penelitian yang dilakukan Jamaluddin *et al.,* (2022) yang memperoleh hasil tidak ada perbedaan yang signifikan pada terapi kombinasi oral dan insulin terhadap kadar glukosa darah GDS. Penelitian lain yang menjelaskan terkait efektivitas terapi kombinasi oral dan insulin menjelaskan bahwa tidak ada perbedaan yang signifikan pada 2 kelompok obat terhadap GDS yang menggunakan metformin-insulin dan metformin-sulfonilurea dengan p 0,330 (>0,05) (Gebrie *et al*., 2021). Hal ini dikarenakan pengobatan dengan terapi antidiabetik tidak dapat mencapai target efektivitasnya jika tidak disertai dengan pengaturan pola hidup (Jamaluddin *et al*., 2022), serta nilai GDS akan berubah tergantung dari asupan makanan dan aktifivitas yang dilakukan (Siregar *et al*., 2020). Faktor di atas pada penelitian ini belum dilakukan, sehingga menjadi keterbatasan penelitian ini.

Berdasarkan penelitian yang telah dilakukan dari parameter GDS, kedua kelompok obat tidak menunjukkan perbedaan efektivitas terkait terapi yang diberikan. Minimnya pasien yang menggunakan terapi obat antidiabetik vildagliptin karena termasuk terapi baru serta data rekam

medis yang tidak lengkap sehingga mengakibatkan jumlah sampel penelitian menjadi terbatas. Penelitian mengenai faktor yang mempengaruhi profil glikemik seperti pola hidup, makanan yang dikonsumsi serta aktivitas juga menjadi keterbatasan dalam penelitian ini. Diharapkan pada penelitian selanjutnya dapat menambah jumlah sampel dan memperluas tempat penelitian serta menganalisis faktor yang mempengaruhi efektivitas profil glikemik pasien.

4. KESIMPULAN

Efektivitas terapi antidiabetik dari dua kelompok obat, kombinasi metformin-insulin dan kombinasi metformin-vildagliptin tidak menunjukkan perbedaan yang signifikan terhadap profil glikemik HbA1c dan GDS dari pasien Diabetes Mellitus tipe 2.

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Formulasi *Tea Tree Oil* sebagai Pengawet dalam Masker Gel *Peel-Off* Ekstrak Etanol Beras Merah (*Oryza rufipogon* Griff.)

Tea Tree Oil Formulation as a Preservative in Brown Rice Ethanol Extract Peel-Off Gel Mask (Oryza rufipogon Griff.)

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Abstrak

Bahan pengawet merupakan eksipien yang sangat dibutuhkan dalam sediaan cair seperti masker gel *peel-off* ekstrak etanol beras merah. Penggunaan paraben diyakini memiliki efek samping jangka panjang pada sistem endokrin dan reproduksi sehingga perlu dikembangkan pengawet alami seperti *tea tree oil*. Selain faktor kualitas sediaan, faktor harga produk juga seringkali menjadi pertimbangan konsumen dalam membeli produk. Tujuan penelitian ini adalah untuk mendapatkan komposisi optimum bahan pengawet metil paraben, propil paraben, dan *tea tree oil* dalam sediaan masker gel *peel-off* ekstrak etanol beras merah. Metode optimasi *simplex lattice design* (SLD) digunakan untuk optimasi kombinasi pengawet metilparaben, propilparaben, dan *tea tree oil* dalam 8 *run* formula. Formula optimum ditentukan dengan parameter pH, daya sebar, waktu mengering, Angka Lempeng Total (ALT), Angka Kapang Khamir (AKK), dan biaya produksi. Hasil penelitian menunjukkan kombinasi *tea tree oil*, metilparaben, dan propilparaben dapat menjaga stabilitas masker gel *peel-off* ekstrak etanol beras merah. Berdasarkan optimasi metode numerik diperoleh komposisi optimum pengawet dalam sediaan terdiri dari metilparaben 0,119%, propilparaben 0,151%, dan *tea tree oil* 0,13%.

Kata kunci: Masker gel *peel-off*; Metilparaben; *Preservative*; Propilparaben; *Tea tree oil*

Abstract

Preservatives, such as brown rice ethanol extract peel-off gel masks, are excipients needed in liquid preparations. The use of parabens is believed to have long-term side effects on the endocrine and reproductive systems, so it is necessary to develop natural preservatives such as tea tree oil. Apart from the product quality factor, the product price factor is often a consumer consideration when purchasing products. This research aimed to obtain the optimum composition of the preservatives methylparaben, propylparaben, and tea tree oil in preparing a brown rice ethanol extract peel-off gel mask. The Simplex Lattice Design (SLD) optimization method was used to optimize the combination of methylparaben, propylparaben, and tea tree oil preservatives in 8-run formulas. The optimum formula was determined by pH, spreadability, drying time, Total Plate Number (ALT), Yeast Mold Number (AKK), and production costs. The research results showed that the combination of tea tree oil, methylparaben, and propylparaben could maintain the stability of the brown rice ethanol extract peel-off gel mask. Based on

numerical method optimization, the optimum composition of the preservative in the preparation consisted of 0.119% methylparaben, 0.151% propylparaben, and 0.13% tea tree oil.

Keywords: *Methylparaben; Peel-off gel mask; Preservative; Propylparaben; Tea tree oil*

1. PENDAHULUAN

Kontaminasi mikrobiologi adalah salah satu masalah paling parah dalam sediaan dermatologis. Mikrobiologi dapat mengurangi keefektifan sediaan dan menimbulkan risiko infeksi bagi pengguna (Bupphatanarat *et al*., 2020). Kosmetik yang mengandung air tidak dapat menghindari penggunaan bahan pengawet. Berbagai bahan dan zat yang larut dalam air pada kosmetik dapat meningkatkan pertumbuhan mikroorganisme sehingga bahan pengawet penting untuk mengurangi risiko kontaminasi yang tinggi (Kim, 2019) termasuk dalam pengembangan masker gel *peel-off* ekstrak etanol beras merah. Beras merah mengandung fitokimia utama yang mengurangi radikal bebas dan stres oksidatif, mencegah peroksidasi lipid (Dwiputri *et al.*, 2022; Mulya Lestari *et al*., 2015).

Masker gel *peel-off* merupakan sediaan dengan kandungan air yang tinggi sehingga memberikan rasa sejuk dan lembab untuk kulit kering. Sediaan masker gel *peel-off* umumnya menggunakan paraben sebagai bahan pengawet dalam formulasinya (Sutarna *et al*., 2013). Paraben adalah pengawet umum dalam kosmetik, propilparaben dan metilparaben menjadi agen sinergis yang menghambat pertumbuhan bakteri dan jamur (Fransway *et al*., 2019). Namun, paraben memiliki efek samping jangka panjang pada sistem endokrin dan reproduksi, sehingga penggunaan *essential oil* seperti *tea tree oil* lebih aman. *Tea tree oil* memiliki aktivitas antibakteri, tetapi memiliki aktifitas rendah dalam fase kosmetik berair, harganya tinggi, dan habitatnya di Australia membuatnya tidak digunakan secara luas (Kim, 2019; Mitra *et al.*, 2021; Patterson *et al*., 2019; Sarkic dan Stappen, 2018).

Tea tree oil sebagai *essential oil* murni (100%) dikomersial dengan harga sekitar $2-5 USD per 10 ml. Mayoritas produksi *tea tree oil* diproduksi di Australia, meskipun Tiongkok, Afrika Selatan, Zimbabwe, dan Kenya juga memproduksi *tea tree oil* untuk penjualan komersial. Nilai komersial yang cukup tinggi dari *tea tree oil* diakibatkan karena proses pemurnian *tea tree oil* setelah distilasi. Untuk menurunkan biaya produksi maka penggunaan *tea tree oil* sebagai bahan pengawet maka perlu dikombinasikan dengan metilparaben dan propilparaben yang harganya masih terjangkau dan mudah ditemukan (Huynh *et al.*, 2012; Kairey *et al.*, 2023; Rathee *et al.*, 2023; Sarkic dan Stappen, 2018)**.** Kombinasi *tea tree oil*, metilparaben, dan propilparaben diharapkan dapat memperkecil biaya yang dibutuhkan dan meningkatkan efektifitas antibakteri dan antijamur karena bersifat sinergis dalam menghambat pertumbuhan bakteri dan meminimalkan efek samping yang ditimbulkan paraben dalam penggunaan jangka panjang.

Penelitian kombinasi antara *tea tree oil*, metilparaben dan propilparaben dalam masker gel *peel-off* ekstrak etanol beras merah yang memasukkan aspek biaya produksi belum pernah dilakukan sebelumnya. Pada artikel (Kim, 2019), kombinasi *lavender oil, tea tree oil* dan *lemon oil* dengan pengawet sintetis (*1-3-dimetylol-5,5-dimethylhylantoin, 3-iodo-2- propynnyl butyl*

carbamate) menunjukkan hasil positif, dan dimungkinkan untuk mengurangi jumlah pengawet sintetis hingga 8,5 kali. Efek antiseptik tinggi juga diamati dalam percobaan *lavender oil* dan *tea tree oil* (masing-masing 0,5%) dan pengawet sintetis dicampur pada konsentrasi 0,1%. Penggunaan *tea tree oil* saja menurunkan daya antiseptik, tetapi penambahan pelarut 5% (polisorbat 80) ke *tea tree oil* 0,5% meningkatkan aktivitas bakteri, tetapi tidak menunjukkan efek antijamur yang cukup. Namun, ketika pengawet sintetis (0,3%) dikombinasikan bersama-sama, sifat antiseptik terpenuhi. Pada penelitian (Kulaksiz PiŞkiN & Seyhan, 2021), *tea tree oil* hanya diuji *Minimum Inhibitory Concentration* yang menghasilkan nilai MIC tercatat sebesar 0,4% untuk *Escherichia coli* dan *Bacillus subtilis* yang membuktikan bahwa efektifnya sama dengan paraben dalam kisaran konsentrasi 0,08%-0,8.

Tujuan penelitian ini untuk mendapatkan komposisi optimum bahan pengawet metil paraben, propil paraben, dan *tea tree oil* dalam sediaan masker gel *peel-off* ekstrak etanol beras merah berdasarkan aspek kualitas sediaan dan biaya produksinya. Formula diperoleh dengan menggunakan metode *simplex lattice design* (SLD). SLD merupakan salah satu metode yang terdapat pada *software Design Expert*. Metode ini dapat menentukan formula lebih cepat dan efektif, menghindari pemilihan formula secara *trial and error* (Rejeki *et al*., 2021).

2. BAHAN DAN METODE

2.1. Alat

Alat-alat yang digunakan dalam penelitian ini yaitu *rotary evaporator*, pH meter, neraca timbang (Ohaus), bejana maserasi, *Memmert Constant climate chamber* HPP110eco, Shimadzu GC-MS-QP2010, kaca bulat alat uji daya sebar, dan alat-alat gelas lainnya.

2.2. Bahan

Bahan-bahan yang digunakan dalam penelitan ini yaitu beras merah, etanol 96%, etanol 96% p.a, HPMC grade farmasi, propilenglikol grade farmasi, PVA grade farmasi, propilparaben grade farmasi, metilparaben grade farmasi, akuades grade farmasi, *tea tree oil* (Intan Chemical).

2.3. Ekstraksi

Tiga ratus gram beras merah dihaluskan, diayak, dan dimaserasi dengan etanol 96% selama 2 hari dengan pengadukan, lalu disaring dengan kertas saring untuk menghasilkan filtrat. Ampas yang tersisa kemudian dimaserasi lagi selama 2 hari, lalu kembali disaring. Filtrat dipekatkan dengan alat *rotary evaporator* hingga diperoleh ekstrak kental (Rejeki *et al*., 2021). Ekstrak kental yang didapatkan diamati secara organoleptik, dihitung rendemen dan kadar airnya.

2.4. Identifikasi *tea tree oil* dengan GC-MS (Poudel *et al.*, 2023)

Tea tree oil dianalisis menggunakan GC-MS Shimadzu GC-MS-QP2010. Kolom kapiler yang digunakan untuk analisis adalah SH-RTX-5MS (60 m - 0,32 mm - 0,25 µm) dengan ikatan silang 5% difenil/95% dimetil polisiloksan sebagai fase diam. Analisis GC dilakukan pada kondisi temperatur oven kolom, 60°C; suhu injeksi, 200°C; ion source temperature, 200°C;

interface temperature, 250°C; mode injeksi split dengan rasio split 130; Helium dengan tekanan 36,2 kPa; aliran gas total, 101,3 mL/menit; aliran kolom, 0,75 mL/menit. Sistem GC-MS dimulai dengan suhu oven awal 60°C selama 1 menit, kemudian meningkat menjadi 200°C. Deteksi spektral massa dilakukan dalam mode ionisasi elektron dengan memindai pada 40 hingga 400 m/z. Total waktu yang diperlukan untuk menganalisis sampel tunggal adalah 14 menit. Identifikasi setiap puncak yang muncul dengan menganalisis hasil spektrum massa yang diperoleh dibandingkan dengan spektrum massa pada *library index* MS.

2.5. Formulasi masker gel *peel-off*

Empat belas formula masker gel *peel-off* ekstrak beras merah (Tabel 1) dipilih dari hasil *running software design expert* 3 variabel dan 3 replikasi dengan rentang masing-masing variabel metilparaben, propilparaben dan *tea tree oil* adalah 0-0,4% (BPOM RI, 2019; Kunicka-Styczyńska *et al.*, 2009). Pembuatan sediaan masker wajah *peel off* dilakukan dengan menambahkan akuades panas ke dalam PVA hingga mengembang (wadah A). Kemudian, HPMC ditambah akuades lalu diaduk hingga mengembang (wadah B). Propilenglikol dilarutkan dengan metilparaben dan propilparaben (wadah C). Selanjutnya, wadah B dan C dimasukkan secara berurutan ke dalam wadah A. Setelah menggabungkan ekstrak beras, diaduk hingga homogen, kemudian tambahkan akuades dan aduk hingga homogen.

Tabel 1. Komposisi formula masker gel *peel-off* ekstrak beras merah sesuai desain optimasi pada *Simplex Lattice Design*.

Komposisi	***Run***													
(%)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Ekstrak beras merah	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PVA	13,75	13,75	13,75	13,75	13,75	13,75	13,75	13,75	13,75	13,75	13,75	13,75	13,75	13,75
HPMC	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Propilenglikol	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Metilparaben	0,067	0,4	0,267	0	0,2	0	0	0,2	0,067	0,4	0	0,133	0,2	0
Propilparaben	0,267	0	0,067	0	0,2	0,2	0,4	0	0,067	0	0,4	0,133	0,2	0
Tea tree oil	0,067	0	0,067	0,4	0	0,2	0	0,2	0,267	0	0	0,1	0	0,4
Etanol	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Akuades	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100

2.6. Evaluasi sediaan masker gel *peel off*

Evaluasi sediaan masker gel *peel-off* meliputi organoleptik, pH, daya sebar, waktu pengeringan serta menghitung biaya yang dibutuhkan setiap formula. Uji organoleptik mengamati secara langsung warna, bentuk, dan bau. Pengukuran pH menggunakan pH meter. Daya sebar ditentukan dengan mengukur diameter sebaran sampel antara dua pelat kaca setelah satu menit (Apriani *et al*., 2018). Waktu pengeringan diamati selama 30 menit hingga kering. Biaya dihitung dari harga pembelian setiap bahan.

2.7. Uji stabilitas (*cycling test*)

Sediaan masker gel *peel-off* disimpan dalam suhu 40±2°C dengan RH 70% selama 21 hari. Kemudian diamati organoleptik dan pengujian pH, waktu sediaan mengering, daya sebar pada hari ke-0 dan ke-21 untuk semua formula (Butler, 2000).

2.8. Uji kontaminasi mikroba

2.8.1. Uji Angka Lempeng Total (ALT)

Sebanyak 5 mL masker gel *peel-off* dipindahkan secara aseptis ke dalam erlenmeyer berisi 45 mL *pepton dilution fluid* (PDF: 1 g pepton dalam 1000 mL akuades). Dari pengenceran 10^{-1} diambil 1 mL dimasukkan ke dalam tabung reaksi berisi 9 mL PDF (pengenceran 10^{-2}). Pengenceran dilakukan sampai dengan 10^{-5}. Hasil pengenceran dipindahkan 1 mL sampel ke cawan petri (duplo), setelah itu dituang media PCA berisi 0,5% TTC dengan temperatur 45°C sebanyak 20 mL per cawan. Cawan petri tersebut dihomogenkan dengan membentuk angka delapan, biarkan beku. Kemudian, inkubasi pada temperatur 35±1°C selama 24 jam. Jumlah koloni yang tumbuh pada cawan dihitung.

2.8.2. Uji Angka Kapang Khamir (AKK)

Masker gel *peel-off* dibuat pengenceran seperti pada uji angka lempeng total. PDA yang sudah steril dimasukkan ke dalam cawan petri @20 mL/cawan dan dibiarkan membeku. Sampel yang sudah dilakukan pengenceran, diambil sebanyak 0,5 mL dimasukkan ke dalam cawan petri berisi PDA steril dan diratakan menggunakan batang bengkok. Dilakukan hal yang sama di setiap pengenceran serta dibuat duplo. Setelah beku di inkubasi pada temperatur 25±1°C selama 3-5 hari. Jumlah koloni yang tumbuh pada cawan dihitung.

2.9. Optimasi formula masker gel *peel-off* ekstrak etanol beras merah

Respon yang digunakan dalam penentuan formula optimum adalah daya sebar, waktu pengeringan, pH dan biaya yang dianalisis menggunakan metode *SLD* pada *software Design Expert versi 13* untuk mengetahui pengaruh faktor dan interaksi faktor. Formula optimum diperoleh dengan melihat nilai *desirability* tertinggi.

3. HASIL DAN PEMBAHASAN

3.1. Ekstraksi

Hasil ekstraksi beras merah berupa ekstrak kental berwarna merah pekat dengan bau beras khas dengan rendemen ekstrak sebesar 1,715%. Rendemen yang dihasilkan lebih besar daripada penelitian sebelumnya, yaitu sebesar 1,053% (Kangwan *et al*., 2023). Hal ini kemungkinan disebabkan karena perlakuan remaserasi yang dalam prosesnya ada siklus pergantian pelarut sehingga efektivitas penarikan akan lebih maksimal (Ningsih *et al*., 2018). Kadar air ekstrak kental beras merah yang dihasilkan adalaha 13,69%. Ekstrak kental yang baik memiliki kadar air 5-30% (Voight, 1995).

3.2. GC-MS (*Gas chromatography–mass spectrometry)*

Senyawa yang teridentifikasi pada *tree tea oil* (Tabel 2) menunjukkan bahwa hasil dari 5 senyawa dengan peak tertinggi memiliki area % yang tidak berbeda jauh dengan penelitian milik (Le *et al*., 2021) dan sesuai dengan standar ISO (ISO 4730:2017). Terdapat satu senyawa pada peak tertinggi yang dengan nilai *retention area* 30,72% yaitu 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (CAS) 4-Terpineol atau bisa disebut terpinen-4-ol dengan CAS: 562-74-3.

Tabel 2. Hasil analisis Gas Chromatography Mass Spectrometry (GC-MS) dari *tea tree oil*.

Senyawa	Area % GCMS	Area % (Le *et al*., 2021)	ISO 4730:2017
terpinen-4-ol	30,72	39,23	30,0 – 48,0
γ-terpinene	26,73	28,69	10,0 – 28,0
ά-pinene	9,42	2,71	1,0 – 6,0
ά-terpinene	7,15	14,64	5,0 – 13,0
ά-terpinolene	7,15	4,36	1,5 – 5,0

Sesuai dengan penelitian *tea tree oil* adalah campuran dari sekitar 100 senyawa, termasuk monoterpen terpinen-4-ol (30% dari minyak), 1,8-cineole dan terpinolene (Huang *et al*., 2021; Nogueira *et al*., 2014). Pada penelitian lainnya, terpinen-4-ol juga menjadi komponen utama pada *tree tea oil* dengan nilai *retention area* 39,23% yang dibandingkan dengan ISO (ISO 4730:2017) standard yaitu 30.0 – 48.0, (Le *et al*., 2021). Oleh karena itu komponen terpinen-4-ol komposisinya masih sesuai dengan kisaran standardnya.

3.3. Evaluasi sediaan masker gel *peel-off* ekstrak etanol beras merah

Empat belas formula yang dipilih dari hasil *running software design expert* metode *simplex lattice design* kemudian dihitung biaya yang dibutuhkan setiap formula dan dievaluasi secara fisik dan cemaran mikroba.

3.3.1. Organoleptik

Hasil pengamatan organoleptik selama 21 hari menunjukkan bahwa dari keempat belas formula tersebut berwarna merah dengan aroma beras yang khas. Masker gel *peel-off* ekstrak etanol beras merah merupakan sediaan yang homogen, meskipun ada perbedaan dalam aroma dan konsistensi, khususnya, formula yang mengandung *tea tree oil* akan memiliki aroma yang lebih kuat dan konsistensi yang lebih encer.

3.3.2. Daya sebar

Hasil evaluasi masker gel *peel-off* ekstrak etanol beras merah pada hari ke-0 (Tabel 3) dan hari ke 21 (Tabel 4) menunjukkan adanya perubahan pada daya sebar (Kurnianto *et al.*, 2017). Setelah 21 hari formula run 4, 8, 9, dan 14 tidak memenuhi syarat daya sebar masker gel *peel-off*, yaitu 5-7 cm. Hal ini diduga karena air menguap selama penyimpanan pada suhu 40°C. HPMC kehilangan airnya karena suhu yang tinggi, sehingga mengakibatkan penurunan

viskositas sediaan. Semakin rendah viskositas sediaan, semakin rendah nilai daya sebar (Abbas *et al*., 2013; Apriani *et al*., 2022). Pemodelan respon daya sebar menggunakan Persamaan 1.

$$Y= 17,24\ A + 17,62B + 24,28C + 11,957AB – 1,1AC – 64,61BC$$

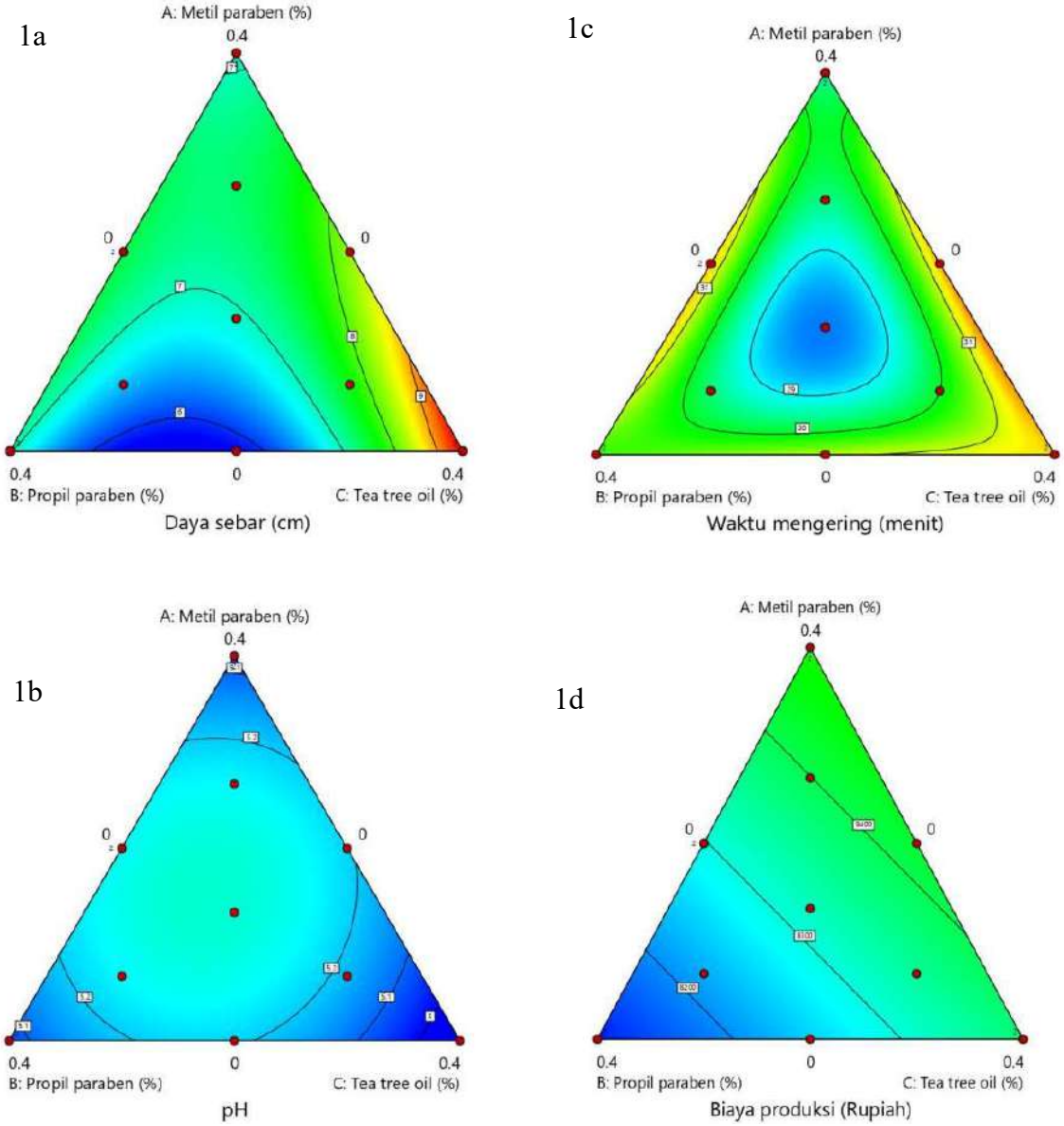
Persamaan 1. Pemodelan respon daya sebar masker gel peel-off ekstrak etanol beras merah. Keterangan: Y = Daya sebar, A = Metilparaben (%), B = propilparaben (%), C = Tea tree oil (%).

Tabel 3. Hasil evaluasi masker gel *peel-off* ekstrak etanol beras merah hari ke-0.

Run	pH	Waktu mengering (menit)	Daya sebar (cm)	Angka lempenng total	Angka kapang khamir
1	7± 0,000	20,37 ± 0,001	7,667 ± 0,557	Negatif	Negatif
2	8± 0,000	18,45 ± 0,001	8,300 ± 0,2	Negatif	Negatif
3	7± 0,000	18,50 ± 0,001	8,400 ± 0,458	Negatif	Negatif
4	8± 0,000	19,50 ± 0,002	8,300 ±0,245	Negatif	Negatif
5	7± 0,000	16,43 ± 0,000	8,433 ± 0,513	Negatif	Negatif
6	7± 0,000	17,08 ± 0,001	7,967 ± 0,058	Negatif	Negatif
7	7± 0,000	18,53 ± 0,000	8,267 ± 0,252	Negatif	Negatif
8	7± 0,000	23,97 ± 0,001	8,033 ± 0, 058	Negatif	Negatif
9	7± 0,000	25,62 ± 0,002	8,100 ± 0,1	Negatif	Negatif
10	8± 0,000	16,38 ± 0,000	8,733 ± 0,569	Negatif	Negatif
11	7± 0,000	16,92 ± 0,001	8,800 ± 0,264	Negatif	Negatif
12	7± 0,000	22,75 ± 0,001	8,767 ± 0,251	Negatif	Negatif
13	7± 0,000	17,13 ± 0,001	8,067 ± 0,971	Negatif	Negatif
14	8± 0,000	17,53 ± 0,000	8,067 ± 0,208	Negatif	Negatif

Tabel 4. Hasil evaluasi masker gel *peel-off* ekstrak etanol beras merah setelah 21 hari.

Run	pH	Waktu mengering (menit)	Daya sebar (cm)	Angka lempenng total	Angka kapang khamir
1	6 ± 0,000	27,92 ± 0,001	7,267 ± 0,252	Negatif	Negatif
2	5 ± 0,000	29,48 ± 0,001	6,967 ± 0,153	Negatif	Negatif
3	6 ± 0,000	29,07 ± 0,001	6,733 ± 0,208	Negatif	Negatif
4	5 ± 0,000	30,52 ± 0,000	9,6 ± 0,529	Negatif	Negatif
5	5 ± 0,000	32,28 ± 0,001	7,633 ± 0,152	Negatif	Negatif
6	5 ± 0,000	31,03 ± 0,001	5,8 ± 0,265	Negatif	Negatif
7	5 ± 0,000	28,75 ± 0,001	6,9 ± 0,100	Negatif	Negatif
8	5 ± 0,000	31,33 ± 0,001	8,7 ± 0,265	Negatif	Negatif
9	5 ± 0,000	30,80 ± 0,004	8,2 ± 0,100	Negatif	Negatif
10	5± 0,000	29,35 ± 0,000	6,967 ± 0,153	Negatif	Negatif
11	5± 0,000	32,32 ± 0,001	6,9 ± 0,100	Negatif	Negatif
12	5± 0,000	29,13 ± 0,001	5,8 ± 0,265	Negatif	Negatif
13	5± 0,000	31,25 ± 0,001	7,63 ± 0,153	Negatif	Negatif
14	5± 0,000	31,83 ± 0,001	9,6 ± 0,529	Negatif	Negatif



Gambar 1. *Contour plot* respon evaluasi sediaan masker gel *peel-off* ekstrak etanol beras merah. Keterangan: daya sebar (1a), pH (1b), waktu mengering (1c), dan biaya produksi (1d) masker gel *peel-off* ekstrak etanol beras merah.

Metilparaben, propilparaben, dan *tea tree oil* memiliki nilai penyebaran yang lebih tinggi (Persamaan 1). Area warna merah disekitar *tea tree oil* pada *contour plot* (Gambar 1A) menunjukkan bahwa *tea tree oil* paling dominan meningkatkan daya sebar. Interaksi metilparaben dan propilparaben memiliki dampak yang berbanding lurus terhadap besar daya sebar (Persamaan 1). Sebaliknya, interaksi propilparaben dan metilparaben dengan *tea tree oil* memiliki dampak yang berbanding terbalik. Paraben belum terbukti mempengaruhi daya sebar, tetapi Persamaan 1 menunjukkan penambahan metilparaben dan propilparaben mempengaruhi daya sebar. Penurunan daya sebar disebabkan interaksi antara *tea tree oil* dengan propil paraben dan metil paraben. *Contour plot* (Gambar 1A), warna biru menunjukkan nilai daya sebar paling

kecil adalah area kombinasi *tea tree oil* dan propilparaben. Formulasi *essential oil* bunga cengkih dalam sediaan topikal lain menunjukkan bahwa peningkatan konsentrasi *essential oil* dalam formula dapat menghasilkan nilai daya sebar yang lebih besar (Kurnianto *et al.*, 2017).

3.3.3. pH

Formula masker gel *peel-off* pada pH 14 menunjukkan tetap stabil selama 21 hari (Tabel 3 dan Tabel 4) karena masih memenuhi kriteria pH kulit yaitu dalam interval pH 4,5-8 (SNI 16-4399-1996). Pemodelan respon pH menggunakan Persamaan 2.

$$Y = 12{,}67A + 12{,}67B + 12{,}28C + 4{,}77AB + 5{,}43AC + 5{,}43BC$$

Persamaan 2. Pemodelan respon daya sebar masker gel *peel-off* ekstrak etanol beras merah. Keterangan: Y = pH, A = Metilparaben (%), B = Propilparaben (%), C = *Tea tree oil* (%).

Metilparaben, propilparaben, dan *tea tree oil* memiliki dampak positif terhadap respons pH (Persamaan 2). Interaksi antara paraben dan *tea tree oil* juga memiliki dampak positif. Hal ini terlihat pada *contour plot* (Gambar 1B), area tengah berwarna biru menunjukkan bahwa sediaan memiliki pH yang besar. Belum ada artikel yang melaporkan terkait pengaruh paraben dan *tea tree oil* terhadap pH, namun persamaan dapat dipakai untuk memprediksi respon terhadap tingkat tertentu dari masing-masing faktor (Stat-Ease, Inc, 2023).

3.3.4. Waktu mengering

Waktu yang dibutuhkan untuk mengering seluruh formula meningkat setelah penyimpanan selama 21 hari (Tabel 3 dan Tabel 4). Faktor lingkungan seperti udara dapat memengaruhi waktu mengering. Kemasan yang kurang kedap dapat menyebabkan peningkatan volume air dalam sediaan karena penyerapan uap dari luar. Waktu mengering dapat dipengaruhi oleh kandungan propilenglikol sebagai humektan dalam sediaan. Propilenglikol dapat menarik dan menahan air karena afinitas dan higroskopisitasnya yang tinggi. Hal ini dapat menghilangkan lembap dari lingkungan dan mengurangi air yang menguap dari sediaan untuk menjaga stabilitas (National Center for Biotechnology Information, 2023; Tanjung & Rokaeti, 2020). Pemodelan respon waktu mengering menggunakan Persamaan 3.

$$Y = 73{,}44A + 75{,}72B + 78{,}6C + 41{,}220AB + 30{,}81AC + 1{,}73BC - 1350{,}85ABC$$

Persamaan 3. Pemodelan respon daya sebar masker gel *peel-off* ekstrak etanol beras merah Keterangan: Y = Waktu mengering, A = Metilparaben (%), B = Propilparaben (%), C = *Tea tree oil* (%).

Metilparaben, propilparaben, dan *tea tree oil* memiliki dampak positif terhadap respon waktu mengering (Persamaan 3). Interaksi paraben dan *tea tree oil* juga menunjukan dampak positif terhadap waktu mengering di mana penambahan konsentrasi paraben dan *tea tree oil* diprediksi meningkatkan waktu mengering, namun kombinasi ketiganya diprediksi menurunkan waktu mengering. *Tea tree oil* sebagai fase minyak memiliki lapisan yang mampu mencegah penguapan air karena tidak bersentuhan dengan permukaan air, sehingga menaikkan nilai waktu mengering (Sriram *et al*., 2020).

Contour plot (Gambar 1C), menunjukkan bahwa warna biru menunjukkan waktu mengering yang paling kecil, dengan warna biru muda, hijau, kuning, dan merah yang menunjukkan nilai daya sebar yang paling besar. Daerah biru menunjukkan bahwa komposisi interaksi paraben dan *tea tree oil* dapat menurunkan nilai daya sebar dan mengurangi nilai waktu mengering. Di sisi lain, warna kuning kemerahan menunjukkan konsentrasi *tea tree oil* yang paling dominan.

3.3.5. Biaya produksi

Formula dengan kandungan *tea tree oil* yang dominan (formula 4, 9 dan 14) membutuhkan biaya lebih besar daripada yang hanya menggunakan metilparaben ada/atau propilparaben (Tabel 5). Pemodelan respon biaya menggunakan Persamaan 4.

$$Y = 21188{,}31A + 20297{,}91B + 20930{,}78C$$

Persamaan 4. Pemodelan respon daya sebar masker gel *peel-off* ekstrak etanol beras merah. Keterangan: Y = Biaya, A = Metilparaben (%), B = Propilparaben (%), C = *Tea tree oil* (%).

Metilparaben, propilparaben, dan *tea tree oil* masing-masing diprediksi meningkatkan nilai biaya secara *linear* dan tidak memiliki interaksi antara variabel karena model yang disarankan adalah *linear* (Persamaan 4). Hal ini juga terlihat pada *countout plot* (Gambar 1D), tidak menunjukkan interaksi antara paraben dan *tea tree oil* karena garis pada *contour plot* membentuk garis *linear*; selain itu, Gambar 1D juga terlihat warna biru pada area propilparaben menunjukkan biaya yang lebih rendah.

Tabel 5. Data biaya produksi masker gel *peel-off* ekstrak etanol beras merah.

Run	**Biaya produksi (Rupiah)**
1	8218,72
2	8100
3	8218,72
4	8812,32
5	8100
6	8456,16
7	8100
8	8456,16
9	8574,881
10	8100
11	8100
12	8337,438
13	8100
14	8812,32

3.2.6. Uji kontaminasi mikroba

Tea tree oil sebagai bahan pengawet dapat dibuktikan dengan formula 4 dan 14 yang hanya menggunakan *tea tree oil* sebagai bahan pengawet memiliki hasil uji cemaran negatif (<10 koloni). Hal ini sesuai dengan (Dreger & Wielgus, 2013), bahwa *tea tree oil* dapat menjadi alternatif pengawet alami untuk meminimalisir efek samping dari paraben. Selain itu dapat menjadi pendukung bahwa *tea tree oil* mempunyai aktivitas antibakteri yang sama dengan

kombinasi metilparaben dan propilparaben seperti data yang dilaporkan oleh (Matos & Cruz, 2018) karena memiliki hasil cemaran negatif. Kombinasi *essential oil* sebagai bahan pengawet alami dan paraben sebagai bahan pengawet sintetis dapat menjadi negatif untuk mengurangi efek samping dari penggunaan paraben dengan cara meminimalisir konsentrasi yang digunakan (Bello *et al*., 2022; Herman *et al*., 2013).

3.4. Penentuan formula optimum

Respon pH, daya sebar, waktu mengering dan biaya digunakan untuk menentukan formula optimum dengan pemberian nilai dan bobot pada setiap respon (Tabel 6). *Goal*, nilai *lower limit* dan *upper limit* dari masing-masing respon pada Tabel 6 ditentukan sesuai dengan persyaratan sediaan masker gel masker *peel-off* yang baik. Daya sebar sediaan mengacu pada Standar Nasional SNI 16-4955-1998 yaitu 5,54-6,08 cm (Hanum *et al*., 2023) Nilai pH yang baik yaitu dalam rentang 4,5 – 8 (SNI 16-4399-1996). Nilai waktu mengering masker gel *peel-off* terbaik adalah 15 menit setelah diaplikasikan ke kulit (Partuti *et al*., 2021). Optimasi metode numerik dengan *software design expert* menunjukkan kombinasi optimum bahan pengawet yaitu metilparaben 0,119%, propilparaben 0,151%, dan *tea tree oil* 0,13%, dengan *desirability* 0,270 (Stat-Ease, Inc, 2023).

Tabel 6. Parameter optimasi dalam penentuan formula optimum masker gel peel-off ekstrak etanol beras merah

Parameter	*Goal*	*Lower Limit*	*Upper Limit*	*Importance*
Metilparaben	*is in range*	0	0,4	3
Propilparaben	*is in range*	0	0,4	3
Tea tree oil	*is in range*	0	0,4	3
Daya sebar (cm)	*is in range*	5	7	3
pH	*is in range*	4,5	8	3
Waktu mengering (menit)	*Minimize*	15	30	3
Biaya produksi (rupiah)	*Minimize*	8100	8812,32	3

Nilai *desirability* dipengaruhi oleh jumlah respon dan target yang ingin dicapai untuk memperoleh formula optimum (Saryanti *et al.*, 2019). Nilai *desirability* yang dihasilkan kurang mendekati 1 karena waktu mengering tidak mendekati dari *goal* yang diinginkan. Formula optimum hasil prediksi mempunyai nilai pH 5,294, daya sebar 6,8 cm, waktu mengering 28,456 menit, dan biaya produksi Rp. 8.307.

4. KESIMPULAN

Hasil optimasi menggunakan *software Design Expert version 13* menunjukkan formula optimum masker gel *peel-off* ekstrak etanol beras merah dengan komposisi metilparaben 0,119%, propilparaben 0,151%, dan *tea tree oil* 0,13%, (*desirability* 0,270).

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Cost Effectiveness Analysis Jamu Saintifik Antihipertensi di Wisata Kesehatan Jamu Kalibakung dan Terapi Kombinasi Antihipertensi Konvensional di Puskesmas Kalibakung

Cost-Effectiveness Analysis of Anti-Hypertension Scientific Jamu in Kalibakung Health Tourism and Conventional Anti-Hypertension Combination Therapy in Kalibakung Puskesmas

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Abstrak

Hipertensi menjadi masalah utama bagi masyarakat di Indonesia dengan prevalensi mencapai 32,2%. Berbagai jenis terapi pengobatan sudah banyak direkomendasikan salah satu yang direkomendasikan dari JNC VIII adalah penggunaan kombinasi antihipertensi konvensional. Kementerian kesehatan melalui B2P2TOOT juga berhasil memproduksi jamu antihipertensi tersaintifikasi yang ditujukan sebagai terapi komplementer. Saat ini masalah efektivitas dalam mencapai target tekanan darah masih menjadi masalah untuk terapi antihipertensi konvensional maupun jamu saintifik. Selain itu besarnya biaya yang dikeluarkan untuk masing-masing terapi juga menjadi pertimbangan bagi pasien dalam menentukan jenis terapi yang akan dipilih. Berdasarkan permasalah tersebut, makan akan di teliti *cost effectiveness analysis* antara jamu saintifik antihipertensi dengan kombinasi antihipertensi konvensional. Rancangan penelitian yang digunakan yaitu observasional analitik dan analisis data menggunakan metode *Incremental Cost Effectiveness Ratio* (ICER) dan diinterpretasikan menggunakan *Cost Effectiveness Plane* (CEP). Pengambilan data biaya menggunkan perspektif *provider* sebagai penyedia layanan kesehatan. Responden sebanyak 44 pasien, terdiri dari 18 pasien dengan terapi kombinasi antihipertensi konvensional di Puskemas Kalibakung, dan 26 pasien dengan terapi jamu saintifik antihipertensi di Wisata Kesehatan Jamu Kalibakung. Hasil persentase efektivitas keberhasilan terapi kombinasi antihipertensi konvensional mencapai 47%, sedangkan persentase efektivitas keberhasilan terapi jamu saintifik antihipertensi mencapai 53,8%. Total biaya rata rata per pasien untuk terapi kombinasi antihipertensi konvensional sebanyak Rp 115.561, dan untuk terapi jamu saintifik antihipertensi sebanyak Rp 102.138. Data tersebut kemudian dianalsis dengan ICER dan didapatkan nilai ICER Rp -2.029/pasien. Interpretasi nilai ICER menggunakan CEP menyimpulkan bahwa terapi jamu saintifik antihipertensi lebih *cost effective* dibandingkan terapi kombinasi antihipertensi konvensional karena nilai ICER berada di area *SouthEast.*

Kata kunci: *Cost Effectiveness Analysis* (CEA); Hipertensi; Jamu saintifik antihipertensi; Kombinasi antihipertensi konvensional

Abstract
Hypertension has become the main problem for Indonesian society, with 32,2% of prevalence. There are many recommendations for treatment therapy. The combined usage of conventional anti-hypertension is one of the recommendations from JNC VIII. The Ministry of Health, through B2P2TOOT, is successfully producing anti-hypertension jamu saintifik that is intended for complementary therapy. Today, the effectiveness of reaching blood pressure targets is still a problem for conventional anti-hypertension therapy as well as jamu saintifik (certified jamu). Moreover, the high cost of each therapy is still a consideration for the medical patient in determining the kind of therapy they will choose. Based on the problem mentioned before, there will be research about the cost-effectiveness analysis between anti-hypertension jamu saintifik and a combination of conventional anti-hypertension. The analytic observational was used as the research design, and the Incremental Cost-Effectiveness Ratio (ICER) method was used as the data analysis and interpreted by the Cost-Effectiveness Plane (CEP). The cost data is taken using the provider's perspective as a health service provider. The successful effectiveness of combination therapy conventional anti-hypertension reached 47%, while anti-hypertension jamu saintifik therapy reached 53,8%. As a result, ICER was used to analyze the data and gain the ICER value Rp -2.029/medical patient. The interpretation of the ICER value by CEP concluded that anti-hypertension jamu saintifik therapy is more cost-effective than conventional anti-hypertension combination therapy because the ICER value is in the southeast area.

Keywords: *Anti-hypertension jamu saintifik; Cost Effectiveness Analysis (CEA); Hypertension*

1. PENDAHULUAN

Hipertensi hingga saat ini masih menjadi penyakit yang memberikan masalah besar bagi dunia dan khususnya di Indonesia. WHO memprediksi persentase pravalensi hipertensi di dunia telah mencapai 11% dan 50 % diantaranya di negara berkembang. Sedangkan untuk efektivitas dari terapi yang saat ini digunakan baru mencapai 34% (World Health Organization, 2012). Indonesia sebagai negara berkembang memiliki pravalensi cukup tinggi untuk penyakit hipertensi mencapai 32,2% dan belum seluruh pasien hipertensi dapat terdiagnosa dan ditangani secara baik oleh tenaga kesehatan (Kemenkes.RI, 2014).

Tujuan terapi pada pasien hipertensi sejatinya untuk mengurangi kejadian kematian akibat kondisi perburukan dari pasien serta mengurangi angka kesakitan bagi pasien (Gunawan, 2007). Terapi yang dianjurkan pertama kali adalah terapi non-farmakologi yaitu memperbaiki pola hidup pasien. Jika cara ini tidak efektif maka baru digunakan terapi farmakologi untuk membantu menurunkan dan menstabilkan tekanan darah. Penggunaan antihipertensi di Indonesia pada umumnya sudah sesuai dengan *guideline* yang tersedia, mulai dari kombinasi Diuretik dengan *Beta Blocker*, kombinasi Diuretik dengan *Calcium Channel Blocker*, kombinasi Diuretik dengan ACE Inhibitor, kombinasi *Beta Blocker* dengan *Calcium Channel Blocker*.

Jauh sebelum ditemukannya obat konvensional untuk hipertensi, masyarakat Indonesia sudah terlebih dahulu menggunakan jamu untuk mencegah dan menyembuhkan penyakit. Jamu meliputi segala bahan alam yang diolah dan diracik menurut cara tradisional untuk memperkuat badan manusia, mencegah penyakit atau menyembuhkan manusia yang menderita penyakit (Siswanto, 2012). Pemerintah melalui Permenkes No.003/Menkes/Per/2010 yang mengatur

program saintifikasi jamu ingin mendorong adanya peningkatan penelitian yang berkaitan penggunaan jamu di masyarakat. Jamu saintifik merupakan ramuan jamu yang sudah lolos uji efektivitas dan uji toksik yang aman dikonsumsi dalam jangka waktu yang panjang. Pengambilan keputusan untuk memilih strategi terapi mana yang memberikan *outcome* terapi rasional yang terbaik, penting melakukan analisis dan evaluasi yang membandingkan antara biaya yang dibutuhkan dengan *outcome* yang dihasilkan (Chen, 2022). *Decision maker* atau pengambil keputusan akan memilih terapi antihipertensi yang mampu memberikan *value for money* atau terapi tersebut memiliki nilai yang berkhasiat, *safety,* bermutu dan memiliki nilai ekonomi yang tinggi. Nilai ekonomi yang tinggi dapat dikatakan terapi yang *cost effective*, yaitu terapi yang dipilih memiliki biaya yang lebih affordable atau terjangkau bagi penyedia maupun pengguna dan memiliki nilai efektivitas klinis yang tinggi.

Tujuan penelitian ini untuk membandingkan *cost effectiveness* terapi kombinasi antihipertensi (kaptopril dan amlodipin) dengan jamu saintifikasi antihipertensi sebagai terapi komplementer.

2. BAHAN DAN METODE

Rancangan penelitian yang digunakan yaitu model observasional analitik (non eksperimental). Penelitian menggunakan pendekataan retrospektif, yang merupakan suatu penelitian dengan mengambil data rekam medis pasien untuk menilai efektivitas terapi dari jamu saintifik antihipertensi dan terapi kombinasi antihipertensi konvensional. Data biaya dihitung berdasarkan perspektif provider sebagai penyedia layanan. Jamu saintifik antihipertensi di Kabupaten Tegal hanya tersedia dan diresepkan di Wisata Kesehatan Jamu dan berisikan 3 kombinasi tanaman herbal yaitu seledri 5 g, daun kumis kucing 3 g, pegagan 3 g. Terapi antihipertensi diambil dari pasien yang menerima kombinasi kaptopril dan amlodipin di Puskesmas Kalibakung. Kombinasi tersebut terbukti efektif menurunkan tekanan darah dan memiliki efek samping yang dapat ditoleransi. JNC 8 juga merekomendasikan penggunaan dua terapi tersebut pada pasien tanpa dan dengan komplikasi tertentu.

Populasi adalah pasien dengan hipertensi umum di Wisata Kesehatan Jamu Kalibakung dan Puskesmas Kalibakung tahun 2017. Pengambilan sampel menggunakan metode *purposive sampling* dengan kriteria inklusi pasien, yaitu pasien dengan tekanan darah > 140/90 mmHg dengan usia > 18 tahun, dan tanpa penyakit komorbid. Pasien yang telah menggunakan jamu saintifik minimal 3 bulan dan pasien yang menggunakan 2 kombinasi antihipertensi konvensional selama minimal 3 bulan. Berdasarkan *expert* opinion dari dokter yang meresepkan jamu saintifik, efektivitas jamu saintifik dapat di evaluasi setelah penggunaan minimal 3 bulan. Efektivitas masing-masing terapi dihitung dari persentase keberhasilan dalam mencapai target tekanan darah. Analisis efektivitas biaya dihitung menggunakan metode *Incremental Cost Effectiveness Ratio* (ICER) dan interpretasikan mengunakan *Cost Effectiveness Plane* (CEP).

3. HASIL DAN PEMBAHASAN

3.1. Karakteristik responden

Pasien hipertensi di Puskesmas Kalibakung yang menggunakan terapi 2 kombinasi antihipertensi konvensional selama minimal 3 bulan sebanyak 18 pasien. Mayoritas pasien di Puskesmas Kalibakung adalah perempuan sebanyak 11 pasien, dan 7 pasien laki-laki. Karakteristik usia pasien di Puskesmas Kalibakung mayoritas adalah pasien dengan rentan usia <50 tahun (50%), sebanyak (50%) pasien dengan usia >50 tahun. Usia menjadi faktor resiko yang tidak dapat dimodifikasi pada hipertensi karena terjadi gangguan fungsi endotelia serta pembuluh darah arteri mulai mengalami kekakuan. Pekerjaan pasien yang menjadi responden mayoritas adalah ibu rumah tangga (33,0%) dan buruh (27,7%), disusul wiraswasta (27,7%), dan pegawai negeri sipil (11,1%). Karakteristik pekerjaan pasien di Wisata Kesehatan Jamu juga didominasi oleh ibu rumah tangga, hal tersebut sejalan dengan penelitian sebelumnya bahwa mayoritas pengguna jamu merupakan pekerja dengan pendapatan dibawah UMR. Secara statistik karakteristik pasien di Wisata Kesehatan Jamu Kalibakung tidak memiliki perbedaan yang signifikan dengan pasien di Puskesmas Kalibakung, karena hasil analisis menggunakan *chi-square* menunjukan nilai *p-value* >0,05 (Tabel 1).

Tabel 1. Karakteristik responden di Puskesmas Kalibakung dan Klinik Wisata Kesehatan Jamu.

Karakteristik	Puskesmas		Klinik Kesehatan Jamu		Total		p *value*
	N	%	N	%	N	%	
Jenis Kelamin							
Laki-laki	7	38,8%	15	57,7%	22	50%	0,037
Perempuan	11	61,2%	11	42,3%	22	50%	
Usia							
<50	9	50,0%	16	61,5%	25	56,8%	0,120
>50	9	50,0%	10	38,5%	19	43,1%	
Pekerjaan							
Ibu rumah tangga	6	33,0%	8	30,8%	14	31,8%	
Buruh			3	11,5%	8	18,8%	
Pegawai negeri sipil	5	27,7%	2	7,7%	4	9,09%	0,117
Wiraswasta	2	11,1%	8	30,8%	13	29,5%	
Petani			4	15,3%	4	9,09%	
Sopir	5	27,7%	1	3,9%	1	2,27%	

3.2. Efektivitas terapi

Keberhasilan atau efektivitas terapi antihipertensi adalah mampu menurunkan dan mengontrol tekanan darah pasien dengan target yang sudah ditentukan. Menurut JNC VII keberhasilan terapi kombinasi antihipertensi pada pasien hipertensi umum dengan usia <60 tahun adalah apabila tekanan darah sistolik dan diastolik mencapai target <140/90 mmHg, dan usia >60 tahun adalah <150/90 mmHg.

Dokter yang meresepkan jamu saintifik hipertensi dan sekaligus melakukan monitoring tekanan darah pasien mengatakan bahwa target penurunan darah mengikuti pedoman yang digunakan oleh terapi antihipertensi konvensional. Berdasarkan hasil observasi data rekam

medik di Wisata Kesehatan Jamu didapatkan sampel sebanyak 26 pasien yang memenuhi kriteria inklusi yaitu minimal 3 bulan menggunakan ramuan jamu saintifik antihipertensi pada periode tahun 2017. Sedangkan jumlah sampel pada Puskesmas Kalibakung sebanyak 18 pasien dengan kriteria minimal 3 bulan menggunakan terapi kombinasi antihipertensi kaptopril dan amlodipin pada periode tahun 2017.

Sebanyak 8 pasien yang diberikan terapi kombinasi antihipertensi konvensional berhasil mencapai target tekanan darah (47%) dan 10 pasien tidak berhasil mencapai target tekanan darah (53%) (Tabel 2). Sebanyak 10 pasien dengan outcome tidak berhasil mencapai target tekanan darah memiliki riwayat tidak patuh dalam mengonsumsi obat. Hal tersebut sejalan dengan hasil penelitian (Kisa, 2003) yang menyatakan bahwa tingkat kepatuhan pasien <50% maka akan menurunkan efektivitas terapi.

Tabel 2. Efektivitas biaya jamu saintifik pasien hipertensi di Wisata Kesehatan Jamu dan Puskesmas Kalibakung.

Variabel	Kombinasi Amlodipin dan Kaptopril (N=18)	Jamu Saintifik (N=26)	Total (N=56)
Berhasil	8 (47%)	14 (53,8%)	26(46,4%)
Tidak berhasil	10 (53%)	12 (46,3%)	30(53,6%)

Jamu saintifik memiliki persentase keberhasilan mencapai target tekanan darah lebih baik dari penggunaan kombinasi antihipertensi konvensional. Dalam suatu penelitian dilaporkan bahwa formula jamu saintifik antihipertensi yang terdiri dari ramuan pegagan, kumis kucing, dan seledri memiliki efek menurunkan tekanan darah lebih besar dari kaptopril (Hussaana *et al.*, 2016). Efek sinergis seledri, pegagan, dan daun kumis kucing telah terbukti menurunkan tekanan darah. Seledri memiliki efek hipotensif, memiliki efek negatif terhadap inotropik dan khronotropik melalui stimulus reseptor musakarinik (Brankovi *et al.*, 2010). Apigenin dalam seledri memiliki efek vasodilator dan diuretik dengan mengurangi simpanan natrium tubuh, sehingga mengurangi volume darah dan *cardiac output* (Zhang *et al.*, 2000). Daun kumis kucing mampu menghambat aktivitas *angiotensin converting enzyme* (ACE) (Saputri *et al.*, 2015). Daun kumis kucing juga mengandung kalium yang dapat meningkatkan aliran ginjal dan ekskresi natrium, sehingga tekanan darah menurun (Adam *et al.*, 2009). Efek hipotensif pegagan dari kandungan triterpenoidnya yang mempengaruhi sistem reninangiotensin-aldosteron (RAAS) dan menghambat ACE dan vasokontriksi (Adaramoye *et al.*, 2009). Efektivitas yang sama baiknya juga didapatkan oleh jamu saintifik pada pasien osteoarthritis ketika dibandingkan dengan piroksikam sebagai terapi konvensional (Atikasari *et al*., 2023).

3.3. Biaya langsung medis

Biaya yang dihitung dalam penelitian ini berdasarkan perspektif *provider* sebagai pemberi pelayanan kesehatan (Nuzulia, 2024) sehingga yang dimaksud biaya langsung medis adalah keseluruhan biaya yang dibutuhkan oleh puskesmas untuk memberikan terapi hipertensi meliputi biaya obat antihipertensi, biaya administrasi, pelayanan pemeriksaan lainnya seperti

pemeriksaan tekanan darah tidak dipungut biaya sedangkan biaya pemeriksaan laboratorium tidak dilakukan pada seluruh pasien yang masuk dalam penelitian ini. Total biaya langsung medis yang dikeluarkan oleh Puskesmas Kalibakung sebesar Rp2.080.100 untuk 18 pasien, dan rata rata biayanya adalah Rp115.561 tiap pasien. Wisata Kesehatan Jamu memiliki total biaya langsung medis sebesar Rp2.653.000 dengan rata rata biaya lebih kecil yaitu Rp102.038,46 dibandingkan kelompok kombinasi antihipertensi konvensional yang ada di Puskesmas Kalibakung (Tabel 3).

Tabel 3. Komponen biaya langsung medis pasien hipertensi di Wisata Kesehatan Jamu dan Puskesmas Kalibakung.

Jumlah Biaya	Kombinasi Antihipertensi Konvensional (N=18)	Jamu Saintifik Antihipertensi (N=26)
Biaya obat	Rp1.995.100	Rp2.445.000
Biaya admistrasi	Rp85.000	Rp208.000
Total biaya	**Rp2.080.100**	**Rp2.653.000**
Rata-rata biaya	Rp115.561	Rp102.038

3.4. *Cost Effectiveness Analysis* (CEA)

Metode *Incremental Cost Effectiveness Ratio* (ICER) dalam penelitian ini untuk mengetahui terapi mana yang lebih *cost effective*. ICER sendiri merupakan metode yang menghitung biaya yang harus dikeluarkan untuk menaikan efektivitas dengan beralih dari suatu pengobotan ke pengobatan lain (Wells *et al.,* 2009). Komponen biaya yang dihitung hanya biaya langsung medis karena perspektif yang yang digunakan adalah perspektif provider. Prinsip dari ICER adalah membandingkan selisih input dan output dari 2 program. Pengukuran nilai ICER dalam penelitian ini membandingkan antara jamu saintifik antihipertensi dengan kombinasi antihipertensi konvensional. Perhitungan untuk ICER, menggunakan Persamaan 1.

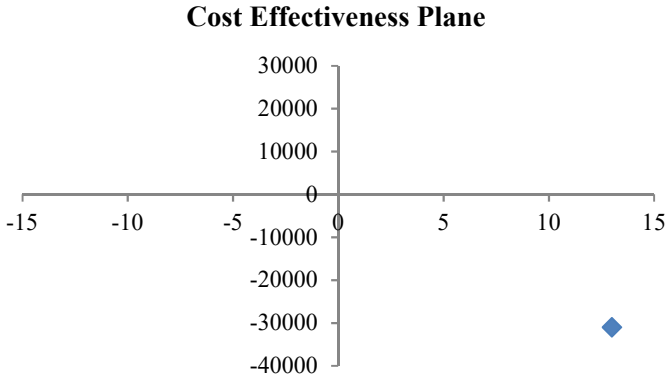
$$\text{ICER}=\frac{(\text{Biaya jamu saintifik - Biaya kombinasi antihipertensi })}{(\text{Efektivitas jamu saintifik - Efektivitas kombinasi antihipertensi })}$$

$$\text{ICER}=\frac{(\text{Rp 102.038 - Rp115.561})}{(53{,}8\% - 47\%)} \quad \text{ICER= -2.029/\% keberhasilan}$$

Persamaan 1. Rumus pengukuran nilai ICER antara terapi jamu saintifik antihipertensi dan kombinasi antihipertensi konvensional.

Hasil perhitingan ICER adalah Rp -2.029/% keberhasilan, semakin kecil nilai ICER atau mencapai negatif maka alternatif terapi jamu saintifik antihipertensi dianggap *cost saving*, artinya untuk menaikan 1% keberhasilan mencapai target terapi, pasien dapat menyimpan uang sebesar Rp 2.029. Hasil ICER tersebut kemudian dapat diplotkan pada suatu gambar 2 dimensi yang disebut *Cost Effectiveness Plane* (CEP), dengan tujuan memudahkan dalam membaca nilai ICER. Nilai ICER yang dihasilkan berada di area *SouthEast* (SE) atau di kuadran II (Gambar 1). Interpretasi dari CEP tersebut adalah jamu saintifik antihipertensi memiliki

efektivitas yang lebih baik namun dengan biaya yang lebih murah, sehingga dapat dikatan *cost effective*.



Gambar 1. Gambaran nilai ICER pada *Cost Effectiveness Plane* (CEP).

4. KESIMPULAN

Hasil penelitian jamu saintifik antihipertensi memiliki persentase keberhasilan lebih baik dibandingkan kombinasi antihipertensi konvensional. Hasil analisis efektivitas biaya atau *cost effectiveness analysis* menggunakan ICER menunjukan hasil Rp -2.029/% keberhasilan, sehingga dapat disimpulkan jamu saintifik antihipertensi dinggap *cost saving* dan setelah diinterpretasikan menggunakan *cost effectiveness plane* menggambarkan hasil ICER berada di kuadran *southeast* sehingga dianggap *cost effective.*

Keterbatasan penelitian adalah belum melakukan pengukuran biaya langsung non medis dan biaya tidak langsung sehingga belum mampu menggambarkan secara menyeluruh dan *real* biaya yang dikeluarkan oleh pasien selama menggunakan jamu saintifik di Wisata Kesehatan Jamu. Saran untuk penelitian selanjutnya adalah mengambil data biaya menggunakan perspektif pasien, dan memperluas jangkauan sampel.

UCAPAN TERIMAKASIH

Penelitian ini dapat dilakukan dengan baik berkat bantuan dan dukungan dari berbagai pihak, untuk itu saya mengucapkan terimakasih kepada kepala Klinik Wisata Jamu Kalibakung, kepala Puskesmas Kalibakung yang telah bersedia bekerjasama untuk melaksanakan penelitian ini.

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Semua penulis menyatakan tidak ada konflik kepentingan terhadap naskah ini.

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Cholecalciferol Effects on Lipid Profile of Experimental Animals: A Scoping Review

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Abstract
Vitamin D is an essential nutrient that has various beneficial effects on the human body. The results of cholecalciferol supplementation are varied, and there has yet to be a comprehensive review regarding its effect on animal models. Therefore, this scoping review aims to summarize the evidence regarding the effect of cholecalciferol (vitamin D3) supplementation on the lipid profiles of animal subjects. PubMed, Scopus, and DOAJ were searched for original research articles published until 2022. Studies were included if they were experimental studies, cholecalciferol was used as a supplement, and the changes in the lipid profile were analyzed. A total of 260 articles were collected, of which 250 articles were excluded, and 10 articles were included for qualitative synthesis. All studies used oral routes to supplement cholecalciferol with various doses and duration ranging from several weeks to several months. Most studies reported reduced lipid parameters in serum or organ-specific animals supplemented with cholecalciferol. As conclusion, cholecalciferol reduces lipid content in animal subjects and may have a beneficial effect on populations with metabolic diseases such as diabetes and dyslipidemia. Further research is required to explore the mechanism of how cholecalciferol affects the lipid profiles of experimental animals.

Keywords: Cholecalciferol; Cholesterol; Lipid

1. INTRODUCTION

Dyslipidemias are quantitive changes in cholesterol concentrations, respective fractions, or triglycerides in the plasma. This condition may increase the risk of atherosclerosis in adults and lead to cardiovascular diseases (Mosca et al., 2022). Approximately 50% of the adult population has dyslipidemia (Hedayatnia et al., 2020). The primary causes of dyslipidemia are gene mutations causing overproduction or inadequate clearance of triglycerides (TG) and low-density lipoprotein (LDL) or underproduction or excessive clearance of high-density lipoprotein (HDL). Secondary causes of dyslipidemia may be caused by various diseases such as hypothyroidism, nephrotic syndrome, chronic kidney disease, diabetes, obesity, and lifestyle causes such as abnormal alcohol intake and smoking (Yanai and Yoshida, 2021).

Vitamin D has been shown in various studies to play a role in various diseases, including dyslipidemia, in which observational studies showed that low vitamin D levels were associated with lipid abnormalities (Surdu et al., 2021; Kim and Jeong, 2019). Cholecalciferol (Vitamin

D3) is a native vitamin D obtained by food ingestion that has various roles in the metabolism of the musculoskeletal, respiratory, endocrine, renal, cardiovascular, and immune systems (Sosa and Gómez, 2020). Previous scoping studies have analyzed the effect of vitamin D on lipid profiles, which found that vitamin D and calcium have a beneficial effect on total cholesterol (TC), triglycerides (TG), and HDL-C using placebo-controlled randomized controlled trials (RCTs) (Morvaridzadeh et al., 2021). Another review found that vitamin D administration in postmenopausal women reduces triglycerides, but LDL-C, HDL-C, and TC changes are negligible (Zhang et al., 2022).

Numerous research studies regarding the effect of cholecalciferol supplementation on lipid profiles in animal models have varying results. However, there still needs to be a comprehensive review of its effect and what parameters of lipid profiles are affected. The purpose of this review is to address the knowledge gap by reviewing the literature on cholecalciferol supplementation in healthy or disease-induced animal models which reported changes in lipid profiles

2. MATERIAL AND METHODS

This scoping review was designed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) guidelines (Tricco et al., 2018).

2.1. Eligibility criteria

To be included in the review, peer-reviewed journal papers needed to (1) involve supplementation of cholecalciferol, (2) analyze the changes in lipid profiles which comprised of either total cholesterol, triglycerides, low-density lipoprotein or high-density lipoprotein, (3) experimental studies using mice as animal subjects. Studies were excluded if they (1) did not report cholecalciferol dose and (2) did not report quantitative changes in the lipid profile. All the studies had to be fully completed and published; abstract-only, presentation-only, and unpublished studies were excluded.

2.2. Information sources

Searches were conducted on the electronic databases of PubMed, Scopus, and the Directory of Open Access Journals (DOAJ) for studies published from its inception until February 2023. (The last search was conducted in February 2023). The databases are chosen considering their advantages, and Pubmed is considered one of the most frequently updated websites with a comprehensive follow-up of a specific subject. Scopus provides more citations, and with proper search strategies, false positives can be eliminated from the search. DOAJ is one of the leading multidisciplinary open-access databases and may provide additional articles that would otherwise not be published under a paid-model database. The search was restricted to papers written or translated into English. Reference lists of all retrieved articles and the profiles of authors with extensive experience in dietary supplement research were scanned for

additional relevant articles. All databases were searched for the earliest known published articles in the database up until the articles were published in December 2022.

2.3. Search strategy

The search strategy involved a combination of keywords limited to the title and abstract. For different databases, the search strategy was adapted according to the search feature of each database. Search strategy in PUBMED includes (Vitamin D3 OR cholecalciferol OR hydroxycholecalciferol OR dihydroxy vitamin D3), AND (Lipid OR cholesterol OR lipid panel OR VLDL OR HDL OR VLDL OR IDL OR very low-density lipoprotein OR intermediate-density lipoprotein OR low-density lipoprotein OR high-density lipoprotein). Search strategy in DOAJ and Scopus includes (Cholecalciferol) AND (Lipid).

2.4. Selection process

Two of the authors screened the studies for eligibility. Any disagreements on study selection or data extraction were resolved by consensus.

2.5. Data charting and items

The first author developed the data charting table and further improved and approved by the other two authors to finally include the authors' names, year of publication, animal model, amount of cholecalciferol supplementation, route of supplementation, comparison group, duration, results analyzed, the health status of animal model, comparison with controls, and comparison with other treatment.

3. RESULTS AND DISCUSSION

3.1. Selection of studies

In total, 260 articles were identified by database searches, whereas manual searches identified no articles. After removing duplicates, 245 articles remained; 221 articles were removed during the initial screening, and 24 articles were removed during full-text screening. Ten articles were included in the analysis (Figure 1).

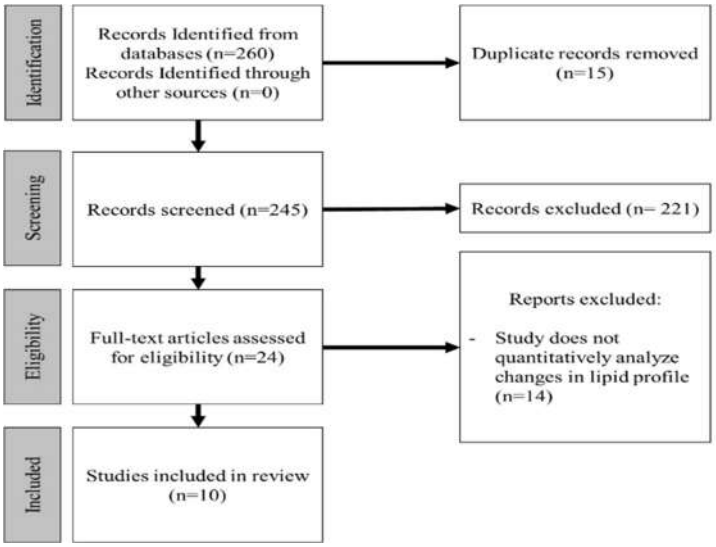

Figure 1. Flow chart for the study selection that searched in PubMed PMC, Scopus, and DOAJ.

3.2. Characteristics of vitamin D3 supplementation

All studies used the oral route to administer cholecalciferol using various vehicles (Tsuruki et al., 1986; Quach et al., 2018; Lee et al., 2020; Lim et al., 2021; Surdu et al., 2021; Wahba et al., 2021; Abd and Sallam, 2022; Atia et al., 2022; Elseweidy et al., 2022; Philouze et al., 2022). Cholecalciferol was either administered as a single treatment or with other supplements. Tsuruki et al. (1986) administered vitamin D3 orally, dissolved in 0.1 ml of propylene glycol and ethanol solution. Philouze et al. (2022) used a vitamin D3-enriched (15000 IU/kg) HFS diet for over 15 weeks. Lee et al. (2020) administered vitamin D3 300 ng/kg orally for 12 weeks. Lim et al. (2021) fed HFD supplemented with vitamin D3 in 300 ng/kg or 600 ng/kg dissolved in olive oil over 12 weeks by oral gavage. Wahba et al. (2021) administered vitamin D3 orally for six weeks in a dose of 10 µg/kg/day along with the same fructose/salt feeding concentration. Abd and Sallam (2022) administered cholecalciferol in two groups, one in 500 IU/KG/day dose by oral route for six weeks and the other group by pretreatment of cholecalciferol 500 IU/Kg/day orally for four weeks, followed by Tamoxifen and cholecalciferol oral administration for the remaining two weeks. Atia et al. (2022) administered vitamin D3 in 500 IU/kg using corn oil via orogastric lavage. Elseweidy et al. (2022) supplemented 170 IU cholecalciferol weekly for eight weeks. Quach et al. (2018) treated hypercholesterolemia mice using 1625 nmol/kg cholecalciferol for eight days. Calgaroto et al. (2015) supplemented 90 µg/kg/day of oral cholecalciferol for 30 days on diabetic rats.

3.2. Changes in lipid profile

Most of the studies reported a significant decrease in lipid parameters, whether it is total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), or high-density lipoprotein (HDL) (10,13,14,15,16,17,19). Cholecalciferol supplementation was reported to impair lipid metabolism, which resulted in a significant decrease of LDL-c ($P<0.0001$) and triglycerides ($P=0.001$) by 140 and 74%, respectively, along with a decrease in HCL-c ($P=0.00002$) by 18% in saline-treated diabetic rats (Calgaroto et al., 2015). Another study plotted plasma and liver cholesterol levels against plasma 1,25(OH2) D3 and liver concentration, which showed an inverse correlation. A significant correlation was found when liver cholesterol was plotted against 1,25(OH2) D. However, the study showed a very shallow and insignificant correlation between plasma 1,25(OH2) D and plasma cholesterol (Quach et al., 2018).

Other studies also reported a significant decrease in lipid-related parameters. A study reported that vitamin D3 administration significantly reduced plasma TG levels, with no significant alterations in TC levels. There was also an elevation of plasma HDL-C levels following a high dose of vitamin D3 in type 2 diabetic mice. The study also showed that vitamin D3 supplementation reduced liver TG content, while hepatic TC levels did not significantly change (Lim et al., 2021). Atia et al. (2022) showed a significant decrease ($p<0.001$) in TC and TG levels in diabetic rats treated with eight weeks of cholecalciferol compared with untreated diabetic rats. Wahba et al. (2021) showed that vitamin D3 significantly improved the markers of obesity and serum lipids such as serum TG, TC, and TC/HDL-C ratio profile compared to

untreated metabolic syndrome rats ($p<0.05$). In the study, vitamin D3 supplementation in normal animals almost exhibited non-significant parameters of the previous parameter compared to the control group. Philouze et al. (2022) showed that cholecalciferol supplementation in HFS-fed mice decreased cholesterol levels. However, there was no effect on TG, other sphingolipids, or cholesteryl esters.

A study by Abd and Sallam (2022) supplemented cholecalciferol as pretreatment analyzes lipid profiles in Tamoxifen (TAM)-induced steatohepatitis in female ra; the study showed that pretreatment using cholecalciferoalleviatedte TAM-induced alterations in lipid profiles. Cholecalciferol also mitigated hepatic TG elevation by TAM and normalized hepatic cholesterol levels. Cholecalciferol alone for six weeks was also reported in the study to reduce serum TG ($F(3, 21)= 3.464$, $p= 0.0346$) and hepatic TG ($F(3,20)= 788.7$, $p<0.0001$). Compared with lipid-reducing drugs, cholecalciferol supplementation was also found to produce the same results, such as the study by Elseweidy et al. (2022), which showed that in diabetic hyperlipidaemic rats, rats treated with atorvastatin and vitamin D3 showed similar results where TC, TG, LDL-C were significantly decreased ($p<0.001$) compared with ¬¬diabetic hyperlipidemic rat control group.

There are also changes in lipid composition on several specific organs of rats supplemented with cholecalciferol. Tsuruki et al. (1986) reported the effect of vitamin D3 and cadmium on the lipid composition of rat intestinal brush border membranes. The total lipid content was found to be increased. Phospholipid duodenal content was increased significantly in vitamin-D3-treated rats without cadmium. Cholesterol and glycolipid levels were not significantly altered in these rats by vitamin D3 treatment. Lee et al. (2020) showed no significant reduction of serum TH and TC levels in mice supplemented with vitamin D3. However, there was a decrease in TG and TC levels in the kidney, while there was still no significant decline in renal LDL by vitamin D3 supplementation. No significant changes were found in renal HDL in vitamin D3-supplemented mice.

Cholecalciferol, or vitamin D3, is synthesized in the skin as the primary source in humans and increases proportionally with the intensity of ultraviolet radiation. The synthesis of vitamin D3 depends on the 7-dehydrocholesterol pathway to control the synthesis of cholesterol in cells. Radiation of ultraviolet B (UV-B) light ionizes the 7-dehydrocholesterol into pre-vitamin D3 thus converting to cholecalciferol. Vitamin D3 is transported with a serum protein vitamin D-binding protein (DBP) and hydroxylated into calcidiol. The calcidiol binds with the vitamin D receptor (VDR). The vitamin D-VDR complex is a primary circulating form in the blood. In the liver, vitamin D3 converts to an active form as calcifediol (25-hydroxycholecalciferol) and is hydroxylated into calcitriol (1,25-dihydroxycholecalciferol) in the kidney (Surdu et al., 2021).

Numerous studies suggest the active form, vitamin D, as a predictor of increased lipid by the adipogenesis process. It promotes adipogenesis by the differentiation of human and mouse adipose tissue-derived stem cells (ASCs) and bone marrow-derived mesenchymal stem cells (BM-MSCs) from pigs (Felicidade et al., 2018). Calcidiol will elevate the expression of the

enzyme CYP24A1 and promote adipogenesis. Atmani et al. (2003) observed calcitriol increase adipocytes by about 180%, presenting calcitriol has a stimulatory effect on proliferation in rat bone marrow-derived mesenchymal stem cells (BM-MSCs) stimulated with calcitriol for 14 days.

One of the significant forms of vitamin D is cholecalciferol, or vitamin D3, which is human-made and can be found in food (Sosa and Gómez, 2021). However, contrary to the study of vitamin D, this primary form improved the lipid profile and enhanced the adipogenesis process. A study reported a significant decrease in LDL-c by cholecalciferol supplementation in diabetic Wistar rats. There is a negative correlation between serum vitamin D3 and LDL-C levels. The same study also reported that cholecalciferol administration ameliorated lipid metabolism in healthy non-diabetic rats. (Calgaroto et al., 2015). Similarly, Aon et al. (2021) documented lower serum vitamin D3 in the type 2 diabetes mellitus with cardiovascular disease group. This group has a high-level lipid profile, and it has been considered among the important risk factors for CVD in type 2 diabetes mellitus.

Type 2 diabetes mellitus is associated with elevated triglyceride, decreased HDL-c level, and increased LDL-c level (Elmi et al., 2021). Cholecalciferol supplementation on healthy subjects also resulted in beneficial results on lipid profiles, in which Abd and Sallam (2022) reported a significant decrease in serum TG of healthy Wistar rats, and another study by Tsuruki et al. (1986) showed a significant decrease of cholesterol and glycolipids in healthy Wistar rats supplemented with cholecalciferol.

Consistent with Atia et al. (2022), the study by Lim et al. (2021) reported lower plasma triglyceride and TC levels in the diabetic mice group after 12 weeks supplemented with 300 mg and 600 mg of vitamin D3. This study also showed that vitamin D3 supplementation lowers lipogenesis and inhibits lipid accumulation by improving β-oxidation. Moreover, the study subsequently showed elevated HDL-c levels after 600 mg vitamin D administration. Similarly, Abeer and Suzan (2019), vitamin D3, also known as significantly improved plasma HDL-c in the gestational diabetes mellitus-induced rat group that injected IM with 20.000 IU/kg of vitamin D3. The mechanism of these actions is also observed in another study (Ivkovic et al., 2022). They found that cholecalciferol did not directly modify the nuclear content of β-oxidation but improved β-oxidation by affecting the ACC/MCD expressions in malonyl-CoA-mediated regulation of β-oxidation (Wang et al. 2019). Ruiz-Ramírez et al. (2016) also noted the upregulating gene expression named uncoupling protein 3 (UCP3) proteins that act as lipotoxicity, reduce ROS production, and oxidative stress in 1000IU/kg of vitamin D3 injected rat group.

In addition, Riek et al. (2013) documented that vitamin D3 could inhibit formatting foam cells by reducing cholesterol deposition and improving cholesterol efflux in macrophages. Marino et al. (2022) found in their experiment that macrophages incubated with free fatty acid will increase PPAR-γ1. Augmentating vitamin D3 was able to downregulate PPAR-γ1. Meanwhile, the deficit of PPAR-γ1 could prevent intracellular lipid droplet accumulation in endothelial cells.

Table 1. Summary of articles on animal studies regarding cholecalciferol supplementation and results of lipid changes were searched in PubMed PMC, Scopus, and DOAJ

First author (year)	Animals	Amount of cholecalciferol supplementation	Route of cholecalciferol supplementation	Comparison group	Duration	Data presented	Health status	Results compared with controls	Results compared with other treatment
Elseweidy et al., 2022	Male Wistar rats	170 IU/week	Oral	Atorvastatin	8 weeks	TC, TG, HDL, LDL	Diabetes, hyperlipidemia	TC, TG, and LDL decreased significantly. HDL increased significantly	No significant difference
Lim, 2021	C57BL/6J mice	300 ng/kg (low cholecalciferol) or 600 ng/kg (high cholecalciferol)	Oral	- (Control only with HFD)	12 weeks	Plasma and hepatic TC, TG, HDL	Diabetes	Significant decrease in plasma TG levels. Significant increase in HDL levels. No significant change in TC levels.	-
Philouze et al., 2022	C57BL/6JRj mice	15.000 IU/kg	Oral	- (Control only with HFS)	15 weeks	Plasma TG	Diabetes	No significant change in TG levels	-
Lee et al., 2020	C57BL/6 mice	300 ng/kg	Oral	- (Control only with Streptozocin)	12 weeks	Plasma TG, TC, LDL, HDL	Diabetes	No significant change in TG, TC, LDL and HDL levels.	-
Wahba et al., 2021	Wistar rats	10 µg/kg/day	Oral	- (Control only with fructose)	6 weeks	Plasma TG, TC, TC/HDL ratio, HDL/LDL ratio	Metabolic Syndrome	Significant decrease in TG levels, TC levels, and TC/HDL ratio. Significant increase in HDL/LDL ratio	-
Abd and Sallam., 2022	Wistar rat	500 IU/kg/day	Oral	- (Control only)	6 weeks	Plasma TG, TC	Healthy rats	Significant decrease in serum TG	-

Table 1. Summary of articles of animal studies regarding cholecalciferol supplementation and results of lipid changes that were searched in PubMed PMC, Scopus, and DOAJ (*Continued*).

First author (year)	Animals	Amount of cholecalciferol supplementation	Route of cholecalciferol supplementation	Comparison group	Duration	Data presented	Health status	Results compared with controls	Results compared with other treatment
Quach et al., 2018	C57BL/6 mice	1625 nmol/kg	Oral	Vitamin D analogues ($1\alpha(OH)D_2$, $1\alpha(OH)D_3$, $1,25(OH)_2D_3$, $25(OH)D_3$)	8 days	Plasma TC	Hypercholesterolemia	No significant change in TC levels	-
Calgaroto et al., 2015	Wistar rat	90 µg/kg/day	Oral	Metformin	30 days	Plasma TG, LDL, HDL	Diabetes	Vitamin D3 and Metformin + Vitamin D3: Significant decrease in LDL and TC levels. Significant increase in HDL levels and ameliorated lipid metabolism in non-diabetic rats	Not compared
Atia et al., 2022	Wistar rat	0.6 mg/kg	Oral (Orogastric lavage)	Glibenclamide	8 weeks	Plasma TC, TG	Diabetes	Significant decrease in TC and TG levels	Better result with Glibenclamide + Vitamin D than GLB-only
Tsuruki et al., 1986	Wistar rat	100 IU 5 times	Oral	- (Control only with cadmium)	2 weeks	Intestinal brush border cholesterol, phospholipid, glycolipid.	Healthy rats	Partial recovery of phospholipid contents. Cholesterol and glycolipids significantly decreased.	-

Most studies included in this review have shown that various doses and duration of cholecalciferol supplementation have beneficial effects on one or more lipid parameters, such as total cholesterol, triglycerides, LDL, and HDL. One study also showed that cholecalciferol supplementation has a similar effect with lipid-lowering medication, which is atorvastatin, resulting in a comparable beneficial effect on total cholesterol, triglycerides, HDL, and LDL. This review has several limitations. The first one is that the study is limited to experimental studies, which may reduce its applicability in a natural clinical setting. The second is language limitation, which limits the studies to only English. The third is that the study was not assessed for risk of bias, which can affect the quality of evidence. Lastly, all of the studies used an oral route, which cannot delineate the effect of cholecalciferol supplementation by other routes. Nevertheless, this review provides a comprehensive overview of the effect of cholecalciferol supplementation on the lipids of experimental animals.

4. CONCLUSION

Cholecalciferol administration ranging from 8 days to 15 weeks in animal subjects has beneficial effects on lipid profile, low-density lipoprotein, and high-density lipoprotein in most experimental studies. The addition of vitamin D3 showed a reduction of TC, TG, and LDL-c levels and also increased HDL-c levels. The lack of vitamin D3 showed vice versa. Several studies have provided the mechanism of vitamin D that alters lipid plasma. However, the direct mechanism of cholecalciferol on lipid profile changes was still unspecific and poorly understood. By reducing lipid content, cholecalciferol supplementation may benefit populations with various health risks, such as metabolic syndromes.

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CONFLICT OF INTEREST

The authors state that there is no conflict of interest in implementing this research from start to finish.

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Use of Favipiravir in Covid-19 Patients: A Narrative Review

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Abstract
COVID-19 is an acute respiratory disease resulting from the infection of SARS-COV-2 viruses and causes high morbidity, which requires appropriate treatment targets. Favipiravir is an antiviral that selectively inhibits RNA-dependent RNA polymerase (RdRp) of virus. This review aimed to identify several studies that prove the effectiveness and safety of using Favipiravir for COVID-19 patients. The search method used the electronic media PubMed and ScienceDirect with the keywords "Efficacy", "Favipiravir", "Treatment", "Safety", SARS-COV-2", and "Favipiravir induced", accompanied by the addition of the affixes "AND" and "OR" and selection by the publication date starting December 2019. The literature search resulted in eight (8) published articles that met the exclusion and inclusion criteria. The results of the review showed that concurrent administration of Favipiravir and Lopinavir/Ritonavir or Chloroquine, with a dosage of Favipiravir of 3200 mg/day followed by 1200 mg/day each in 2 divided doses, was considered adequate for improving the clinical symptoms of COVID-19 patients with mild-moderate symptoms in early administration. Meanwhile, administering Favipiravir with anti-IL-6 Tocilizumab for patients with severe symptoms showed a fairly good effect. The most frequently reported ADE (adverse drug events) in the use of Favipiravir were hyperuricemia and elevated alanine aminotransferase (ALT) levels. This review concluded that the best clinical response to Favipiravir is shown in COVID-19 patients with mild-to-moderate early symptoms.

Keywords: COVID-19; Favipiravir; Treatment

1. INTRODUCTION

COVID-19 is a disease caused by the SARS-COV-2 viral infection, which can produce several symptoms of severe acute respiratory syndrome. This viral infection is deemed to cause high morbidity and, therefore, requires prompt treatment. COVID-19 first appeared in Wuhan, China, in December 2019. It can disrupt the respiratory system by causing severe symptoms and fatality, especially in elderly patients aged 70-80 years (Onder et al., 2020). The existence of SARS-COV-2 began with the emergence of the Severe Acute Respiratory Syndrome Coronavirus (SARS-COV) in 2002 and the Middle East Respiratory Syndrome Coronavirus (MERS-COV) in 2012. The incidence of SARS-COV and MERS-COV has triggered the emergence of the SARS-COV-2 virus, which is more dangerous than its two predecessors. It has also been known that most of the genome sequence of the SARS-COV-2 virus is

homologous with the genome of the SARS-COV virus, whereas some others are homologous with the genome of the MERS-COV virus (Lu et al., 2020; Zhou et al., 2020).

The treatment for COVID-19 focuses on two targets, which include reducing the number of viruses in the body and improving the health status of the patients (Inoue et al., 2020). Previous research shows that several antiviral agents are deemed effective against COVID-19 in the general population. Among these antiviral groups, Favipiravir acts as an antiviral agent previously developed to treat influenza (Koshi et al., 2021).

Favipiravir (T-705; 6-fluoro-3-hydroxy-2-pyrazinecarboxamide) is a selective, powerful antiviral agent inhibiting the RNA-dependent RNA polymerase (RdRp) found in viruses. Favipiravir was discovered through a chemical screening based on a specific antiviral activity against influenza viruses conducted by Toyama Chemical Co., Ltd. Favipiravir goes through intracellular phosphoribosylation to obtain its active form, Favipiravir-RTP (Favipiravir ribofuranosyl-5B-triphosphate), which is known as a substrate for RNA-dependent RNA polymerase (RdRp) that inhibits the activity of RNA polymerase (Udwadia et al., 2021a). Favipiravir has a broad antiviral spectrum against other RNA viruses, such as bunyavirus, influenza, and filovirus. It is identified as having in-vitro activities against SARS-COV-2, although it requires higher concentrations compared to such other drugs as Chloroquine or Remdesivir, with a value of EC50 of 61.88 μM (Wang et al., 2020).

The initial clinical experience of Favipiravir against SARS-COV-2 shows promising results, but it remains necessary to further study its effectiveness and safety, especially in specific patient groups, such as geriatrics and pediatrics. Although the WHO only authorizes emergency use and clinical trials of Favipiravir exclusively for COVID-19 patients, several studies have reported that their trial results effectively improve the clinical symptoms of COVID-19 patients. In this review, the effectiveness of Favipiravir is discussed according to the patient's symptoms, including those with mild and moderate to severe symptoms, through a narrative review presented in a narrative study.

2. MATERIALS AND METHODS

The data collection from 2020-2021 was conducted through literature searches using the electronic media, PubMed and ScienceDirect, with the search keywords "Efficacy", "Favipiravir", "Treatment", "Safety", "SARS-COV-2", and "Favipiravir induced" supported by the addition of "AND" and "OR" affixes. The specified eligibility criteria were articles published in English, presenting research, case reports, as well as prospective and retrospective observational studies, and addressing a research topic of the treatment of Favipiravir in COVID-19 patients. The exclusion criteria were review articles and articles that discussed Favipiravir for non-COVID-19 patients.

The quality of the article selection was evaluated using the indicator parameters for effectiveness and safety studies, and the content integrity of each article with the RCT method was evaluated using the Consort 2010. In contrast, articles with case reports were assessed using the Care Checklist 2013. Based on the search results, a total of 86 articles were obtained, 43

articles were selected based on an assessment of the abstracts and titles, 35 articles discussed Favipiravir for non-COVID-19 patients, and the result was eight articles to be reviewed.

3. RESULTS AND DISCUSSION

3.1. Favipiravir mechanism of action

SARS-COV-2 is a type of positive-sense single-stranded RNA virus belonging to the subfamily "coronavirinae" in the family "coronaviridae" which is part of the order "nidovirales" and has four main structural proteins, including the spike, envelope, membrane, and nucleocapsid. The spike protein plays a crucial role in the binding of the virus to the host cell membrane (Tortorici & Veesler, 2019). Transmission of SARS-COV-2 can occur through a patient's droplets/cough, and the virus will then infect healthy cells through a direct interaction with a specific receptor, or the ACE2 receptor, found on the SARS-COV-2 host-cell surface (Hoffmann et al., 2020). SARS-COV-2 typically infects body organs with many ACE2 cells, including the pulmonary epithelial cells (Cevik et al., 2020). The detailed pathogenesis of SARS-COV-2 remains to be known, but it is estimated to be similar to its predecessor, SARS-COV (Cevik et al., 2020).

SARS-COV-2 initially enters the body and moves toward the pulmonary epithelial cells, and then the spike protein of the virus will bind itself to the primary receptor ACE2. In the cells, the virus structure will be cleaved with the aid of the enzyme TMPRSS2 to allow fusion between the cell membrane and the virus envelope, followed by the formation of a genome ready to release into the cytoplasm. In the cytoplasm, the ribosome will translate the viral RNA into proteins. The viral RNA is propagated by the enzyme RNA-dependent RNA polymerase (RdRp), after which the constituent proteins and RNA combine to form virions in the Golgi apparatus and prepare for exocytosis and replication in other cells (Allen et al., 2020).

The mechanism of action of Favipiravir against SARS-COV-2 is known to play a role in terminating the viral RNA chain (Shannon et al., 2020). Favipiravir acts as a guanosine analog to prevent viral RNA synthesis (Figure 1). Favipiravir is an antiviral therapy since its guanosine analog resembles the natural structure of the body's nucleoside or nucleotide. As a result, the RNA polymerase of the virus will mistakenly recognize Favipiravir-RTP as a purine nucleotide (Furuta et al., 2013). Like other positive-strand RNA viruses, the nucleus of the viral replication is present in RNA-dependent RNA polymerase (RdRp).

It has been known that SARS-COV-2 utilizes the viral RNA-dependent RNA polymerase (RdRp) to replicate and express its genome, and RdRp will join the non-structural proteins (NSPs) to form a replication-transcription complex that plays a role in the synthesis up to the extension of RNA strands (Hillen, 2021). The RNA-dependent RNA polymerase (RdRp) of SARS-COV-2 is known to be 10-fold more active than that of any other virus. It has an extraordinarily high nucleotide incorporation rate, allowing Favipiravir or other nucleotide analogs to be inserted easily into viral RNA (Shannon et al., 2020). Therefore, RNA-dependent RNA polymerase (RdRp) becomes one of the antiviral targets which is considered promising to overcome COVID-19 (Subissi et al., 2014).

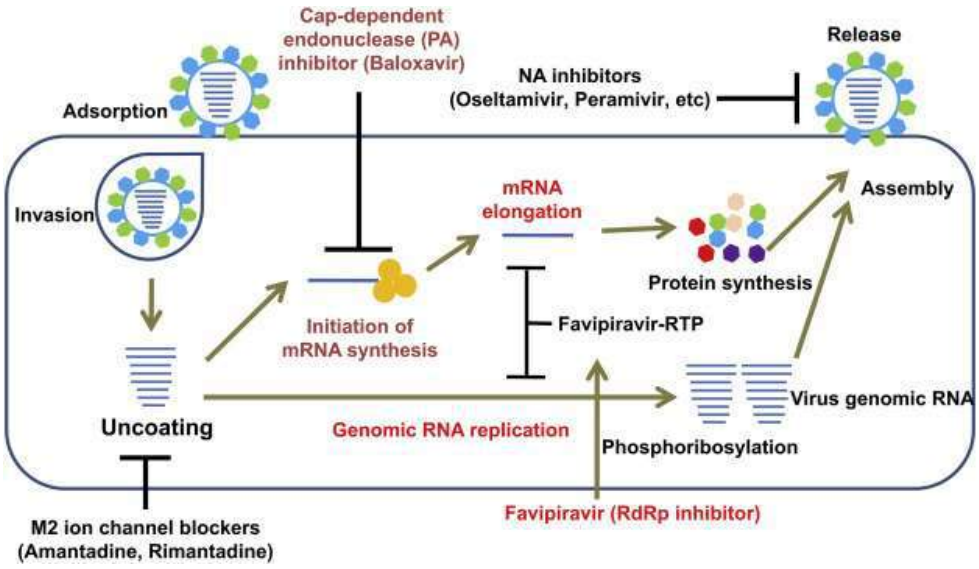

Figure 1. Mechanism action of favipiravir (modified from (Fang and Wang, 2020)).

Favipiravir is among the antivirals targeting the inhibition of RNA-dependent RNA polymerase (RdRp) in viruses. Favipiravir is a prodrug that will undergo an intracellular metabolic process to convert into its active form. First, Favipiravir enters the body and has active phosphoribosylation catalyzed by the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT) to form an active compound called Favipiravir-ribofuranosyl-50-triphosphate (Favipiravir-RTP). Favipiravir-RTP will then bind to the active site of RdRp (RNA-dependent RNA polymerase) when the viral replication process occurs to inhibit RNA replication (Allen et al., 2020).

3.2. Effectiveness of Favipiravir

A total of 8 articles are to be reviewed for the effectiveness of favipiravir (Table 1 and Table 2). In the articles to date, there have been no specifically approved drugs for the treatment of COVID-19. However, some antiviral agents, including Favipiravir, are considered effective in improving the clinical symptoms of COVID-19 patients. Favipiravir is currently used in the treatment of COVID-19 by some countries, including Indonesia, and in various trials as a promising clinical intervention to combat SARS-COV-2 infection. Favipiravir was initially used to treat influenza RNA viruses (Guo et al., 2020). Reports on the effectiveness of Favipiravir in COVID-19 still need to be made. In an in-vitro clinical trial conducted on Vero E6 cells, it was found that the EC50 of Favipiravir was 61.88 mM (Wang et al., 2020). The absorption time of Favipiravir in the body is approximately 0.5 to 1 hour (Nguyen et al., 2017). The dose of Favipiravir used in the treatment of COVID-19 remains unspecified. However, some studies have determined that it is adjusted to the initial administration for the influenza virus, in which on the first day of treatment, patients are given 3200-3600 mg Favipiravir orally followed by a dosage of 1200-1600 mg divided into two doses per day until day 10 or day 14 (Ivashchenko et al., 2021; Udwadia et al., 2021b).

The main characteristic of the patients in this study is COVID-19 patients who were confirmed positive by the result of the RT-PCR method in a laboratory and had varied symptoms from mild-moderate to severe. Mild symptoms are characterized by a fever of < 38ºC and a respiratory rate of 12 to ≤ 20 breaths/minute (Cai et al., 2020). Moderate symptoms include cough, sore throat, body aches, and nasal congestion (Ivashchenko et al., 2021; Udwadia et al., 2021b). Meanwhile, severe symptoms include lung conditions showing pneumonia positive from a chest CT scan, cough, difficulty breathing, and infections in the lower respiratory tract (Khamis et al., 2021; Lou et al., 2021). The patients' average age in all the literature discussed in the study was 18-89 years in both groups with mild-moderate and severe symptoms. The comorbidities commonly experienced by the patients with mild-moderate to severe symptoms were diabetes mellitus (22.7%), hypertension (34.9%), liver disorder (16%), pulmonary disease (6.8%), mild-severe chronic kidney disease (25.1%), and conjunctivitis (5.17%).

At the early treatment stage, the patients had a variety of symptoms from mild-moderate to severe, and a drug combination was therefore administered comprising antivirals and such other drugs as analgesics-antipyretics, antibiotics, and vitamins (Chen et al., 2021; Udwadia et al., 2021b). Many studies have been conducted to assess the effectiveness of Favipiravir for COVID-19 patients. Studies of COVID-19 patients with initial symptoms show that the administration of Lopinavir/Ritonavir and anti-malarial Chloroquine together with Favipiravir improved lung conditions based on the observation of average chest CT scan results on day 10-15 with 91.43% vs. 62.22% (P = 0.004) as well as a relatively faster viral clearance (4 days with IQR 2.5-9 vs. 11 days with IQR 8-13; P < 0.001). In addition, the combination of Favipiravir and Chloroquine was also studied in groups of patients with severe symptoms. However, the findings reported that Favipiravir and Chloroquine did not significantly improve patients' inflammatory response (P= 0.785) (Cai et al., 2020; Ivashchenko et al., 2021; Udwadia et al., 2021b).

Other studies were also conducted to examine the effects of Favipiravir administration on patients during initial treatment and when the symptoms deteriorated. They also investigated the effects of adding a combination of Favipiravir and Baloxavir for patients given standard care. The results showed no significant differences in the two treatment groups (initial treatment and deteriorated symptoms) since the hazard ratio [aHR] was 1.42 with a 95% confidence interval [95% CI] of 0.76 to 2.62, and the time to reduce fever was relatively similar in both groups, which was three days (aHR = 1.88, 95% CI = 0.81 to 4.35). Meanwhile, adding Baloxavir and Favipiravir is perceived as providing insignificant clinical benefits to patients (Doi et al., 2020; Lou et al., 2021).

Of all the articles reviewed, administering Favipiravir in COVID-19 patients with mild-moderate symptoms is considered acceptable to reduce clinical symptoms, accelerate viral clearance time, and improve lung conditions, as evidenced by the chest CT scan if administered for early symptoms. However, administering Favipiravir to COVID-19 patients immediately after the onset of symptoms or approximately around the sixth day after the first onset of

symptoms is deemed less effective in improving the clinical symptoms. This is likely caused by the growing number of viruses, the concentration of the administered drug, and the duration of Favipiravir administration. Meanwhile, in patients with severe symptoms, the administration of Favipiravir in combination with Arbidol does not improve the patient's clinical symptoms, nor does the administration of other drugs, such as Baloxavir marboxil and Chloroquine. However, the administration of Favipiravir and Anti-IL-6 Tocilizumab is considered effective in improving patients' lung inflammation, although their use for COVID-19 patients should be reevaluated since they are considered to carry the risk of cytokine storm response (Zhou et al., 2020).

Furthermore, inadequate improvement of COVID-19 patients with severe clinical symptoms is likely due to the disease severity characterized by the viral load, comorbidity, age, and early clinical symptoms that are too severe to treat with an only standard of care (Gonçalves et al., 2020).

Meanwhile, the viral clearance and clinical symptom improvement in patients with mild-moderate symptoms do not differ much from those with severe symptoms. On average, Viral clearance occurred from day 4 to day 10 (Cai et al., 2020; Ivashchenko et al., 2021). Improvement in the lung conditions observed from the chest CT scan results was typically noticed starting from day 14 and day 15 (Ivashchenko et al., 2021; Zhao et al., 2021). In addition, patients' fever was reduced on the third day of the drug administration (Udwadia et al., 2021b). In essence, evaluating the effectiveness of Favipiravir requires further clinical research with a larger population of patients. Various clinical characteristics, ages, physical conditions, and comorbidities are some of the factors that influence such evaluation. In addition, drug concentration and pharmacology of drugs also affect their effectiveness (Lou et al., 2021).

3.3. Favipiravir safety treatment

Adverse drug events (ADE) are also frequently found in COVID-19 treatment. Physiological and pathological factors can influence adverse reactions. As indicated in this study, each patient has different clinical characteristics. The main characteristic is that COVID-19 patients were confirmed positive by the laboratory using the RT-PCR method, with mild-moderate to severe early symptoms. The average age of the patients was 18-89 years, combined with all patients with mild-moderate to severe symptoms. Some patients had one or more comorbidities, with the most common being diabetes mellitus (22.7%), hypertension (34.9%), liver disorder (16%), pulmonary disease (6.8%), mild-severe CKD/chronic kidney disease (25.1%), and conjunctivitis (5.17%). These comorbidities were generally found in patients aged 50-80 years or above.

According to the articles collected in this study, the most frequent incidence of ADE (adverse drug event) was hyperuricemia (Table 3), affecting almost half of the patient population (48.9%) (Chen et al., 2021; Doi et al., 2020). Favipiravir is likely to decrease the excretion of uric acid into the urine and, therefore, result in elevated uric acid concentration in the blood.

Table 1. Effectiveness of Favipiravir in patients with mild-moderate symptoms.

Reference	Country	Study Design	Patient Population	Dosage and Route of Administration		Clinical Outcome	Information
Favipiravir (FVP) vs. Lopinavir/Ritonavir (LPV/RTV)							
(Cai et al., 2020).	China	Non-randomized control trial	Non-critical COVID-19 patients FVP (n=35) vs. LPV/RTV (n=45)	FVP, peroral Day 1 = 1600 mg, 2x1 day Day 2-14 = 600 mg, 2x1 day	LPV/RTV, peroral Day 1-14 = 400mg/100 mg, 2x1day	Improved chest CT scan on day 14: FVP = 32/35 (91.4%) LPV/RTV = 28/45 (62.2%) Viral load drop: FVP = 4 days LPV/RTV = 11 days FVP showed an excellent clinical response to COVID-19 for viral load drop and improved chest CT scan.	All patients were given 5 million IU Interferon, 2x1 day via inhalation
Favipiravir (FVP) vs. Hydroxychloroquine (HCQ) and Lopinavir/Ritonavir (LPV/RTV)							
(Ivashchenko et al., 2021)	Russia	Multicenter randomized control trial phase II/III	COVID-19 patients with mild-moderate symptoms FVP = 1600 mg, n=20 FVP = 1800 mg, n=20	FVP peroral Day 1 = 1600 mg Day 2-14 = 600 mg FVP n=20 Day 1 = 1800 mg Day 2-14 = 800 mg FVP n = 20	Standard of Care (SOC): HCQ and LPV/RTV followed the COVID-19 treatment guidelines in Russia	Viral clearance: FVP = 37/40 (92.5%) SOC = 16/20 (80.0%) Improved chest CT scan: FVP = 36/40 (90%) SOC = 16/20 (80.0%) On days 10 and 15, Favipiravir showed a quite good response for viral clearance and an improved chest CT scan.	Standard of Care: Hydroxychloroquine for 15/20 patients (75%), Lopinavir/Ritonavir for 1/20 patients (5%), no etiotropic therapy for 4/20 patients (20%)

Table 1. Effectiveness of Favipiravir in patients with mild-moderate symptoms (*Continued*).

Reference	Country	Study Design	Patient Population	Dosage and Route of Administration	Clinical Outcome	Information
Favipiravir (FVP)						
(Doi et al., 2020).	Japan	Prospective, Randomized, Open-label Trial of Early vs. Late Favipiravir	Inpatients with confirmed COVID-19 were given Favipiravir in the early treatment and late treatment of the symptom stage Early treatment group, n=36 Late treatment group, n=33	FVP peroral Day 1 = 1800mg 2x1 day followed by 800 mg 2x1 day with a total of 19 doses for 10 days	N/A	Viral clearance on day 6: Early treatment group = 66.7% Late treatment group = 56.1% The average time to reduce fever was 3 days. Administration of Favipiravir did not significantly improve the viral clearance in the first six days of the administration.	N/A
Favipiravir (FVP) vs. Control							
(Udwadia et al., 2021b).	India	Randomized, Comparative, Open-label, Multicenter Phase 3 Clinical Trial	COVID-19 patients with mild-moderate symptoms Favipiravir n=70 Control n = 68	FVP peroral Day 1 = 1800mg 2x1 day Day 2-14 = 800 mg 2x1 day	N/A	Improved clinical outcome was indicated by a decreased body temperature, higher oxygen saturation, and reduced respiratory rate on day 3 (FVP) and day 5 (control group). Favipiravir might reduce the duration of clinical signs and symptoms in patients with mild-moderate COVID-19 symptoms, as indicated by the shorter clinical recovery time.	The control group was administered with antipyretics, cough suppressants, antibiotics, and vitamins.

Table 2. Effectiveness of Favipiravir in patients with severe symptoms.

Ref.	Country	Study Design	Patient population	Dosage and Route of Administration		Clinical Outcome	Information
Favipiravir (FVP) vs. Arbidol				Dosage of Compared Drug			
(Chen et al., 2021)	China	Multicenter Randomized Control Trial	COVID-19 pneumonia patients FVP n = 116 Arbidol n=120	FVP, peroral Day 1 = 1600 mg, 2x1day Day 2-14 = 600 mg 2x1 day for 10 days	Arbidol, peroral = 200 mg 3x1 day	Clinical recovery on day 7 = 62/120 patients (51.67%) in the Arbidol group and 71/116 patients (61.21%) in the Favipiravir group Favipiravir showed no superior efficacy compared to Arbidol in improving patients' clinical recovery until day 7.	The administration of Arbidol was combined with standard of care by using traditional Chinese medicine, antibiotics, supplementary antivirals, immunomodulatory drugs, steroids, psychotic drugs, nutritional support, cardiovascular drugs, and oxygen support.
Favipiravir (FVP) vs. Baloxavir marboxil (BAL)							
(Lou et al., 2021).	China	Open control trial	COVID-19 patients FVP n=9 BAL n=10 Control group n = 10	FVP, peroral = 1600 mg 2x1 day followed by 600 mg 3x1 day, duration of administration = no more than 14 days	BAL, peroral = Day 1 and day 4 = 480 mg 1x1 day readministered on day 7 if the test result remained positive	Viral shedding time of 14 days FVP = 7/9 (77%) BAL = 7/10 (70%) Control group = 10/10 (100%) Adding Favipiravir and Baloxavir marboxil to the standard of care did not provide significant clinical benefits.	FVP and BAL were followed by administration of previous antivirals = Lopinavir/Ritonavir (400mg/100mg, BID, peroral) or Darunavir/Cobicistat and Arbidol (200mg, TID, peroral); all with Interferon-α inhalation (100,000 IU, TID or QID)

Table 2. Effectiveness of Favipiravir in patients with severe symptoms (*Continued*).

Ref.	Country	Study Design	Patient population	Dosage and Route of Administration		Clinical Outcome	Information
Favipiravir (FVP) vs Hydroxychloroquine (HCQ)							
(Khamis et al., 2021).	Oman	Randomized, open-label controlled trial	COVID-19 patients FVP n = 44 HCQ n = 45	FVP, peroral Day 1 = 1600 mg followed by 600 mg 2x1 day for a maximum of 10 days FVP combined with 8 million IU (0.25mg) Interferon beta-1b 2x1 day for 5 days	HCQ, peroral Day 1 = 400 mg 2x1 day followed by 200 mg 2x1 day for 7 days	Clinical recovery was assessed based on the patient's clinical outcome: **Transferred to ICU** FVP = 8/44 (18.2%) HCQ = 8/45 (17.8%) **Discharged** FVP = 29/44 (65.9%) HCQ = 31/45 (68.9%) **O2 saturation when discharged** FVP = 94 HCQ = 95 **Died** FVP = 5/44 (11.4%) HCQ = 6/45 (13.3%) Favipiravir + IFN1B and hydroxychloroquine showed no significant differences in the inflammatory markers/clinical outcomes in COVID-19 patients with moderate to severe pneumonia.	N/A
Favipiravir (FVP) vs. Tocilizumab							
(Zhao et al., 2021).	China	Multicenter random trial (3:1:1)	Combined COVID-19 adult patients (FVP vs. Tocilizumab) N = 14 FVP n = 7 Tocilizumab n = 5	FVP peroral Day 1 = 1600 mg, 2x1 day Day 2-7 = 600 mg 2x1 day	Tocilizumab, I.V = 4-8 mg/kg (400 mg) Tocilizumab was administered into 100 ml of 0.9% normal saline	On day 14, FVP vs. Tocilizumab significantly reduced lung lesions compared to the FVP group. In the FVP vs. Tocilizumab group, nearly all patients experienced clinical recovery of fever, muscle pain, shortness of breath, and diarrhea (14/14 or 100%). The number of deaths/ventilators in the FVP group was higher (2/7 or 28.5%) as opposed to the combination group and Tocilizumab monotherapy group (0/14 and 0/5 or 0%). Tocilizumab vs. Favipiravir and Tocilizumab monotherapy improved pneumonia in COVID-19 patients and inhibited the symptom severity.	N/A

This is because Favipiravir is metabolized into inactive M1 metabolites by aldehyde oxidase and xanthine oxidase and excreted into the urine. In the kidneys, uric acid treatment is regulated by the balance between tubule reabsorption and secretion in the proximal tubules. Favipiravir and M1 act as moderate inhibitors of organic anion transporters 1 and 3 (OAT1 and OAT3), which are involved in the excretion of uric acid in the kidneys. M1 increases the reabsorption of uric acid through urate transporter 1 (URAT1) in the proximal tubules of the kidneys (Mishima et al., 2020). There was a case report on a COVID-19 male patient aged 42 years who had a history of hyperuricemia without recurring gout for more than one year, diabetes, or hypertension. The patient was administered with Favipiravir at a dosage of 3600 mg/day followed by 1600 mg/day, each in two divided doses until day 14. On the 13th day, it was reported that the patient's uric acid level increased by 2.3 mg/dl, and the patient was then given NSAID treatment until the complaint declined. The patient recovered on day 20 and was discharged two days later (Hase et al., 2020). This report suggests that a patient's medical history can affect the incidence of hyperuricemia in Favipiravir administration (Hase et al., 2020). The second ADE (adverse drug event) widely reported in several articles was elevated ALT (Alanine Transaminase) levels in the liver experienced by approximately 22 patients or 16.38% of the patient population in all the articles (Chen et al., 2021; Doi et al., 2020).

A case report on Favipiravir for COVID-19 treatment revealed that some patients in the 50-80 age range (11.4%) experienced a significant increase in liver enzymes after 12 days to 2 weeks of treatment. Several patients were then treated with ursodeoxycholic acid (15mg/kg), after which the symptoms and results of laboratory examinations of liver enzyme levels gradually improved within 4 up to 10 weeks (Kumar et al., 2021; Wu and McGoogan, 2020). Another incidence has also been reported as respiratory failure in 2/7 (28.5%) in the Favipiravir group, which was higher than the findings (0/14 and 0/5 patients) in other study groups (Zhao et al., 2021). The other study on Remdesivir also reported a similar finding, which needs careful monitoring of adverse reactions in patients with comorbid conditions and pregnancy (Siada et al., 2022; Kurniawan et al., 2022).

Table 3. Adverse drug events associated with Favipiravir (FVP).

Reference and Year	Study Design	Patient Category	Findings
(Kumar et al., 2021)	Case report	3 COVID-19 patients aged 70 years, 52 years, and 50 years	Elevated liver enzymes or ALT after using Favipiravir
(Magpie et al., 2021)	Case report	1 COVID-19 patient aged 64 years	Hypersensitivity in the form of fever after 14 days of Favipiravir administration
(Doi et al., 2020)	Prospective, Randomized, Open-Label Trial	82 COVID-19 patients in the safety group	• Hyperuricemia in 69/82 (84.1%) • Elevated triglycerides in 9/82 (11%) Increased Alanine aminotransferase in 7/82 (8.5%)

Table 3. Adverse drug events associated with Favipiravir (FVP) (*Continued*).

Reference and Year	Study Design	Patient Category	Findings
(Chen et al., 2021)	RCT multicenter trial	240 COVID-19 patients randomized for FVP administration (120) and control group (120)	• ADE was reported in 37% of the Favipiravir group and 28% of the control group • The most common ADE of Favipiravir were abnormal LFT/liver function tests (10 or 10%), elevated serum uric acid (16 or 13.79%), psychiatric symptoms (5 or 4.31%), and gastrointestinal reactions (16 or 13.79%).
(Cai et al., 2020)	Open control trial nonrandomized, before- after controlled study	80 patients tested COVID-19 positive by RT-PCR = 35 patients given FVP, 45 patients in the control group	• Four (4) patients given FVP (11.43%) reported ADE: • 2 (5.71%) with diarrhea • 1 (2.86%) with liver injury • 1 with poor diet
(Doi et al., 2020)	Prospective, Randomized, Open-Label Trial	82 COVID-19 patients in the safety group	• Hyperuricemia in 69/82 (84.1%) • Elevated triglycerides in 9/82 (11%) • Increased Alanine aminotransferase in 7/82 (8.5%)
(Ivashchenko et al., 2021)	Multicenter Randomized Control Trial Phase II/III	60 COVID-19 patients with moderate pneumonia, randomized with different dose regimens of Favipiravir versus standard of care	• 7/40 (17.5%) in the Favipiravir group experienced adverse drug reactions, including diarrhea, nausea, vomiting, chest pain, and elevated levels of liver transaminase. • Mild-moderate adverse drug reactions leading to drug discontinuation occurred in 2/40 patients (5%).
(Lou et al., 2021)	Open control trial	29 COVID-19 patients confirmed positive by RT-PCR randomized for 1:1:1	• Some patients in the Favipiravir group experienced more than one side effect, including: • Respiratory failure and elevated alanine aminotransferase (4 or 44%), lymphopenia and lower hemoglobin (7 or 77%), increased lactate dehydrogenase and D-dimer (5 or 55%), decreased albumin (8 or 88%), and higher triglycerides (6 or 66%).

Table 3. Adverse drug events associated with Favipiravir (FVP) (*Continued*).

Reference and Year	Study Design	Patient Category	Findings
(Doi et al., 2020)	Prospective, Randomized, Open-Label Trial	82 COVID-19 patients in the safety group	• Hyperuricemia in 69/82 (84.1%) • Elevated triglycerides in 9/82 (11%) • Increased Alanine aminotransferase in 7/82 (8.5%)
(Khamis et al., 2021)	Randomized, open-label controlled trial	Covid-19 patients with moderate-severe symptoms randomized for Favipiravir (45) and standard of care (45)	• No significant side effects, such as hyperuricemia, liver enzyme disorder, or QTc prolongation, were reported from using Favipiravir in this study.

4. CONCLUSION

A limitation was noted in the included studies. The standard of care varied across studies, and other antiviral therapies were or have not been included during treatment. Based on this case, analyzing the result of the treatment effect of a specific drug can be difficult. The use of Favipiravir for COVID-19 patients has a noticeable effect on clinical improvement only among patients with mild-moderate symptoms when administered early at a dosage of 3200 mg/day followed by 2400 mg/day, each in two divided doses up to day 14, in combination with Lopinavir/Ritonavir antivirals or with Chloroquine. In the group of patients with severe symptoms, Favipiravir was administered at the same dose as that for the previous group and, in some studies, was given in combination with Arbidol, Chloroquine, and Interferon beta-1b. However, its combination with anti-IL-6 Tocilizumab was regarded as having a good response. The frequent ADE (adverse drug events) due to Favipiravir use reported in some studies were hyperuricemia and elevated alanine aminotransferase (ALT).

CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

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Efektivitas *Stick* Herbal Usada Bali sebagai Antioksidan dan Anti-Inflamasi pada Mencit Putih Jantan yang Diinduksi Karagenan

Effectiveness of Usada Bali Herbal Stick as an Antioxidant and Anti-Inflammatory in Male White Mice Induced with Carrageenan

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Abstrak

Inflamasi disebabkan oleh terlepasnya mediator pro inflamasi di dalam tubuh, seperti TNF-α, caspase-3, dan prostaglandin. Tujuan penelitian ini adalah untuk mengetahui aktivitas antioksidan dan antiinflamasi dari stick herbal Usada Bali yang mengandung rimpang kencur, bunga cengkeh, dan daun kelor pada mencit putih jantan yang diinduksi karagenan. Penelitian dilakukan dengan rancangan penelitian eksprimental laboratorium, dimana dalam pengujian antioksidan digunakan penelitian *in-vtiro* DPPH dengan instrument spektrofotometri UV-Vis, sedangkan pengujian antiinflamasi berupa penelitian *in-vivo* dengan rancangan *randomize pre test and postest with control group design* dan menggunakan hewan coba dengan rincian pembagian kelompok sebagai berikut: Kelompok 1 (P1) adalah kelompok perlakuan plasebo, kelompok 2 (P2) adalah kelompok perlakuan yang diberikan natrium diklofenak, kelompok 3 (P3) adalah kelompok perlakuan yang diberikan *Stick* Herbal Usada Bali. Pengujian antioksidan memberikan hasil bahwa sampel memiliki nilai IC_{50} 82,285 µg/mL dengan kategori (kuat) dan nilai *Antioxidant Activity Index* 0,469 dengan katagori (sedang). Pengujian antiinflamasi berdasarkan analisis SPSS menunjukkan bahwa terdapat perbedaan bermakna antara kelompok kontrol negatif dengan kelompok perlakuan *Stick* Herbal Usada Bali dengan nilai p=0,000 (p<0,05), serta tidak terdapat perbedaan bermakna antara kelompok positif dengan kelompok perlakuan *Stick* Herbal Usada Bali p=0,222 (p>0,05). Efektivitas antioksidan dan antiinflamasi *Stick* Herbal Usada Bali dipengaruhi oleh senyawa yang terkandung di dalamnya yaitu flavonoid dan saponin yang diketahui memiliki khasiat sebagai antioksidan dan antiinflamasi. Sehingga, dapat disimpulkan bahwa *Stick* Herbal Usada Bali berpotensi sebagai alternatif terapi antiinflamasi dan memiliki efektivitas sebagai antioksidan.

Kata kunci: Antiinflamasi; Antioksidan; DPPH; Karagenan; *Stick* Herbal Usada Bali

Abstract
Inflammation is caused by the release of pro-inflammatory mediators in the body, such as TNF-α, caspase-3, and prostaglandins. This study aimed to determine the activity of the Usada Bali herbal Stick, which contains kencur rhizome, clove flower, and Moringa leaves as an antioxidant using the DPPH method and anti-inflammatory in carrageenan-induced male white mice. The research was carried out using a laboratory experimental research design, wherein the antioxidant test, the in-vitro DPPH study, was used with the UV-Vis spectrophotometry instrument, while the anti-inflammatory test was in the form of an in-vivo study with a randomized pre-test and posttest with control group design and using mice samples. Antioxidant testing showed that the sample had an IC50 value of 82.285 µg/mL with the (strong) category and an Antioxidant Activity Index value of 0.469 with the (moderate) category. Anti-inflammatory testing based on SPSS analysis showed that there was a significant difference between the negative control group and the Usada Bali Stick Herbal treatment group p=0.000 (p<0.05), and there was no significant difference between the positive group and the Usada Bali Stick Herbal treatment group p=0.222 (p>0.05). The antioxidant and anti-inflammatory effectiveness of the Usada Bali stick was influenced by its compounds, namely flavonoids and saponins, which are known to have antioxidant and anti-inflammatory properties. Thus, it can be concluded that the Usada Bali stick has the potential as an alternative to anti-inflammatory and antioxidant therapy.

Keywords: *Anti inflammation; Antioxidant; Carrageenan; DPPH; Usada Bali Stick Herbal*

1. PENDAHULUAN

Indonesia merupakan salah satu negara yang menganggap kesehatan sebagai masalah yang cukup serius. Masyarakat Indonesia menggunakan sebagian besar waktunya untuk melakukan aktivitas di luar ruangan. Faktor lingkungan dan iklim di Indonesia seperti polusi udara, asap rokok, intensitas sinar ultraviolet (UV) yang tinggi, dan suhu mengakibatkan tingginya paparan radikal bebas (Haryoto dan Priyanto, 2018). Radikal bebas merupakan molekul dengan satu atau lebih elektron tidak berpasangan yang memiliki peran dalam rusaknya jaringan, serta proses terjadinya suatu penyakit tidak menular dalam tubuh manusia (Pratama dan Busman, 2020). Antioksidan merupakan senyawa atau zat yang dapat menghambat, menunda, mencegah atau memperlambat reaksi oksidasi yang merupakan reaksi kimia pembentuk radikal bebas (Haryoto dan Priyanto, 2018).

Radikal bebas diketahui berdampak pada meningkatkanya kadar IL-6, dan berkolerasi dengan meningkatnya serum TNF-α yang menyebabkan inflamasi (Suryadinata, 2018). Inflamasi merupakan suatu respon protektif normal terhadap luka jaringan yang disebabkan oleh trauma fisik, zat kimia yang merusak atau zat-zat mikrobiologik (Agustina *et al*., 2015). Tanda-tanda utama dari inflamasi yaitu *rubor* (kemerahan) terjadi pada tahap pertama, *calor* (panas) pada tahap kedua, dan *tumor* (pembengkakan) pada tahap ketiga. Inflamasi biasanya diobati dengan menggunakan obat antiinflamasi. Obat antiinflamasi dapat dibagi menjadi 2 golongan yaitu golongan obat antiinflamasi steroid dan obat antiinflamasi golongan non steroid (AINS), obat antiinflamasi golongan steroid bekerja dengan cara menghambat pelepasan prostagladin dari sel-sel sumbernya, dan untuk obat antiinflamasi golongan non steroid merupakan obat analgetik lemah, antiflogistik, yang bekerja melalui mekanisme lain seperti

inhibisi siklooksigenase (Pramitaningastuti dan Anggraeny, 2017). Obat antiinflamasi nonsteroid memiliki sejumlah efek samping yang signifikan bila digunakan dalam jangka waktu yang panjang, seperti gangguan gastrointestinal, kardiovaskular, ginjal, hati, serebral, dan paru-paru (Bindu *et al*., 2020).

Pengobatan tradisional berbasis bahan alam atau herbal telah berkembang sejak zaman dahulu di Indonesia. Bali merupakan salah satu provinsi di Indonesia yang memiliki kekayaan budaya berupa pengobatan tradisional. Usada Bali merupakan salah satu pengobatan tradisional Bali yang dipercaya memiliki berbagai manfaat kesehatan, salah satunya adalah sebagai antioksidan dan antiinflamasi (Sutomo dan Iryadi, 2019). Beberapa bahan obat yang tertuang dalam Lontar Usada Bali yang diyakini berfungsi sebagai antioksidan dan antiinflamasi yaitu tumbuhan kelor (*Moringa oleifera* L.), kencur (*Kaempferia galanga* L.), dan cengkeh (*Syzygium aromaticum* L.). Ulfa *et al*., (2016) melaporkan bahwa berdasarkan hasil analisis fitokimia yang telah dilakukan kelor mengandung flavonoid, saponin dan senyawa polifenol yang diketahui memiliki aktivitas sebagai antiinflamasi. Flavonoid pada kelor bekerja dengan cara menghambat asam arakhidonat dan sekresi enzim lisosom dari endothelial sehingga menghambat proliferasi dan oksidasi dari proses radang. Terhambatnya pelepasan asam arakhidonat dari sel inflamasi akan menyebabkan substrat arakhidonat bagi jalur siklooksigenase dan jalur lipooksigenase berkurang. Lisosom mengandung protease dan enzim lain. Protease lisosom merupakan salah satu mediator kimiawi inflamasi yang memiliki aktivitas enzimatis langsung sehingga penghambatan enzim ini dapat mengurangi inflamasi (Simorangkir *et al*., 2020). Hal ini senada dengan riset yang dilakukan oleh Zulfa *et al*., (2020) yang mengatakan bahwa senyawa flavonoid pada kelor dan kencur dapat menghambat aktivitas enzim sikloosigenase dan lipoksigenase. Hal ini menyebabkan terhambatnya jalur metabolisme asam arakidonat dengan terjadinya pembentukkan prostaglandin dan pelepasan histamin pada radang. Selain flavonoid, saponin pada tanaman kelor dan kencur diduga berinteraksi dengan banyak membran lipid seperti protaglandin dengan menghambat pelepasan asam arakidonat dari sel radang sehingga substrat arakidonat bagi jalur siklooksigenase dan jalur lipooksigenase akan berkurang (Andriyono, 2019).

Tanaman kencur (*Kaempferia galanga* L.) adalah tanaman yang dimanfaatkan sebagai ramuan obat-obatan tradisional untuk mencegah penyakit tertentu seperti masuk angin, batuk, dan sakit tenggorokan. Selain itu, kencur memiliki manfaat lain sebagai antibakteri, antifungi, anti-inflamasi, antioksidan, antivirus, antihipertensi, atikarsinogenik, antinosiseptif, antituberkulosis, dan larvasida. Sedangkan, Cengkeh (*Syzygium aromaticum* L.) berpotensi sebagai antioksidan dan antiinflamasi dengan adanya kandungan seperti eugenol, flavonoid, asam hidroksibenzoat, asam hidroksisinamat serta vitamin C dan E, pada setiap 100 g cengkeh diketahui memiliki kandungan vitamin C sebesar 80,8 mg dan vitamin E sebesar 8,52 mg, keduanya berperan sebagai antioksidan dalam mencegah reaksi oksidatif. Tingginya aktivitas antioksidan dalam cengkeh berasal dari kandungan asam lemak tidak jenuh yang tinggi, yaitu kadar asam lemak linoleate sebanyak 6,01%, linoleate 8,5%, dan eikosa tetraenoat 12,13%, sehingga membentuk antioksidan alami. Begitupula dengan daun kelor (*Moringa oleifera* L.),

berdasarkan analisis fitokimianya kaya akan kandungan flavonoid dan polifenol sebagai antioksidan dan antiinflamasi (Kaur dan Kaushal, 2019; Ulfa *et al*., 2016). Ekstrak rimpang kencur (*Kaempferia galanga* L.) menghasilkan flavonoid yang memiliki kemampuan sebagai antioksidan yang mampu mentransfer sebuah elektron atau sebuah atom hidrogen ke senyawa radikal bebas sehingga menghambat peroksidasi lipid, menekan kerusakan jaringan oleh radikal bebas, dan menghambat beberapa enzim. Kencur memiliki komponen senyawa utama yaitu Etil-trans-p-metoki sinamat dan trans-etil-sinamat yang bersifat farmakologi sebagai obat ekspektorat, karminatif, obat batuk, rematik, anti kanker, kolera, vasorelaksasi, antimikroba, antialergi, dan penyembuhan luka. Hasil penelitiannnya menunjukkan bioaktivitas kencur dapat sebagai antikanker dan antioksidan (Ali *et al*., 2018). Sedangkan tanaman cengkeh diketahui banyak mengandung minyak atsiri yang berasal dari bagian bunga sebanyak 10-20%, tangkai 5-10% dan daun 1-4%. Kandungan minyak atsiri didominasi oleh eugenol dalam jumlah besar yaitu 70-80%, aktivitas antioksidan eugenol sebanding dengan aktivitas antioksidan sintetik pyrogallol dan BHA (Kaur dan Kaushal, 2019; Nurdjannah, 2004). Minyak cengkeh merupakan minyak atsiri dari tanaman cengkeh *(syzygium aromaticum)* yang memiliki mekanisme menghambat sintesis prostaglandin (Iriani *et al*., 2017). Minyak atsiri yang terkandung pada kencur juga dapat menghambat agregasi platelet dengan menghambat pembentukkan tromboksan sehingga berperan dalam efek antiinflamasi (Andriyono, 2019).

Namun saat ini, data ilmiah mengenai aktivitas antioksidan dan antiinflamasi ramuan tersebut dalam bentuk sediaan *stick* masih terbatas. Berdasarkan latar belakang tersebut, maka dilakukan penelitian bertujuan untuk menguji aktivitas antioksidan dengan metode DPPH (2,2-*diphenyl*-1-*picrylhidrazil*), serta aktivitas anti inflamasinya pada mencit inflamasi yang diinduksi karagenan. Metode DPPH dipilih karena metode ini telah umum digunakan dan memiliki keuntungan diantaranya cepat, sederhana, akurat, dan sampel yang dibutuhkan lebih sedikit (Dontha, 2016; Pardede dan Pardede, 2018).

2. BAHAN DAN METODE

2.1. Bahan

Bahan yang digunakan yaitu simplisia Daun kelor (*Moringa oleifera* L.), rimpang kencur (*Kaempferia galanga* L.), cengkeh (*Syzygium aromaticum* L.) diperoleh dari Desa Yangapi Kecamatan Tembuku Kabupaten Bangli – Bali Indonesia, etanol 70% (Bratachem; Indonesia), etanol 96% (Bratachem; Indonesia), *carnauba wax, cera alba, castor oil, olive oil,* BHT, *metil paraben,* asam stearat, gliserin, setil alkohol, potassium hidroksida, propilen glikol, asam askorbat (Merck; Jerman), DPPH (Merck; Jerman), kloroform (Merck, Jerman), amoniak (Bratachem, Indonesia), asam sulfat (Merck, Jerman), etanol (Bratachem, Indonesia), asam hidroklorida (Merck, Jerman), asam asetat anhidrat (Merck, Jerman), mencit putih jantan (*Mus muculus* L) berumur 6-9 minggu dengan berat badan 18-25 gram, natrium diklofenak, karagenan (Sigma Aldrich; St. Louis, MO), dan pletismometer (Orchid, India). Bahan yang digunakan dalam pembuatan sediaan *Stick* Herbal Usada Bali termuat pada Tabel 1. Ekstrak daun kelor, daun kencur, dan daun cengkeh masing-masing berperan sebagai bahan aktif

dengan bobot 15% w/w. *Carnauba wax* dan cera alba berfungsi sebagai agen penstabil dengan bobot masing-masing 15% dan 10% w/w. *Castor oil* dan *olive oil* hadir sebagai emolien dengan bobot 15% dan 14,85% w/w. Balsem *stick* ini dirancang dengan cermat untuk memberikan manfaat yang diinginkan sambil mempertahankan stabilitas dan keawetan produk (Iswandana *et al*., 2018).

Tabel 1. Formulasi sediaan *Stick* Herbal Usada Bali.

Nama Bahan	Fungsi	Bobot (% w/w)
Ekstrak daun kelor	Bahan aktif	15
Ekstrak daun kencur	Bahan aktif	15
Ekstrak daun cengkeh	Bahan aktif	15
Carnauba wax	Agen penstabil	15
Cera alba	Agen penstabil	10
Castor oil	Emolien	ad 15
Olive oil	Emolien	14,85
BHT	Stabilitator	0,05
Metil paraben	Pengawet	0,1

2.2. Metode

2.2.1. Determinasi tanaman

Tanaman yang digunakan sebagai bahan pada penelitian ini dideterminasi di Lembaga Ilmu Pengetahuan Indonesia yang bertempat di UPT Balai Konservasi Tanaman Kebun Raya Eka Karya Bali untuk memastikan kebenaran identitas tanaman.

2.2.2. Pembuatan ekstrak

Rimpang kencur, bunga cengkeh, dan daun kelor disortasi dan dibersihkan, kemudian dikeringkan di dalam nampan yang ditutup kain hitam dan tidak terkena sinar matahari langsung. Kemudian, sortasi kering dilakukan untuk memisahkan bahan atau benda asing pada simplisia. Simplisia dihaluskan dengan blender hingga menjadi serbuk halus lalu dimasukkan ke dalam wadah terpisah. Proses ekstraksi dilakukan dengan penambahan pelarut etanol dengan perbandingan 1:4 dan selama 3 hari sembari diaduk. Kemudian, hasil maserat dipekatkan dengan *rotary evapotator* dengan suhu 78ºC. Daun kelor (*Moringa oleifera* L.) berat 213,83g menghasilkan 43,3g ekstrak, rimpang kencur (*Kaempferia galanga* L.) berat 218,7g menghasilkan ekstrak 44,3g, cengkeh (*Syzygium aromaticum* L.) dengan berat 258,27g menghasilkan ekstrak 52,3g, Ekstrak kental kemudian disimpan pada wadah tertutup rapat dan terlindung dari cahaya matahari.

2.2.3. Pembuatan sediaan *Stick* Herbal Usada Bali

Ditimbang bahan yang akan digunakan, yakni: ekstrak daun kelor (1,5 g); ekstrak daun kencur (1,5 g); ekstrak daun cengkeh (1,5 g); *carnauba wax* (1,5 g); *cera alba* (1 g); *castor oil* (1,5 g); *olive oil* (1,485 g); BHT (0,005 g); metil paraben (0,01 g). Dilelehkan *carnauba wax* dan *cera alba* di atas penangas air sampai suhu 80ºC (Campuran I). Dilelehkan *castor oil* dan sebagian *olive oil* di atas penangas air sampai suhu 80ºC (Campuran II). BHT dan metil paraben

dicampurkan dengan sebagian *olive oil* lalu tuangkan ke campuran II. Lalu dicampurkan campuran II ke campuran I sambil tetap berada di atas penangas air. Ditambahkan satu per satu ekstrak sambil diaduk sampai homogen. Aduk di atas penangas air selama 5 menit. Turunkan lalu tunggu hingga tidak terlalu panas. Setelah itu campuran dimasukkan ke dalam cetakan dan didiamkan pada suhu ruang sampai mengeras selama kira-kira 30 menit dan dikemas dalam wadah. Prosedur pembuatan sediaan *stick* mengacu pada riset yang dilakukan oleh Iswandana *et al*. (2018) dengan melakukan optimasi penyesuaian bahan yang digunakan pada formula *stick.*

2.2.4. Uji skrining fitokimia

Pengujian skrining fitokimia (Tabel 2) digunakan untuk mengetahui senyawa fitokimia pada ekstrak cengkeh, ekstrak kelor, dan ekstrak kencur.

Tabel 2. Skrining fitokimia dengan pereaksi yang digunakan pada ekstrak cengkeh, kelor, dan kencur.

Senyawa Fitokimia	Pereaksi
Alkaloid	Dragendroff Mayer
Flavonoid	Mg + Alkohol klorhidrat + Amil alkohol
Saponin	Dikocok 10 detik + HCl 2N
Tanin	$FeCl_3$ 1%
Kuinon	NaOH 1N

2.2.4. Uji aktivitas antioksidan

Penelitian ini menggunakan metode DPPH dengan mengujikan *Stick* Herbal Usada Bali sebagai sampel pada konsentrasi 50; 60; 70; 80; 90; 100 µg/ml, dan asam askorbat sebagai pembanding pada konsentrasi 2; 4; 6; 8; 10 µg/ml. Larutan radikal bebas DPPH 40 ppm dibuat dengan pelarut etanol 96% dan diukur panjang gelombangnya dengan spektrofotometer UV-Vis pada panjang gelombang 400-800 nm. Pengukuran peredaman radikal bebas DPPH dilakukan dengan melarutkan larutan sampel uji dan pembanding yang berbeda konsentrasi kemudian dipipet masing-masing sebanyak 4 ml dan dimasukkan ke dalam tabung reaksi yang berbeda dan ditambahkan 4 ml larutan DPPH. Sebagai pembanding, tabung reaksi yang berbeda dimasukkan larutan DPPH 40 ppm sebanyak 4 ml dan etanol 96% sebanyak 4 ml. Tabung reaksi kemudian dihomogenkan dan diinkubasi 30 menit. Lalu, diamati absorbansinya pada panjang gelombang maksimum 518 nm dengan spektrofotometer UV-Vis. Kemudian, masing-masing tingkat konsentrasi diuji lalu dihitung persentase peredaman radikal bebasnya menggunakan Persamaan 1, kemudian hasil tersebut diplotkan dalam sebuah grafik hingga menemukan suatu persamaan y = bx + a dan memperoleh nilai IC_{50} dengan mengganti y = 50 pada persamaan regresi linier dimana x ada konsentrasi (µg/ml) dan y adalah persentase inhibisi.

$$\text{Persentase aktivitas antioksidan} = \frac{A0-A1}{A0} \times 100\%$$

Persamaan 1. Persentase aktivitas antioksidan. Keterangan = A0 adalah absorbansi tanpa sampel dan A1 adalah absorbansi setelah ditambah sampel (Mishra *et al*., 2012).

Analisis hasil pengujian ini dilakukan secara kualitatif dan kuantitatif, dimana secara kualitatif dilakukan dengan melihat perubahan warna masing-masing sampel setelah diinkubasi bersama DPPH. Jika elektron DPPH berpasangan dengan elektron pada sampel maka akan terjadi perubahan warna sampel, sedangkan secara kuantitatif kekuatan antioksidan *Stick* Herbal Usada Bali diklasifikasikan berdasarkan parameter IC_{50} dan AAI (Persamaan 2).

$$\text{Nilai AAI} = \frac{\text{Konsentrasi DPPH}}{\text{IC50}}$$

Persamaan 2. Nilai AAI (*Antioxidant Activity Index)*. Keterangan = IC_{50} adalah kekuatan sampel untuk meredam 50% radikal bebas (Sawiji dan La, 2022).

2.2.5. Uji aktivitas antiinflamasi

Uji aktivitas antiinflamasi yang dilakukan mengacu pada persetujuan etik oleh Komite Etik Hewan Nomor: B/127/UN.14.2.9/PT.01.04/2022, hewan coba mencit putih (*Mus musculus*) jantan dengan berat rata-rata 20g sebanyak 27 ekor dikelompokkan secara acak menjadi 3 kelompok perlakuan. Dari masing-masing kelompok akan diberikan perlakuan sesuai dengan yang dijelaskan pada rancangan penelitian. *Pretest* dan *posttest* dilakukan dengan mengukur telapak kaki mencit menggunakan pletismometer. 27 ekor mencit putih jantan yang diadaptasi selama 7 hari dan kemudian dibuat edema dengan diinduksi karagen sebanyak 0,1 mL secara intra planar, lalu dilakukan pretest dengan cara diukur volume kaki mencit menggunakan pletismometer. Kelompok 1 diberikan plasebo sebagai kontrol negatif, kelompok 2 diberikan *stick* natrium diklofenak sebagai kontrol positif dengan dosis 15 mg dan kelompok 3 diberikan formulasi *stick* sesuai formula pada Tabel 1 selama 5 jam, seluruh perlakuan diberikan pada mencit secara topikal. Lalu dilakukan pengukuran posttest dengan diukur volume kaki mencit menggunakan pletismometer (Sukmawati *et al*., 2015). Analisis data dilakukan secara statistik *Shapiro-Wilk* karena data berjumlah kurang dari 50, dan dilanjutkan dengan uji *Kruskal-Wallis,* serta *Mann Whitney.*

3. HASIL DAN PEMBAHASAN

3.1. Determinasi tanaman komponen *stick* Herbal Usada Bali

Hasil determinasi tanaman yang menjadi komponen dalam *Stick* Herbal Usada Bali (Tabel 3). Tanaman yang digunakan berasal dari suku yang berbeda-beda, yakni *Myrtaceae, Zingiberaceae,* dan *Moringaceae.*

Tabel 3. Identitas spesies dan suku tanaman komponen *Stick* Herbal Usada Bali.

Nama tanaman	Spesies tanaman	Suku	Nomor dokumen determinasi
Cengkeh	*Syzygium aromaticum* L.	Myrtaceae	
Kencur	*Kaemferia galangal* L.	Zingiberaceae	B-. 6361/III/KS.01.14/7/2021
Kelor	*Moringa oleifera* L.	Moringaceae	

3.2. Hasil skrining fitokimia

Hasil skrining fitokimia menunjukkan bahwa seluruh komponen tanaman dalam *Stick* Herbal Usada Bali mengandung senyawa metabolit sekunder (Tabel 4). Hal ini menunjukkan

bahwa jenis pelarut dalam metode ekstraksi tanaman dalam *Stick* Herbal Usada Bali mampu menarik metabolit sekunder tersebut. Flavonoid banyak ditemukan berikatan dengan gula berbentuk glikosidanya yang menyebabkan senyawa golongan flavonoid mudah larut dalam pelarut polar, kelarutan zat ke dalam suatu pelarut ini sangat ditentukan oleh kecocokan sifat dari struktur kimia antara zat terlarut dan pelarut yaitu *like dissolves like,* yang berarti senyawa polar akan lebih mudah larut dalam pelarut polar, sedangkan senyawa non-polar akan lebih mudah larut dalam pelarut non-polar (Suhendra *et al*., 2019). Begitupula dengan saponin yang merupakan glikosida dengan berat molekul tinggi, tersusun dari gula yang berhubungan dengan triterpene atau steroid aglikon, serta memiliki polaritas yang tinggi, karenanya etanol yang merupakan pelarut polar mampu menarik senyawa saponin (Santosa *et al*., 2018).

Tabel 4. Hasil skrining fitokimia ekstrak tanaman komponen *Stick* Herbal Usada Bali. Keterangan = (+) adalah positif mengandung senyawa fitokimia, dan (-) adalah negatif tidak mengandung senyawa fitokimia.

Senyawa Fitokimia	Ekstrak		
	Kelor	Cengkeh	Kencur
Alkaloid	+	-	-
Flavonoid	+	+	+
Saponin	+	+	+
Tanin	+	+	-
Kuinon	-	-	-

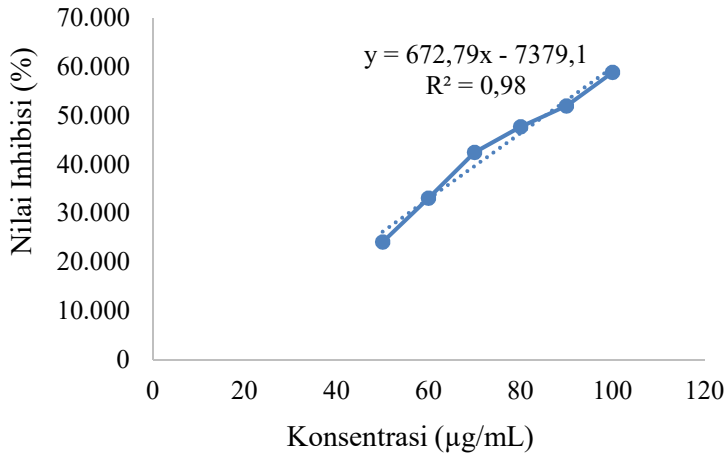
3.3. Hasil pengujian antioksidan

Penelitian ini untuk mengetahui efektivitas *Stick* Herbal Usada Bali sebagai antioksidan. Secara pengamatan kualitatif, sampel asam askorbat mengubah warna radikal DPPH dari warna awal ungu menjadi kuning pucat setelah dilakukkan inkubasi selama 30 menit. Sedangkan, sampel formula *Stick* Herbal Usada Bali setelah dilakukkan inkubasi selama 30 menit mengubah warna radikal DPPH namun terlihat tidak sesignifikan seperti sampel asam askorbat. Berdasarkan hasil yang diperoleh dapat diketahui bahwa formula *Stick* Herbal Usada Bali memiliki aktivitas antioksidan kuat dengan nilai IC_{50} 82,285 ppm, sedangkan sampel asam askorbat memiliki aktivitas antioksidan sangat kuat. Nilai antoksidan dikatagorikam kuat jika nilai IC_{50} 50-100 ppm, katagori sangat kuat dengan nilai $IC_{50} < 50$ ppm. Tristantini, *et al*. (2016) menegaskan bahwa semakin kecil nilai IC_{50} yang diperoleh maka semakin besar aktivitas antioksidannya. Hal ini dapat terjadi karena dalam ekstrak kelor, cengkeh, dan kencur terkandung banyak senyawa yang berperan sebagai antioksidan yang kuat seperti flavonoid dan alkaloid dimana senyawa tersebut termasuk kedalam senyawa polar, jenis pelarut yang dipergunakan dalam ekstraksi berpengaruh sangat nyata terhadap aktivitas antioksidan pada ekstrak. Kemampuan melarutkan yang tinggi ini berhubungan dengan kepolaran pelarut dan kepolaran senyawa yang diekstraksi, semakin mirip kepolaran pelarut dengan kepolaran zat yang terkandungdalam bahan yang diekstraksi maka akan semakin banyak komponen zat yang dapatdiekstraksi sehingga dapat terjadi peningkatan rendemen yang diperoleh (Tan *et al*., 2013).

Pengamatan secara kualitatif tersebut didukung oleh teori mengenai mekanisme kerja metode DPPH dalam pengujian aktivitas antioksidan. Sampel yang memiliki aktivitas antioksidan akan mendonorkan elektronnya kepada radikal DPPH, yang berakibat pada berubahnya warna ungu DPPH menjadi warna kuning pucat dan mengurangi absorbansi DPPH. Semakin kuat sebuah sampel dalam mengubah warna radikal DPPH maka semakin kuat aktivitas antioksidannya. Antioksidan diperlukan untuk meredam radikal bebas yang menyebabkan *stress oksidative* dan berdampak pada kesehatan manusia. Hasil pengukuran persentase inhibisi sampel *Stick* Herbal Usada Bali (Tabel 5) berdasarkan perhitungan dengan Persamaan 1. Hasil perhitungan persentase inhibisi kemudian dibuat dalam bentuk kurva untuk memperoleh persamaan linieritasnya yaitu y = 0,6728x – 7,3796 dengan R^2 sebesar 0,98 (Gambar 1).

Tabel 5. Hasil nilai absorbansi dan persentase Inhibisi *Stick* Herbal Usada Bali pada metode DPPH.

Konsentrasi (µg/mL)	Absorbansi	Persentase Inhibisi (%)
50	0.389	24,172
60	0.343	33,138
70	0.295	42,495
80	0.268	47,758
90	0.246	52,047
100	0.211	58,869

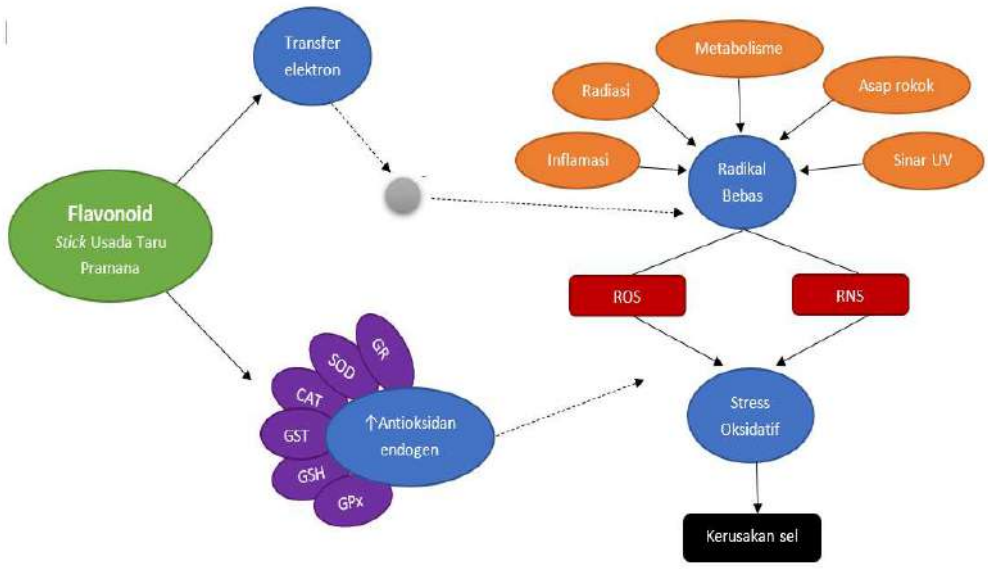


Gambar 1. Kurva regresi linear persentase nilai inhibisi *Stick* Herbal Usada Bali.

Persamaan regreasi linieritas digunakan untuk menghitung IC_{50} yang merupakan konsentrasi sampel untuk meredam 50% radikal bebas DPPH dan nilai AAI yang merupakan indeks aktivitas antioksidan untuk menggolongkan sifat antioksidan (Sawiji dan La, 2022). Nilai AAI digunakan untuk mengklasifikasikan kemampuan antioksidan dari suatu sampel. Nilai AAI dikategorikan lemah jika AAI 0,5-1, sedang 1-2, dan nilai 2 untuk kuat (Dah-Nouvlessounon *et al*., 2023). Sampel *Stick* Herbal Usada Bali memiliki nilai IC_{50} 82,285 µg/mL yang tergolong kuat berdasarkan penggolongan aktivitas antioksidan oleh (Souhoka *et al*.,

2019), dan nilai AAI 0,469 yang tergolong sedang berdasarkan penggolongan aktivitas antioksidan oleh (Sari dan Putra, 2018). Nilai IC_{50} dan nilai AAI dapat memiliki penggolongan aktivitas antioksidan yang berbeda, hal ini disebabkan oleh perbedaan klasifikasi antar kedua parameter tersebut, dimana semakin kecil nilai IC_{50} maka semakin besar aktivitas antioksidannya, sedangkan semakin besar nilai AAI maka semakin besar pula aktivitas antioksidannya (Sawiji dan La, 2022). Nilai AAI dapat dikatakan sebagai indikator universal untuk kekuatan antioksidan, hal ini dikarenakan ketika sampel diuji dengan konsentrasi radikal bebas yang berbeda IC_{50} dapat beragam, sedangkan nilai AAI akan tetap sama (Arsul *et al*., 2022).

Tingginya aktivitas antioksidan *Stick* Herbal Usada Bali diakibatkan oleh kandungan dalam ekstrak kelor, cengkeh, dan kencur terkandung banyak senyawa yang berperan sebagai antioksidan yang kuat seperti flavonoid, dimana senyawa tersebut termasuk kedalam senyawa polar, jenis pelarut yang dipergunakan dalam ekstraksi berpengaruh sangat nyata terhadap aktivitas antioksidan pada ekstrak (Musfiroh *et al*., 2023). Riset ini dikuatkan dengan riset yang dilakukan oleh Ulfa *et al*. (2016) dikatakan bahwa saponin memiliki aktivitas sebagai antiinflamasi, selain sebagai antiinflamasi. Saponin juga merupakan senyawa tersebut juga memiliki efek farmakologis sebagai antioksidan, analgesik, fungisidal, dan bakterisidal serta meningkatkan proses angiogenesis (Fatimatuzzahroh *et al*., 2015).



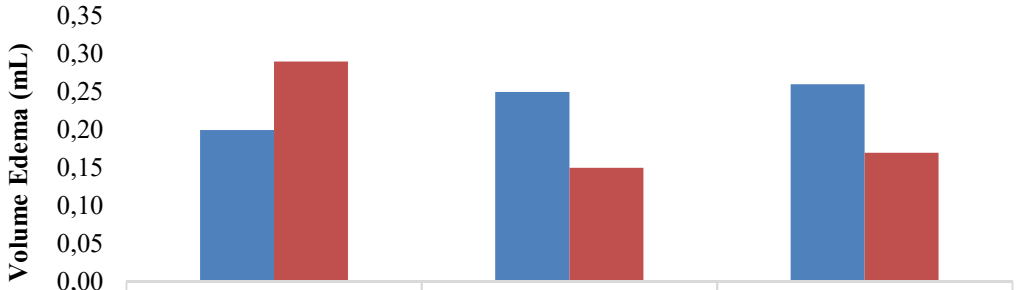
Gambar 2. Potensi mekanisme kerja flavonoid sediaan *Stick* Herbal Usada Bali terhadap radikal bebas (modifikasi dikutip dari (Adrianta *et al*., 2021)).

Flavonoid dapat memberi efek antioksidan dengan mencegah terjadinya ROS (*Reactive Oxygen Species*) dan RNS (*Reactive Nitrogen Species*) dengan cara menangkap radikal bebas, atau secara tidak langsung terjadi peningkatan enzim antioksidan endogen. Mekanisme kerja flavonoid terdiri dari dua yaitu mekanisme kerja secara langsung dan mekanisme kerja secara tidak langsung (Gambar 2). Mekanisme kerja secara langsung dimana flavonoid dapat

memberikan transfer elektron kepada radikal bebas, radikal bebas tersebut dapat disebabkan oleh adanya reaksi inflamasi, radiasi, metabolisme, asap rokok, serta sinar UV. Kondisi tidak keseimbangan antara radikal bebas dengan antioksidan didalam tubuh menyebabkan timbulnya reaksi ROS (*Reactive Oxygen Species*) dan RNS (*Reactive Nitrogen Species*), kedua keadaan ini dapat menyebabkan stres oksidatif yang mampu mengakibatkan terjadinya kerusakan sel. Mekanisme kerja secara tidak langsung dimana flavonoid akan memicu peningkatan antioksidan endogen, antioksidan endogen terdiri dari GR, SOD, CAT, GST, GSH, dan GPx. Antioksidan endogen ini dapat meredam radikal bebas sehingga mencegah terjadinya kerusakan sel (Adrianta *et al*., 2021).

3.4. Hasil pengujian antiinflamasi

Penelitian ini bertujuan untuk mengetahui efektivitas *Stick* Herbal Usada Bali terhadap mencit putih jantan sebanyak 27 ekor yang dibagi secara acak menjadi 3 kelompok perlakuan, setiap kelompok terdiri dari 9 ekor mencit. Kelompok 1 (P1) adalah kelompok perlakuan yang diberikan plasebo, kelompok 2 (P2) adalah kelompok perlakuan yang diberikan natrium diklofenak, kelompok 3 (P3) adalah kelompok perlakuan yang diberikan sediaan *Stick* Herbal Usada Bali. Pengujian antiinflamasi dilakukan dengan mengukur volume kaki mencit 30 menit setelah diinduksi karagenan 1% (*pretest*) dan pada jam ke-1, jam ke-3, dan jam ke-5 (*post test*) menggunakan alat pletismometer.



	kontrol negatif	kontrol positif	formulasi stick usadha taru pramana
pretest	0,20	0,25	0,26
post test	0,29	0,15	0,17

Gambar 3. Grafik perbandingan rata-rata volume edema kaki mencit sebelum perlakuan (*pretest)* dan setelah perlakuan (*posttest)* pada 3 kelompok perlakuan.

Volume rata-rata edema kaki mencit pada kelompok kontrol negatif meningkat hingga jam ke-5, dengan rerata ± SD sebesar 0,29 ± 0,031 (Gambar 3). Sementara pada kelompok kontrol positif, rerata ± SD adalah 0,15 ± 0,027, dan pada kelompok *Stick* Herbal Usada Bali, terjadi penurunan dengan rerata 0,17 ± 0,012. Kelompok uji kontrol positif dan kelompok uji perlakuan sediaan *Stick* Herbal Usada Bali menunjukkan peningkatan sampai jam ke-1, mulai mengalami penurunan pada jam ke-3 dan ke-5. Rata-rata penurunan volume edema pada kontrol negatif -0,09, kontrol positif 0,1, dan kelompok *Stick* Herbal Usada Bali 0,09. Sehingga

kelompok kontrol negatif tidak mengalami penurunan bengkak, sedangkan kelompok kontrol positif dan kelompok *Stick* Herbal Usada Bali mengalami penurunan.

Hasil pengukuran volume edema kaki mencit jantan berdasarkan pengujian *Shapiro Wilk* tidak terdistribusi secara normal karena nilai p<0,05. Pengujian untuk mengetahui perbedaan volume edema pada telapak kaki mencit jantan menggunakan statistika *Kruskal-Wallis* pada setiap kelompok uji yang mendapatkan nilai *significancy* 0,000 (p<0,05). Hal ini menunjukkan bahwa tidak terdapat 2 kelompok yang berbeda bermakna, sehingga analisis statistika ini kemudian dilanjutkan dengan uji *Post Hoc Mann Whitney.* Uji *Post Hoc Mann Whitney* menunjukkan bahwa terdapat perbedaan bermakna antara kelompok negatif dengan kontrol positif, serta antara kelompok negatif dengan kelompok perlakuan *Stick* Herbal Usada Bali dengan nilai 0,000 (p<0,05). Sedangkan, perbandingan antara kelompok kontrol positif dengan kelompok perlakuan *Stick* Herbal Usada Bali menunjukkan bahwa tidak ada perbedaan bermakna dengan nilai p 0,222 (p>0,05). Hal ini menunjukkan bahwa kontrol negatif tidak mempunyai efek antiinflamasi pada mencit dikarenakan adanya peningkatan rekasi inflamasi sesuai dengan gambar 3, sedangkan kontrol positif dan kelompok perlakuan Stick Herbal Usada Bali mempunyai efek sebagai penurunan inflamasi.

Potensi antiinflamasi dari *Stick* Herbal Usada Bali senyawa yang menyebabkan terjadinya penurunan inflamasi yaitu flavanoid, saponin, dan minyak atsiri. Hasil ini dikuatkan oleh penelitian lainnya yang mengatakan bahwa flavonoid dapat menghambat aktivitas enzim sikloosigenase dan lipoosigenase (Zulfa *et al*., 2020). Kandungan Flavonoid ini menyebabkan terhambatnya jalur metabolisme asam arakidonat dengan terjadinya pembentukkan prostaglandin dan pelepasan histamin pada radang (Andriyono, 2019). Selain flavonoid, saponin pada pada tanaman kelor dan kencur menurut hasil riset juga diduga berinteraksi dengan banyak membran lipid seperti protaglandin dengan menghambat pelepasan asam arakidonat yang menyebabkan sel radang akan berkurang tersedianya substrat arakidonat bagi jalur siklooksigenase dan jalur lipooksigenase (Andriyono, 2019). Minyak cengkeh merupakan minyak atsiri yang berasal dari tanaman cengkeh (*Syzygium aromaticum*) yang mempunyai aktivitas mekanisme menghambat sistesis prostaglandin (Iriani *et al*., 2017). Minyak atsiri yang terkadung pada kencur juga dapat menghambat agregasi platelet dengan menghambat pembentukkan tromboksan sehingga berperan dalam efek antiinflamasi (Andriyono, 2019). Dengan demikian, *Stick* Herbal Usada Bali mempunyai potensi sebagai alternatif terapi dalam pengobatan antiinflamasi.

4. KESIMPULAN

Stick Herbal Usada Bali memiliki efek antioksidan dengan kekuatan sedang-kuat dengan metode DPPH dan efek antiinflamasi pada mencit jantan yang diinduksi karagenan. Sehingga, fungsi tanaman tradisional sebagai terapi alternatif antiinflamasi maupun antioksidan perlu ditindaklanjuti dan diperkenalkan kepada masyarakat untuk mengurangi efek samping penggunaan obat kimia.

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Sintesa Nanopartikel Senyawa Bioaktif Daun Pegagan (*Centella asiatica*) dan Uji Pengaruh Pemanasan dan Tekanan Terhadap Diameter dan Indeks Polidispersitasnya

Nanoparticle Synthesis of Bioactive Compounds of Centella Asiatica Leaves and Effect of Heating and Pressure on the Its Diameter and Polydispersity Index

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Abstrak

Senyawa bioaktif daun pegagan memiliki banyak manfaat. Senyawa ini memiliki sifat antioksidan, antimikroba dan antiinflamasi. Senyawa ini telah banyak digunakan untuk menyembuhkan beragam jenis penyakit. Oleh karena itu sangat berpotensi untuk dikembangkan sebagai bahan obat-obatan. Namun sifatnya sangat hidrofobik dan mudah terdegradasi. Untuk mengatasi masalah ini, pendekatan baru yaitu memperkecil ukuran partikelnya berorde nanometer, dapat dilakukan. Tujuan penelitian ini adalah mensintesa partikel senyawa bioaktif daun pegagan berukuran nanometer dan menguji pengaruh pemanasan dan tekanan terhadap diameter dan indeks polidispersitasnya. Senyawa bioaktif daun pegagan diekstrak menggunakan pelarut etanol. Nanopartikelnya disintesa menggunakan surfaktan tween 80. Pengaruh pemberian pemanasan dan tekanan terhadap diameter rata-rata dan indeks polidispersitasnya, diuji. Dari hasil yang diperoleh dengan menggunakan 6 ragam massa konsentrat hasil ekstrak: 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, dan 9 mg yang masing-masing dilarutkan ke dalam 100 mL etanol, diameter rata-rata nanopartikelnya (dan indeks polidispersitas) masing-masing adalah 10,7 nm (0,266); 11,1 nm (0,240); 11,8 nm (0,395); 12,7 nm (0,086); 12,8 nm (0,299); dan 13,2 nm (0,464). Ketika nanopartikel ini dipanaskan pada temperatur 121^{o}C dan tekanan 2 bar selama 15 menit, diameter rata-ratanya menjadi lebih besar: 11,0 nm; 11,3 nm; 12,4 nm; 12,9 nm; 13,5 nm; 14,1 nm; dan indeks polidispersitasnya menjadi lebih kecil: 0,196; 0,202; 0,242; 0,058; 0,274; dan 0,303. Hasil ini menunjukkan bahwa semakin besar massa konsentrat yang digunakan, semakin besar diameter rata-rata nanopartikel yang dihasilkan. Pemberian pemanasan dan tekanan, memperbesar diameter rata-rata nanopartikel dan memperkecil indeks polidispersitasnya.

Kata kunci: *Centella asiatica*; Diameter rata-rata; Indeks polidispersitas; Nanopartikel; Senyawa bioaktif daun pegagan

Abstract

Pegagan leaf compounds have several benefits, such as anti-inflammatory, antibacterial, and antioxidant properties. It has vast potential as a medicinal ingredient, as it has been used to treat many ailments. However, it is pretty hydrophobic and degrades quickly, which can be resolved with a novel strategy: making the particles as small as nanometers. The study aimed

to synthesize nanoscale-sized bioactive compound particles from pegagan leaves and investigate how pressure and heat affect the particles' diameter and polydispersity index. The bioactive compounds in pegagan leaves were extracted using ethanol solvent. The nanoparticles were synthesized using the surfactant Tween 80. The polydispersity index and average diameter were tested for pressure and heat. The average diameter of the nanoparticles (and index polydispersity) derived from the results obtained using six different masses of extract concentrate (4 mg, 5 mg, 6 mg, 7 mg, 8 mg, and 9 mg) dissolved in 100 mL of ethanol are 10.7 nm (0.266); 11.1 nm (0.240); 11.8 nm (0.395); 12.7 nm (0.086); 12.8 nm (0.299); and 13.2 nm (0.464). The average diameter of these nanoparticles increased after 15 minutes of heating at 121oC and 2 bar of pressure: 11.0, 11.3, 12.4, 12.9, 13.5, and 14.1 nm; moreover, the polydispersity index decreased to 0.196, 0.202, 0.242, 0.058, 0.274, and 0.303. These studies demonstrate that the amount of concentrate utilized increases the average diameter of the nanoparticles formed. The average diameter of the nanoparticles increases, and the polydispersity index decreases with pressure and heat.

Keywords: *Average diameter; Bioactive substances of pegagan leaves; Centella asiatica; Nanoparticles; Polydispersity index*

1. PENDAHULUAN

Daun pegagan mengandung material senyawa bioaktif yang memiliki banyak manfaat. Beberapa hasil penelitian menunjukkan senyawa bioaktif daun pegagan dapat digunakan sebagai obat terapi untuk menyembuhkan beragam jenis penyakit, seperti luka (Lopez *et al*., 2022), gatal pada kulit (Park, 2021), epilepsi, kejang-kejang, sakit pada lambung, dan sakit pada paru-paru (Biswas *et al*., 2021). Selain itu, juga ditemukan bahwa material senyawa bioaktif daun pegagan memiliki sifat seperti antioksidan (Buranasudja *et al*., 2021), antimikroba (Wong *et al*., 2021a), antiinflamasi dan neuroprotektif (Wong *et al*., 2021b). Hal ini menunjukkan bahwa daun pegagan memiliki potensi yang sangat besar untuk dikembangkan dan dijadikan sebagai salah satu sumber bahan obat-obatan ke depan untuk mengatasi masalah-masalah penyakit yang berkaitan dengan hal-hal di atas. Sehingga, usaha untuk mempelajari dan mengembangkannya perlu dilakukan, terutama untuk meningkatkan kemanfaatannya sebagai bahan obat-obatan sehingga dapat memiliki efikasi yang tinggi. Senyawa-senyawa bioaktif yang terkandung dalam daun pegagan mudah diekstrak dengan teknik ekstraksi yang telah dikenal saat ini. Setelah diekstrak, senyawa ini dapat langsung digunakan atau dikembangkan lebih lanjut untuk tujuan yang dikehendaki. Namun, dalam proses penggunaan dan pengembangan senyawa bioaktif daun pegagan terdapat beberapa masalah yang menjadi penghambat, seperti sulitnya senyawa ini larut di dalam air (berisfat hidrofobik) dan mudahnya senyawa-senyawa ini terdegradasi (teroksidasi, yang dimediasi oleh kenaikan temperatur dan atau paparan cahaya) (Bazana *et al*., 2019). Oleh karena itu, bioavailabilitasnya menjadi sangat rendah di dalam tubuh dan penggunaannya menjadi sangat tidak efisien dan tidak efektif.

Pendekatan dengan memperkecil ukuran partikel berorde nanometer (nanopartikel) tepat dilakukan untuk meningkatkan bioavailabilitas senyawa aktif (Sahu *et al*., 2021). Ukuran partikel senyawa bioaktif daun pegagan sangat kecil (berorde nanometer) menyebabkan karakteristik kinetisnya di dalam suatu medium air sepenuhnya hanya dikendalikan oleh gerak *Brown*-nya saja sehingga akan mudah didispersi secara homogen dalam medium air tersebut

(Yetisgin *et al*., 2020). Partikel senyawa bioaktif yang telah terdispersi dapat diangkut dan disebar ke dalam organ-organ tubuh sehingga bioavailabilitasnya dalam tubuh akan meningkat. Hasil studi pustaka yang dilakukan, sintesa senyawa bioaktif daun pegagan berorde nanometer sampai saat ini belum dilaporkan. Surfaktan digunakan untuk membantu sekaligus mempermudah pembentukan nanopartikelnya dan untuk menjaga dispersi nanopartikel senyawa tersebut tetap stabil di dalam medium yang mengandung air (dispersi tetap homogen), serta untuk menghindari terjadinya degradasi (Shaban *et al*., 2020).

Kestabilan fisis dari nanopartikel yang telah dihasilkan memerlukan investigasi terkait kestabilan ukuran (diameter rata-ratanya) dan kestabilan distribusi ukuran (indeks polidispersitasnya) selama mendapatkan perlakukan pemanasan dan tekanan. Sebelum produk nanopartikel tersebut dipakai untuk terapan harus melalui proses sterilisasi yang melibatkan pemanasan dan sekaligus pemberian tekanan. Investigasi terkait kestabilan fisis nanopartikel perlu dilakukan untuk mengetahui pengaruh pemanasan dan pemberian tekanan terhadap perubahan diameter rata-rata dan indeks polidispersitas dari produk nanopartikel.

Sintesa nanopartikel senyawa bioaktif daun pegagan dilakukan menggunakan surfaktan tween 80 di dalam suatu ruang berbentuk tabung berdiameter 500 μm dan panjang 50 cm. Pemanasan dan tekanan diberikan pada nanopartikel yang dihasilkan untuk melihat pengaruhnya terhadap perubahan diameter rata-ratanya dan indeks polidispersitasnya. Oleh karena itu, tujuan penelitian ini adalah mensintesa nanopartikel senyawa bioaktif daun pegagan dan menguji pengaruh pemanasan dan tekanan terhadap diameter dan indeks polidispersitas dari nanopartikel yang dihasilkan.

2. BAHAN DAN METODE

2.1. Bahan

Bahan yang digunakan pada penelitian ini adalah: (1) daun pegagan segar hasil budidaya petani Desa Plumpang, Kecamatan Plumpang, Kabupaten Tuban, Provinsi Jawa Timur; (2) pelarut etanol (C_2H_5OH) (*absolute for analysis*); dan (3) surfaktan tween 80. Pelarut etanol dan surfaktan tween 80 masing-masing dibeli dari Merck KGaA Germany dengan kemurnian 99,9%. Kedua bahan ini langsung digunakan tanpa pemurnian tambahan.

2.2. Metode

2.2.1. Ekstraksi senyawa bioaktif daun pegagan

Ekstraksi senyawa bioaktif daun pegagan dilakukan dengan teknik maserasi mengikuti langkah-langkah Rumanti dan Saragih (2023) dengan sedikit modifikasi. Daun pegagan segar dibersihkan menggunakan air mengalir dan digiling sampai membentuk serbuk halus. Sebanyak 300 g serbuk halus kering daun pegagan dilarutkan ke dalam 500 mL etanol, kemudian diaduk menggunakan *magnetic stirrer* (laju 150 rpm pada temperatur ruang) selama 1 jam. Setelah itu didiamkan selama 4 jam. Kemudian kedua proses ini kembali dilakukan sebanyak empat kali dengan kondisi lingkungan yang sama, sehingga total waktu maserasi adalah 20 jam.

Maserat yang dihasilkan dipisahkan dengan cara disaring menggunakan kertas saring (filter paper Whatman No.1). Pelarut etanol yang terdapat di dalam maserat selanjutnya diuapkan menggunakan alat *rotary evaporator* yang dioperasikan pada 80 rpm selama 3 jam.

Proses penguapan (evaporasi) dilakukan pada tekanan 0,5 atm dan temperatur 40ºC. Hasil konsentrat dimasukkan ke dalam suatu wadah botol gelas tertutup dan dibalut dengan *aluminium foil* untuk menghindari paparan langsung cahaya dan disimpan di dalam ruang bertemperatur -10ºC.

2.2.2. Identifikasi senyawa bioaktif daun pegagan

Konsentrat yang diperoleh merupakan kumpulan senyawa-senyawa bioaktif yang berhasil diekstrak dari daun pegagan. Selanjutnya senyawa-senyawa tersebut diidentifikasi menggunakan teknik *gas chromatography - mass spectroscopy* (GC-MS), yaitu teknik identifikasi yang menggabungkan sistem *gas chromatography* dan sistem *mass spectroscopy* secara integratif. Alat yang digunakan adalah Shimadzu GC-MS QP2010 Ultra (Japan). Identifikasi senyawa menggunakan dua besaran yang diperoleh dari hasil pengukuran oleh sistem *gas chromatography* yaitu waktu retensi (menit), dan besaran yang diperoleh dari pengukuran oleh sistem *mass spectroscopy* yaitu spektrum rasio massa terhadap muatan (m/z) dari setiap jenis senyawa yang terukur.

Konsentrat ekstrak daun pegagan sebanyak 1 g dilarutkan ke dalam 50 mL etanol dan diinjeksikan sebanyak 1 mL ke dalam bagian tabung masukan pipa kapiler dari peralatan Shimadzu GC-MS QP2010 Ultra. Temperatur pipa kapiler dinaikkan secara bertahap, yaitu: (1) dari temperatur ruang dinaikkan ke temperatur 80ºC dan ditahan selama 1 menit; (2) dari 80ºC dinaikkan ke 250ºC dengan laju 280ºC/menit; (3) dari 250ºC dinaikkan ke 300ºC dengan laju 270ºC/menit; dan (4) dari 300ºC dinaikkan ke 320ºC dengan laju 260ºC/menit dan ditahan selama 24 menit.

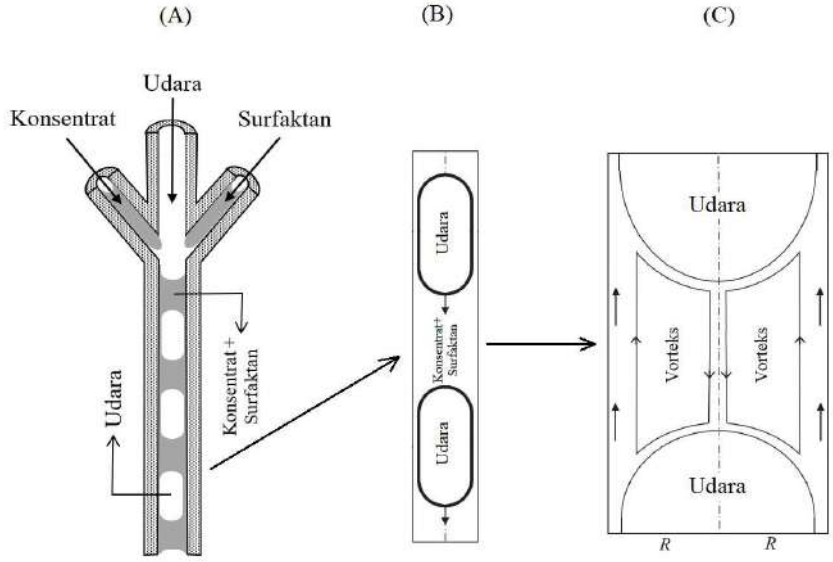
Ketika larutan konsentrat di atas diinjeksi ke dalam bagian masukan tabung pipa kapiler, pada saat yang sama gas pembawa helium dialirkan ke dalam pipa kapiler. Tekanan gas helium pada bagian masukan pipa kapiler dijaga pada 42,3 kP untuk menghasilkan aliran cairan larutan konsentrat ke dalam pipa kapiler dengan laju 0,74 mL/menit. Akibat pemanasan pipa kapiler, molekul-molekul senyawa-senyawa penyusun konsentrat akan menguap. Uap molekul-molekul ini akan mengalir di dalam tabung dibawa oleh gas pembawa. Waktu retensi (menit) dan spektrum rasio massa terhadap muatan (m/z) dari setiap molekul yang mengalir ini akan diukur.

Waktu retensi dan spektrum rasio massa terhadap muatan dari setiap jenis senyawa penyusun konsentrat digunakan untuk mengidentifikasi jenis senyawa tersebut dengan cara mencocokkan kedua nilai besaran tersebut dengan nilai besaran yang sama pada basis data *National Institut of Standards and Technology Mass Spectral Database* (NIST-MS) yang telah terintegrasi pada perangkat lunak sistem peralatan Shimadzu GC-MS QP2010 Ultra.

2.2.3. Sintesa nanopartikel senyawa bioaktif daun pegagan

Senyawa bioaktif daun pegagan yang telah diidentifikasi menggunakan GC-MS selanjutnya disintesa membentuk partikel-partikel berukuran nanometer (nanopartikel). Tween 80 digunakan sebagai surfaktan. Proses sintesa dilakukan di dalam ruang berbentuk tabung yang jari-jarinya sangat kecil (R = 250 μm). Modus alir cair-gas yang terjadi di dalam tabung dimanfaatkan untuk mencampur bahan yang digunakan (Abiev, 2021). Sistem peralatan sintesa

(Gambar 1) dikembangkan sendiri oleh tim penulis. Tabung yang digunakan terbuat dari bahan gelas dengan panjang 50 cm.



Gambar 1. Tabung gelas berjari-jari 250 μm dan panjang 50 cm yang digunakan untuk mensintesa nanopartikel senyawa bioaktif daun pegagan. Keterangan: (A) Sistem masukan prekursor (konsentrat dan surfaktan) dan udara ke dalam tabung. (B) Modus alirnya yang disusun secara berurut dan teratur oleh bagian cair (konsentrat+surfaktan) dan bagian gas (udara). (C) Pola alir molekul-molekul prekursor di bagian cair ketika mengalir di dalam tabung (sirkulatif membentuk vorteks). Pada sistem ini gaya tarik gravitasi terhadap bahan cair yang terdapat di dalam tabung dimanfaatkan sebagai pembangkit aliran.

Injeksi udara ke dalam tabung bertujuan untuk memisahkan campuran konsentrat dan surfaktan menjadi segmen-segmen kecil sehingga membentuk aliran Taylor (*Taylor flow*) (Abiev, 2020). Ketika cairan campuran tersebut berukuran sangat kecil dan membentuk modus alir aliran Taylor, maka jumlah molekul-molekul senyawa bioaktif daun pegagan yang terdapat di dalamnya menjadi sangat terbatas sehingga nanopartikel senyawa bioaktif yang akan terbentuk menjadi sangat kecil. Molekul surfaktan tween 80 akan mengenkapsulasi partikel senyawa bioaktif tersebut dalam ukuran yang sangat kecil (berorde nanometer) ketika proses pencampuran terjadi di dalam tabung.

Enam ragam massa konsentrat digunakan 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, dan 9 mg, masing-masing diencerkan ke dalam 100 mL etanol. Surfaktan tween 80 diencerkan dengan menggunakan perbandingan tween 80: etanol = 50 mL: 150 mL. Kedua jenis prekursor yang telah diencerkan ini diinjeksi ke dalam tabung menggunakan *syringe* dengan debit masing-masing 0,85 mL/menit. Posisi tabung dibuat miring dengan sudut 60^o terhadap bidang datar agar bahan precursor yang terdapat di dalam tabung tertarik oleh gravitasi dan membangkitkan aliran cairan di dalam tabung. Udara dibiarkan masuk secara alami ke dalam tabung untuk membentuk pola alir cair-gas.

Kondisi alir cairan-gas yang terbentuk di dalam tabung ketika menggunakan parameter eksperimen seperti diterangkan di atas adalah: (1) panjang segmen gas di dalam tabung sebesar 500 μm, (2) panjang segmen cairnya sebesar 600 μm, dan (3) laju alir kedua segmen tersebut

sebesar 5 cm/detik. Segmen-segmen ini mengalir sepanjang 50 cm di dalam tabung. Proses pencampuran prekursor dan proses sintesa nanopartikel senyawa bioaktif daun pegagan terjadi di dalam aliran cair-gas tersebut. Hasil sintesa nanopartikel selanjutnya ditampung ke dalam suatu wadah gelas berisi air yang telah didestilasi (*distilled water*) sebanyak 100 mL. Penampungan dilakukan untuk tiap-tiap besar massa konsentrat yang digunakan. Penampungan dihentikan ketika volume total di dalam wadah penampungan telah mencapai 150 mL. Nanopartikel senyawa bioaktif daun pegagan hasil sintesa akan terdispersi di dalam medium air yang terdapat di dalam wadah penampungan.

2.2.4. Pengukuran diameter nanopartikel dan indeks polidispersitasnya

Nanopartikel senyawa bioaktif daun pegagan hasil sintesa selanjutnya dikarakterisasi untuk mengetahui besar diameter rata-rata dan indeks polidispersitas masing-masing. Pengukuran dilakukan dengan teknik hamburan cahaya (*light scattering*) menggunakan sistem peralatan *Particle Size Analyzer* (PSA) merek HORIBA SZ-100 (Horiba Instrument, Inc. USA). Hasil pengukuran yang diperoleh adalah besaran diameter rata-rata nanopartikel, spektrum distribusi diameter-diameter tersebut, dan besar indeks polidispersitas distribusi diameter untuk masing-masing sampel di atas.

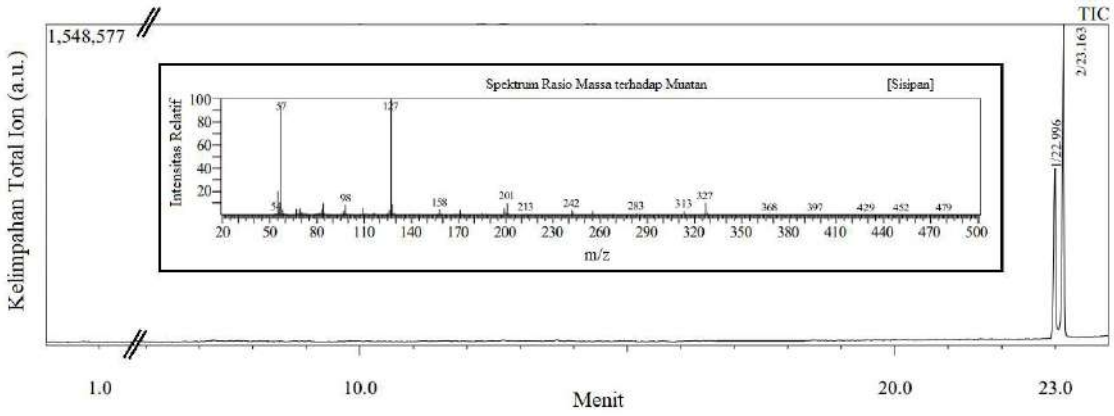
2.2.5. Pemanasan dan pemberian tekanan

Enam ragam sampel hasil sintesa nanopartikel senyawa bioaktif daun pegagan yang telah diukur diameter rata-rata dan indeks polidispersitasnya selanjutnya dipanaskan dan sekaligus secara bersamaan diberi tekanan untuk melihat pengaruhnya terhadap perubahan diameter dan indeks polidispersitasnya. Sebanyak masing-masing 10 mL cairan yang berasal dari masing-masing keempat ragam sampel nanopartikel dipisahkan dan dimasukkan ke dalam masing-masing wadah kaca yang terbuka dan steril. Kemudian keempat wadah kaca yang telah berisi nanopartikel tersebut dimasukkan ke dalam *autoclave* untuk diberi perlakuan panas dan tekanan. Sistem peralatan *autoclave* selanjutnya dipanaskan sehingga mencapai temperatur 121^{o}C dan tekanan 2 bar. Setelah kedua besaran parameter ini dicapai, kemudian dipertahankan selama 15 menit. Penetapan sistem tersebut mengikuti parameter standar yang direkomendasikan oleh *European Pharmacopoeia*. Keempat wadah kaca berisi nanopartikel yang telah diberi perlakuan pemanasan dan pemberian tekanan, diukur kembali diameter rata-rata dan indeks polidispersitasnya menggunakan peralatan *Particle Size Analyzer* (PSA).

3. HASIL DAN PEMBAHASAN

Hasil pengukuran GC-MS, diperoleh suatu kromatogram total ion (*total ion chromatogram*, TIC) dan spektrum rasio massa terhadap muatan (m/z) dari tiap-tiap senyawa bioaktif yang terukur (Gambar 2). Kromatogram total ion (*total ion chromatogram*, TIC) menunjukkan puncak-puncak kelimpahan total ion dan waktu retensi dari masing-masing puncak kelimpahan total ion tersebut. Spektrum rasio massa terhadap muatan dari senyawa bioaktif yang terukur menghasilkan puncak-puncak kelimpahan total ion. Rasio massa terhadap muatan sebesar 127 terukur secara dominan dan diikuti berikutnya oleh rasio massa terhadap muatan sebesar 57. Luas masing-masing puncak yang dihasilkan dan persentase puncak

kelimpahan total ion yang dihasilkan oleh senyawa bioaktif ekstrak etanol daun pegagan (Tabel 1).



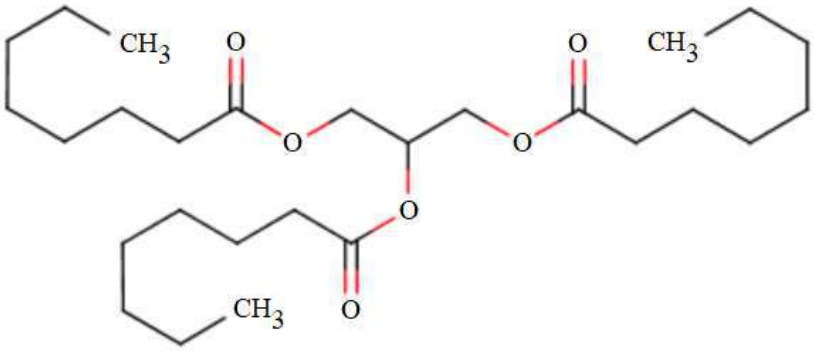
Gambar 2. Kromatogram total ion (*total ion chromatogram*, TIC) senyawa bioaktif ekstrak etanol daun pegagan yang diukur menggunakan sistem peralatan GC-MS QP2010 Ultra. Sisipan adalah spektrum rasio massa terhadap muatannya (m/z).

Hasil sintesa nanopartikel senyawa bioaktif daun pegagan didispersikan ke dalam air. Hasil sintesa terdispersi secara homogen di dalam air, hasil dispersi tersebut selanjutnya diukur menggunakan peralatan *Particle Size Analyzer* (PSA) untuk memperoleh data distribusi diameter, diameter rata-rata (*Z-Average*) dan indeks polidispersitas (PI) nanopartikel (Gambar 3 kolom A). Semua nanopartikel yang telah diukur kemudian disimpan selama seminggu di dalam ruang bertemperatur -5^{o}C. Setelah itu, nanopartikel-nanopartikel tersebut diberi tekanan dan pemanasan dengan cara *autoclaving* menggunakan parameter seperti yang telah diterangkan pada bagian eksperimen. Sebelum dimasukkan ke dalam *autoclave*, temperatur nanopartikel terlebih dahulu dinaikkan ke temperatur ruang dengan cara memaparkannya di ruang terbuka tanpa cahaya. Nanopartikel yang telah mengalami proses *autoclaving* selanjutnya diukur kembali distribusi diameter, diameter rata-rata (*Z-Average*) dan indeks polidispersitas (PI) nanopartikel menggunakan peralatan *Particle Size Analyzer* (PSA) yang sama (Gambar 3 kolom B).

Tabel 1. Hasil pengukuran GC-MS masing-masing puncak kelimpahan total ion yang dihasilkan oleh senyawa bioaktif ekstrak etanol daun pegagan.

Puncak #	Luas Puncak	% Luas Puncak	Waktu Retensi (menit)	Nama Senyawa
1	2005218	33.87	22.996	*octanoic acid,1,2,3-propanetriyl ester*
2	3914729	66.13	23.163	*octanoic acid,1,2,3-propanetriyl ester*

Hasil kromatogram (Gambar 2) terdapat dua puncak kelimpahan total ion yang terjadi, yaitu: puncak pertama terjadi pada waktu retensi 22.996 menit dan puncak kedua terjadi pada waktu retensi 23.163 menit. Dua puncak ini sangat berdekatan. Rasio massa terhadap muatan (m/z) dari senyawa bioaktif dan fragmen-fragmennya yang menghasilkan puncak-puncak kelimpahan tersebut membentuk spektrum massa (Gambar 2). Karakteristiknya menghasilkan dua rasio massa terhadap muatan yang paling dominan, yaitu: sebesar 127 dan sebesar 57.



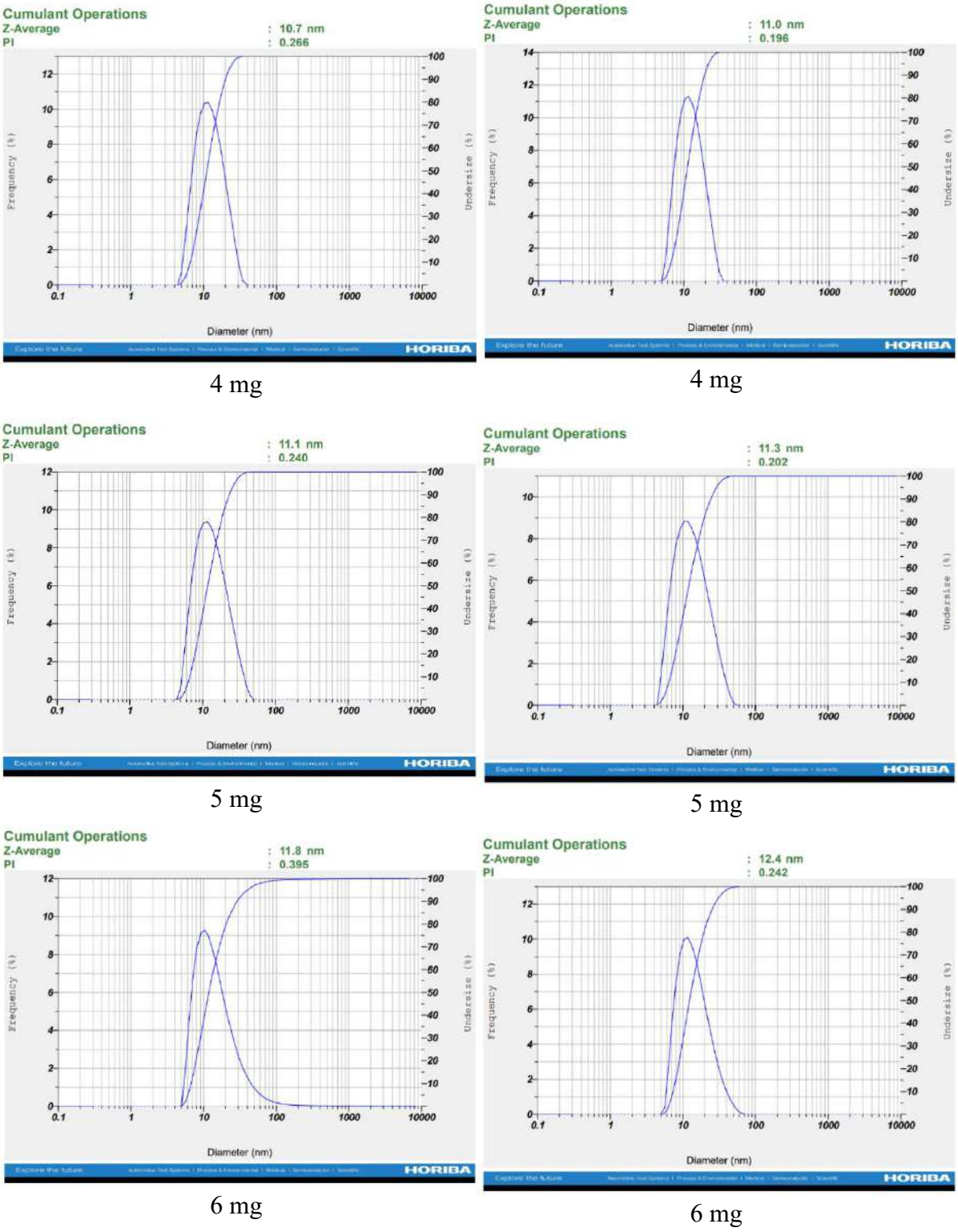
Gambar 3. Struktur molekul *octanoic acid,1,2,3-propanetriyl ester*.

Waktu retensi dan spektrum rasio massa terhadap muatan dari setiap jenis senyawa tersebut diidentifikasi dengan cara mencocokkan kedua besaran ini dengan besaran yang sama pada basis data *National Institut of Standards and Technology Mass Spectral Database* (NIST-MS). Hasil identifikasi kedua puncak, puncak pertama yang terjadi pada waktu retensi 22.996 menit (dengan persen luas puncak = 33.87%) dan puncak kedua yang terjadi pada waktu retensi 23.163 menit (dengan persen luas puncak = 66.13%) adalah sama-sama dihasilkan oleh senyawa bioaktif *octanoic acid,1,2,3-propanetriyl ester* atau dengan nama lain *trioctanoin* dengan rumus molekul $C_{27}H_{50}O_6$ dan berat molekul 470 g/mol (Gambar 3). Hasil identifikasi menunjukkan bahwa konsentrat ekstrak etanol daun pegagan memiliki kandungan senyawa bioaktif: *octanoic acid,1,2,3-propanetriyl ester*.

Senyawa ini kemudian disintesa untuk menghasilkan partikel berukuran nanometer. Diameter rata-rata nanopartikel yang diperoleh setelah disintesa dengan menggunakan 6 ragam massa konsentrat yang berbeda, yaitu: 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, dan 9 mg, tersebar dari 10,7 nm sampai 13,2 nm (Gambar 4A). Ukuran diameter rata-rata nanopartikel yang dihasilkan relatif sangat kecil, di bawah 100 nm seperti yang diperlukan untuk berbagai ragam penggunaan (Gaumet *et al*., 2008). Nanopartikel tersebut terdispersi secara homogen dalam medium air.

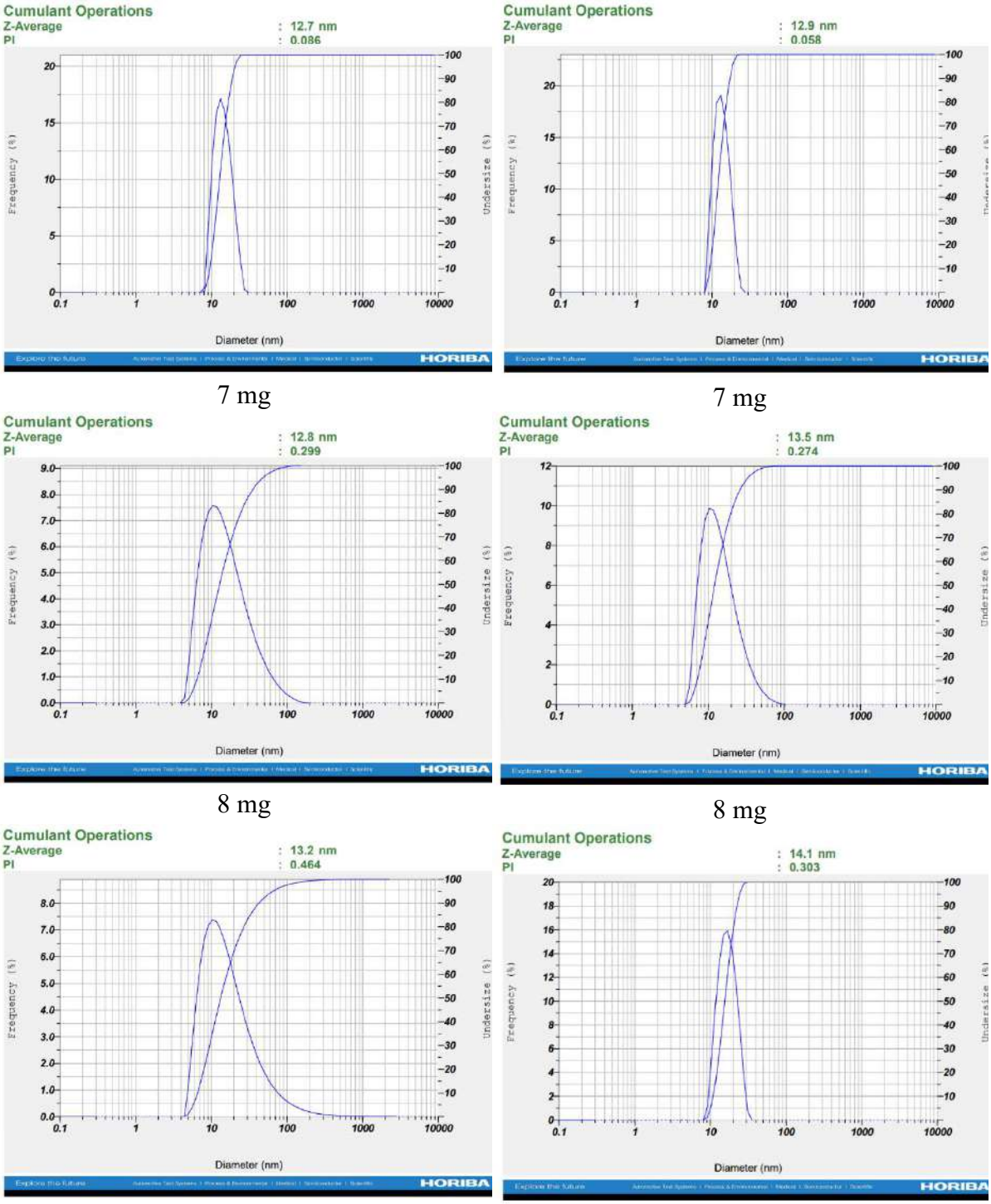
Ukuran nanopartikel yang diperoleh (Gambar 4A), dipengaruhi oleh besarnya massa konsentrat yang digunakan. Hubungan massa konsentrat senyawa bioaktif ekstrak etanol daun pegagan yang digunakan pada proses sintesa nanopartikel dengan diameter rata-rata nanopartikel yang dihasilkan (Gambar 5). Terlihat bahwa semakin besar massa konsentrat senyawa bioaktif yang digunakan, semakin besar pula diameter rata-rata nanopartikel yang dihasilkan. Hubungannya linier. Linieritasnya dapat didekati dengan persamaan $y_1 = 0{,}5286x + 8{,}6143$. Determinasi perubahan massa konsentrat yang digunakan (x) terhadap perubahan besar diameter rata-rata nanopartikel yang dihasilkan (y_1), sangat tinggi yaitu 95,96% ($R_1^2 = 0{,}9596$). Faktor pengali (gradien) pertambahan besar diameter rata-rata nanopartikel (y_1) ketika massa konsentrat senyawa tersebut ditambah (x), juga cukup besar yaitu: 0,5286. Ini menunjukkan bahwa penambahan massa konsentrat yang digunakan sangat mempengaruhi (menambah ukuran nanopartikel) diameter rata-rata nanopartikel yang dihasilkan.

(A) (B)

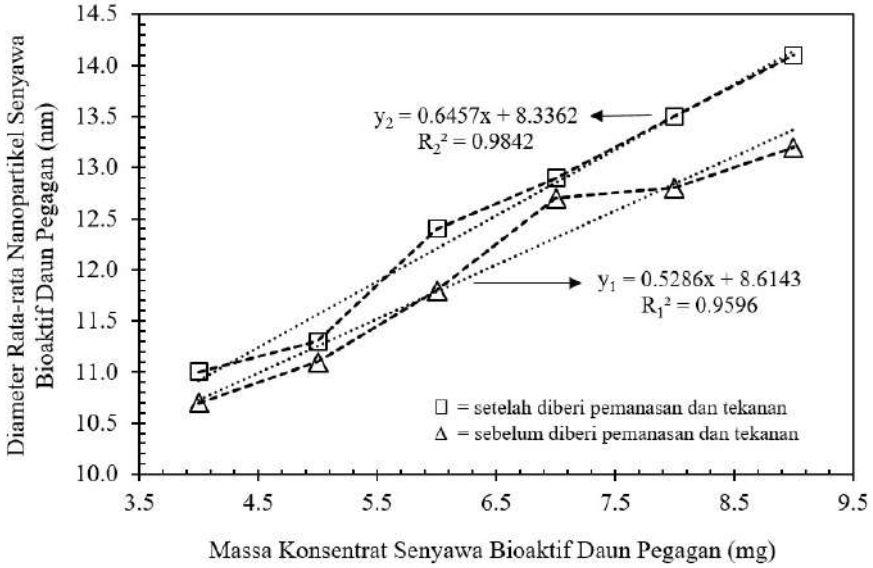


Gambar 4. Distribusi diameter, diameter rata-rata (*Z-Average*) dan indeks polidispersitas (PI) nanopartikel senyawa bioaktif daun pegagan yang disintesa dengan menggunakan 6 ragam massa konsentrat hasil ekstraksi, yaitu: 4 mg, 5 mg, 6 mg, 7 mg, 8 mg dan 9 mg. Keterangan (A) sebelum dan (B) sesudah diberi pemanasan 121^{o}C dan tekanan 2 bar selama 15 menit.

(A) (B)



Gambar 4. Distribusi diameter, diameter rata-rata (*Z-Average*) dan indeks polidispersitas (PI) nanopartikel senyawa bioaktif daun pegagan yang disintesa dengan menggunakan 6 ragam massa konsentrat hasil ekstraksi, yaitu: 4 mg, 5 mg, 6 mg, 7 mg, 8 mg dan 9 mg. Keterangan (A) sebelum dan (B) sesudah diberi pemanasan 121ºC dan tekanan 2 bar selama 15 menit (*Lanjutan*).



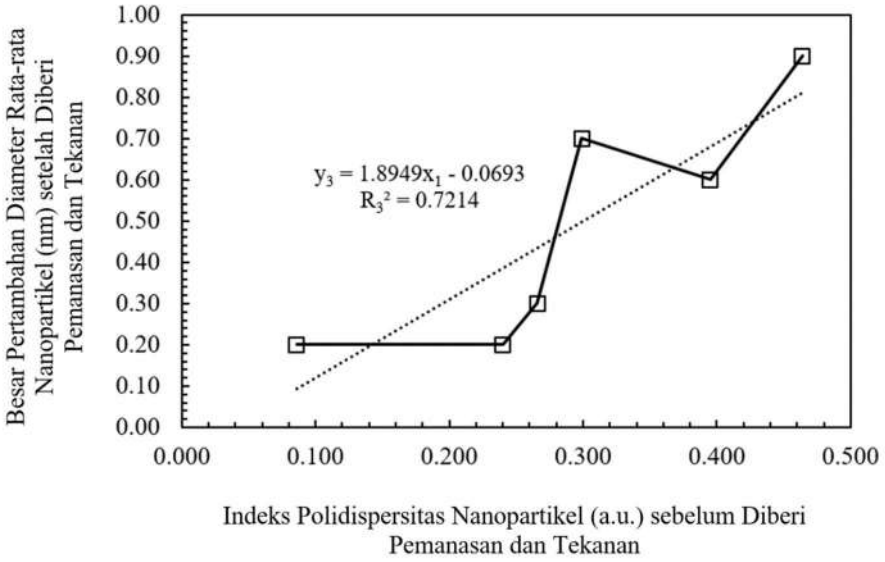
Gambar 5. Hubungan massa konsentrat senyawa bioaktif ekstrak etanol daun pegagan yang digunakan pada proses sintesa nanopartikel dengan diameter rata-rata nanopartikel yang dihasilkan. Keterangan □ = sebelum diberi pemanasan dan tekanan, Δ = setelah diberi pemanasan dan tekanan.

Pada saat proses sintesa nanopartikel dilakukan di dalam ruang berbentuk tabung berjari-jari sangat kecil, yaitu: 250 μm sebagaimana digunakan pada penelitian ini, molekul-molekul senyawa bioaktif daun pegagan akan bercampur dengan molekul-molekul surfaktan dan membentuk nanopartikel di ruang yang volumenya sangat kecil dengan modus alir yang sirkulatif tersebut (Gambar 1C). Volume yang sangat kecil dan modus alir yang sirkulatif, kuantitas senyawa bioaktif yang disintesa sangat terbatas dan transport massanya sangat tinggi. Transport massa yang sangat tinggi akan menghasilkan waktu pencampuran dan pembentukan nanopartikel yang sangat cepat. Kuantitas senyawa bioaktif yang terbatas dan volumenya sangat kecil akan menghasilkan ukuran nanopartikel yang sangat kecil pula. Ukuran panjang segmen cair di dalam tabung dijaga tetap sama yaitu: 600 μm dan kecepatan alirnya juga dijaga tetap sama yaitu: 5 cm/det, serta konsentrasi surfaktan yang digunakan pun juga dijaga tetap sama untuk setiap penggunaan massa konsentrat yang berbeda. Oleh karena itu, penambahan massa konsentrat yang digunakan akan meningkatkan ukuran nanopartikel yang dihasilkan karena kuantitas senyawa bioaktif yang disintesa meningkat.

Proses *autoclaving* dengan suhu 121ºC dan tekanan 2 bar selama 15 menit menyebabkan diameter rata-rata nanopartikelnya berubah menjadi lebih besar. Perubahan ini terjadi untuk semua nanopartikel (Gambar 5). Ini menunjukkan bahwa pemberian pemanasan dan sekaligus tekanan kepada sekumpulan nanopartikel tersebut menyebabkan diameter rata-ratanya bertambah besar. Perubahan ini juga bersifat linier terhadap besarnya massa konsentrat yang digunakan pada saat nanopartikel disintesa. Kelinierannya dinyatakan oleh persamaan y_2 = 0,6457x + 8,3362, dimana determinasi perubahan massa konsentrat yang digunakan (x) terhadap perubahan besar diameter rata-rata nanopartikelnya (y_2), juga sangat tinggi yaitu 98,42% (R_2^2 = 0,9842).

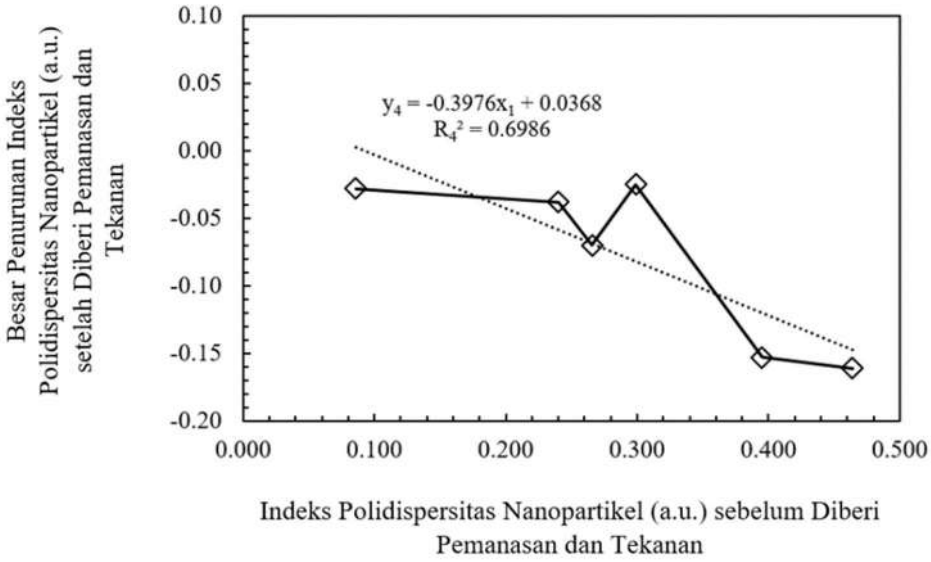
Jika diperhatikan, gradien pada persamaan y_1 dan gradien pada persamaan y_2 juga berbeda, masing-masing adalah 0,5286 dan 0,6457. Gradien pada persamaan y_2 lebih besar dibandingkan dengan gradien pada persamaan y_1. Artinya, diameter rata-rata nanopartikel akan bertambah lebih signifikan bila nanopartikel tersebut dipanaskan dan diberi tekanan. Untuk massa konsentrat yang digunakan sebesar 9 mg saat sintesa dilakukan, perubahan diameter rata-rata nanopartikelnya sesudah dan sebelum diberi pemasanan dan tekanan, sebesar: 14,1 nm – 13,2 nm = 0,9 nm. Nilai ini lebih besar dibandingkan dengan nilai parameter yang sama pada saat menggunakan massa konsentrat yang lebih kecil.

Hubungan indeks polidispersitas nanopartikel sebelum diberi pemanasan dan tekanan dengan besarnya pertambahan diameter rata-rata nanopartikel setelah diberi pemanasan dan tekanan (Gambar 6). Dalam hal ini nilai indeks polidispersitas (pada persamaan ditunjukkan sebagai variable x_1) yang diperoleh di atas menunjukkan lebar-sempitnya distribusi ukuran diameter dari nanopartikel-nanopartikel tersebut, dimana bila nilai indeks polidispersitasnya besar maka distribusi ukuran diameter nanopartikel-nanopartikelnya lebar, dan sebaliknya.



Gambar 6. Hubungan indeks polidispersitas nanopartikel (ditunjukkan oleh variable x_1) sebelum diberi pemanasan dan tekanan dengan besar pertambahan diameter rata-rata nanopartikel setelah diberi pemanasan dan tekanan.

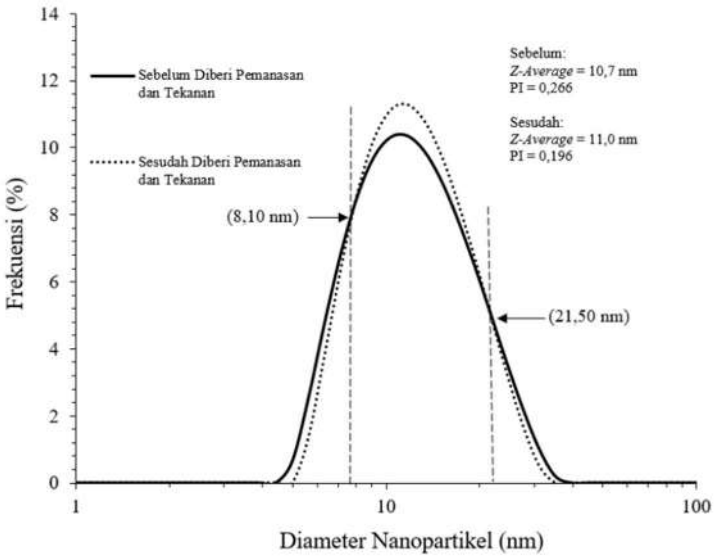
Hubungan indeks polidispersitas nanopartikel sebelum diberi pemanasan dan tekanan dengan besar pertambahan diameter rata-rata nanopartikel setelah diberi pemanasan dan tekanan didekati dengan persamaan linier: $y_3 = 1{,}8949x_1 + 0{,}0693$, dengan $R_3^2 = 0{,}7214$. Hasil ini menunjukkan bahwa semakin besar nilai indeks polidispersitas nanopartikel (x_1), semakin besar pula pertambahan diameter rata-rata nanopartikel setelah diberi pemanasan dan tekanan (y_3). Atau dengan kata lain, semakin lebar distribusi ukuran diameter nanopartikel sebelum diberi pemanasan dan tekanan, semakin besar pertambahan diameter rata-ratanya setelah diberi pemanasan dan tekanan. Ini menunjukkan bahwa distribusi ukuran diameter nanopartikel-nanopartikel tersebut (keragaman) berkontribusi membangkitkan penambahan diameter rata-ratanya ketika diberi pemanasan dan tekanan. Hal ini dipertegas dengan hasil indeks indeks polidispersitasnya setelah diberi pemanasan dan tekanan (Gambar 7).



Gambar 7. Hubungan indeks polidispersitas nanopartikel (ditunjukkan oleh variable x_1) sebelum diberi pemanasan dan tekanan dengan besar penurunan indeks polidispersitas nanopartikel setelah diberi pemanasan dan tekanan.

Hubungan indeks polidispersitas nanopartikel sebelum diberi pemanasan dan tekanan dengan besar penurunan indeks polidispersitasnya setelah diberi pemanasan dan tekanan (Gambar 7). Besar penurunan indeks polidispersitas nanopartikel setelah diberi pemanasan dan tekanan semakin besar ketika indeks polidispersitas awalnya besar (indeks polidispersitas sebelum diberi pemanasan dan tekanan). Besar penurunan ini terlihat bersifat linier terhadap besarnya indeks polidispersitasnya sebelum diberi pemanasan dan tekanan dan didekati dengan persamaan $y_4 = -0{,}3976x_1 + 0{,}0368$ (R_4^2 =0,6986). Ini mempertegas bahwa keragaman ukuran nanopartikel-nanopartikel tersebut sebelum diberi pemanasan dan tekanan berkontribusi pada penambahan diameter rata-ratanya ketika diberi pemanasan dan tekanan. Bila indeks polidispersitas nanopartikel semakin besar sebelum diberi pemanasan dan tekanan maka penurunan indeks polidispersitas tersebut semakin besar pula setelah diberi pemanasan dan tekanan. Koefisien pengalinya sebesar -0,3976.

Khedr dan Striolo (2019) melaporkan suatu populasi nanopartikel (nanoemulsi) yang memiliki ukuran beragam dan terdispersi di dalam suatu medium, dapat mengalami pertambahan ukuran diameter rata-ratanya yang disebabkan koalisi antar nanopartikel. Nanopartikel berukuran lebih kecil memiliki solubilitas yang lebih tinggi di dalam medium dibandingkan nanopartikel berukuran lebih besar sehingga nanopartikel dengan ukuran lebih kecil akan memiliki mobilitas lebih tinggi dalam medium dan memiliki kecenderungan berdifusi menuju nanopartikel yang lebih besar dan berkoalisi sehingga memperbesar diameter nanopartikel yang lebih besar tersebut. Peristiwa ini dipengaruhi oleh temperatur, ketika temperatur sistem (medium dan nanopartikel) naik, laju peristiwa ini semakin tinggi. Proses koalisi yang disebabkan oleh peristiwa seperti ini akan mengurangi jumlah nanopartikel yang memiliki ukuran yang lebih kecil di dalam populasi sehingga lebar distribusinya akan berkurang. Dengan kata lain, besar indeks polidispersitas nanopartikel tersebut akan berkurang.



Gambar 8. Distribusi ukuran nanopartikel senyawa bioaktif daun pegagan sebelum dan sesudah proses *autoclaving* pada temperatur 121^{o}C dan tekanan 2 bar selama 15 menit. Nanopartikel disintesa dengan menggunakan massa konsentrat 4 mg.

Diameter nanopartikel akan cenderung mengecil karena molekul- molekul penyusunnya mengalami pemadatan (terkompresi) akibat tekanan hidrostatis di dalam *autoclave*. Nanopartikel yang memiliki jumlah molekul yang paling besar (ukuran yang paling besar) akan mengalami pengecilan yang paling besar. Sehingga, ketika tekanan telah diberikan, jumlah nanopartikel yang memiliki ukuran yang besar akan berkurang. Pada proses *autoclaving* (pemberian pemanasan dan tekanan), kenaikan temperatur lebih dahulu terjadi, lalu kenaikan temperatur ini membangkitkan kenaikan tekanan di dalam *autoclave*. Kenaikan temperatur menyebabkan peristiwa koalisi antar nanopartikel lebih dahulu terjadi, lalu diikuti oleh proses kompresi. Kedua peristiwa tersebut menyebabkan jumlah nanopartikel yang berukuran sangat kecil dan jumlah nanopartikel yang berukuran sangat besar akan berkurang setelah proses *autoclaving.* Hal ini membuat diameter rata-rata nanopartikel menjadi bertambah dan indeks polidispersitasnya berkurang.

Distribusi ukuran hasil sintesa nanopartikel menggunakan konsentrat sebesar 4 mg sebelum dan sesudah diberi pemanasan dan tekanan (Gambar 8). Setelah mengalami pemansan dan pemberian tekanan, diameter rata-rata (*Z-average*) nanopartikel bertambah menjadi 11,0 nm dari sebelumnya 10,7 nm dan indeks polidispersitas nanopartikel berkurang menjadi 0,196 dari sebelumnya 0,266. Jumlah (frekuensi) nanopartikel yang berukuran lebih kecil (kurang dari 8,10 nm) dan jumlah nanopartikel yang berukuran lebih besar (lebih dari 21,50 nm) terlihat berkurang setelah diberi pemanasan dan tekanan. Pengurangan jumlah nanopartikel yang berukuran lebih kecil terjadi karena adanya peristiwa koalisi. Hasil koalisi tersebut memperbesar diameter rata-rata nanopartikel. Sementara jumlah nanopartikel yang berukuran paling besar berkurang karena terkompresi oleh adanya tekanan. Kedua hal ini menjadikan indeks polidispersitas nanopartikel berkurang dan diameter rata-ratanya bertambah.

4. KESIMPULAN

Nanopartikel senyawa bioaktif daun pegagan telah berhasil disintesa dengan diameter rata-rata terentang dari 10,7 nm sampai 13,2 nm. Besar diameter rata-rata nanopartikel yang diperoleh bergantung pada jumlah massa konsentrat senyawa bioaktif yang digunakan. Semakin tinggi massa konsentrat yang digunakan, semakin besar pula diameter rata-rata nanopartikel yang dihasilkan. Pemberian pemanasan dan tekanan pada nanopartikel melalui *autoclaving* pada temperatur 121^{o}C dan tekanan 2 bar selama 15 menit, menambah besar diameter rata-ratanya dan mengurangi besar indeks polidispersitasnya. Nanopartikel yang memiliki indeks polidispersitas awal yang lebih besar memiliki pertambahan diameter rata-rata yang lebih besar. Pertambahan diameter rata-rata nanopartikel ini disebabkan oleh terjadinya koalisi nanopartikel-nanopartikel yang berukuran lebih kecil dengan nanopartikel yang berukuran lebih besar. Proses koalisi ini membuat populasi nanopartikel yang berukuran lebih kecil menjadi berkurang sehingga menurunkan nilai indeks polidispersitasnya.

DEKLARASI KONFLIK KEPENTINGAN

Tidak ada konflik kepentingan pada penulisan dan publikasi artikel ini.

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Formulasi dan Uji Iritasi Tabir Surya dengan Kandungan Aktif Pati Umbi Porang (*Amorphophallus oncophyllus*)

Formulation and Irritation Test of Sunscreens with Active Content of Porang Tuber Starch (Amorphophallus oncophyllus)

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Abstrak

Tabir surya dapat melindungi kulit manusia dari paparan radiasi ultraviolet penyebab kanker, tetapi beberapa tabir surya yang beredar di pasaran mengandung senyawa karsinogenik. Pemanfaatan pati umbi porang (*Amorphophallus oncophyllus*) sebagai zat aktif dalam formula tabir surya dapat menjadi alternatif sediaan tabir surya yang aman. Tujuan dari penelitian ini adalah mengetahui potensi pemanfaatan pati umbi porang sebagai tabir surya. Tahapan penelitian terdiri atas formulasi sediaan dengan konsentrasi pati umbi porang sebesar 5%, 10%, dan 15%, evaluasi sifat fisik, penentuan nilai SPF, dan uji iritasi terhadap hewan coba. Evaluasi sifat fisik terdiri atas uji organoleptis, uji pH, uji viskositas, uji daya sebar, dan uji daya lekat. Hasil penelitian menunjukkan tabir surya pati umbi porang memiliki penampakan fisik yang baik dan homogen. Nilai pH berada pada rentang 7,24±0,03-7,74±0,03, viskositas 2538,24±213,97-14356±4,3 cPs, daya lekat 0,69±0,23-1,32±0,09 detik, dan daya sebar 6,25±0,43-6,38±0,25 cm. Nilai SPF formula I, II, dan III berturut-turut adalah 5,143; 9,312; dan 17,231. Formula III merupakan formula terbaik dari segi sifat fisik dan tidak menimbulkan iritasi saat diuji pada hewan coba. Pati umbi porang disimpulkan dapat diformulasikan sebagai zat aktif dalam tabir surya.

Kata kunci: Bahan alam; Formulasi; Pati; Porang; Tabir surya

Abstract

Sunscreen can protect human skin from ultraviolet radiation, which is a cause of cancer. However, some sunscreens available on the market contain carcinogenic compounds. Using porang tuber starch (Amorphophallus oncophyllus) as an active ingredient in sunscreen formulas could be a potential option for creating safer sunscreen products. This research aimed to determine the potential use of porang tuber starch (Amorphophallus oncophyllus) in sunscreen. The research method consisted of formulating the sunscreen with concentrations of porang tuber starch at 5%, 10%, and 15%, evaluating physical properties, determining the SPF value, and conducting irritation tests on test animals. The evaluation of physical properties included organoleptic testing, pH testing, viscosity testing, spreadability testing, and adhesion testing. The research showed that porang tuber starch sunscreen has an acceptable and

homogeneous physical appearance. The pH valued range from 7.24±0.03 to 7.74±0.03, viscosity ranges from 2538.24±213.97 to 14356±4.3 cPs, adhesion ranges from 0.69±0.23 to 1.32±0.09 seconds, and spreadability ranges from 6.25±0.43 to 6.38±0.25 cm. The SPF values were formula I, II, and III are 5.143, 9.312, and 17.231, respectively. Formula III was the best formula for physical properties and does not irritate the animals. It was concluded that porang tuber starch can be formulated as an active ingredient in sunscreen.

Keywords: *Formulation; Natural products; Porang; Starch; Sunscreen*

1. PENDAHULUAN

Paparan radiasi sinar ultraviolet dari matahari dalam jangka waktu yang panjang dapat menyebabkan efek buruk pada kulit. Efek buruk tersebut dapat memicu terjadinya melasma atau bercak hitam kecokelatan pada kulit. Selain itu, efek berbahaya yang disebabkan oleh radiasi ini adalah terjadinya melanoma atau kanker kulit. Kanker kulit dapat terjadi karena radiasi sinar ultraviolet yang dapat merusak DNA di mana DNA yang tidak diperbaiki dapat menyebabkan mutasi pada sel onkogen dan supresor tumor (Alcantara *et al.,* 2020; Bernard *et al.,* 2019). Penggunaan tabir surya sangat penting untuk menghindarkan kulit dari pengaruh buruk paparan radiasi sinar ultraviolet yang dipancarkan oleh matahari. Tabir surya dapat digunakan untuk melindungi kulit karena tabir surya dapat memantulkan, menghamburkan, dan menyerap radiasi sinar ultraviolet di area tubuh yang sering terpapar sinar matahari (Minerva, 2019).

Tabir surya memiliki fungsi krusial bagi manusia, tetapi beberapa tahun terakhir ditemukan zat karsinogenik yang terdapat pada tabir surya di pasaran. Pada tahun 2019, Food and Drug Administration (FDA) menarik peredaran tabir surya yang mengandung zat berbahaya bagi tubuh. Beberapa kosmetik tersebut mengandung senyawa turunan benzena yang bersifat karsinogenik, di antaranya *octinoxate*, *dioxybenzone*, *ensulizole*, *homosalate*, *meradimate*, *benzophenone*, *octocrylene*, *sulisobenzone*, dan *avobenzone*. Senyawa-senyawa tersebut dapat terabsorbsi menuju sirkulasi sistemik dan ditemukan pada cairan tubuh, seperti asi, ketuban, urin, dan darah. Senyawa turunan benzena juga telah menunjukkan mekanisme proliferasi seluler pada organ payudara, prostat, dan paru-paru yang dapat memicu kanker (Dinardo dan Downs, 2019; Matta *et al*, 2019). FDA telah melarang adanya kandungan senyawa turunan benzena dalam produk kosmetik, terutama tabir surya. BPOM juga telah menetapkan kadar maksimum beberapa senyawa turunan benzena dalam kosmetik, seperti kadar *benzophenone-4* yang tidak boleh melebihi 5% dan *oxybenzone* yang tidak boleh melebihi 6%. Senyawa benzena dan turunannya dalam konsentrasi berapa pun pada produk kosmetik dapat menimbulkan risiko pada konsumen dan lingkungan (Badan Pengawas Obat dan Makanan, 2019; Hudspeth *et al.*, 2022).

Tabir surya juga dapat menyebabkan kanker Dengan adanya bahan-bahan karsinogenik dalam tabir surya, manusia tidak hanya dapat terkena kanker karena paparan sinar matahari, tetapi juga dapat terserang kanker karena kandungan tabir surya tersebut. Oleh karena itu, diperlukan eksplorasi dan inovasi produk tabir surya baru yang tidak menimbulkan efek karsinogenik. Umbi porang (*Amorphophallus oncophyllus*) adalah bahan yang memiliki potensi

sebagai bahan tabir surya alami. Menurut Aryanti *et al.* (2015), pati umbi porang memiliki kadar amilum atau pati sebesar 47,55% dan kandungan amilosa sebesar 17,54%. Amilum dapat berfungsi sebagai tabir surya secara fisik. Sifat *opaque* amilum yang tidak dapat ditembus cahaya dan dapat memantulkan sinar sangat bermanfaat untuk mencegah penetrasi radiasi sinar ultraviolet dari matahari pada kulit (Oktaviasari dan Zulkarnain, 2017).

Penelitian terkait pemanfaatan pati menjadi tabir surya antara lain, penelitian Infante *et al.* (2021) mengenai tabir surya pati singkong dan jagung dengan komposisi masing-masing 5% dikombinasikan dengan PEG-75 lanolin menunjukkan bahwa terdapat peningkatan aktivitas penghamburan cahaya dan kapasitas antioksidan dari *sunscreen*. Menurut Oktaviasari dan Zulkarnain (2017), pati kentang dengan variasi konsentrasi 15% yang dibuat sediaan lotion juga terbukti memiliki aktivitas sebagai tabir surya sesuai dengan nilai *Sun Protection Factor* (SPF) sebesar 15. Hasil penelitian dari Zulkarnain *et al*. (2013), juga menunjukkan bahwa peningkatan konsentrasi amilum bengkuang berbanding lurus dengan peningkatan nilai SPF dibandingkan dengan kelompok kontrol negatif.

Salah satu tanaman dengan kandungan pati adalah umbi porang yang ketersediaannya melimpah di Indonesia. Produksi umbi porang di Indonesia mencapai 142.000 ton dari luas lahan sebesar 19.950 hektare. Pada tahun 2024, produksi umbi porang direncanakan sebesar 600.000 ton dari luas lahan sebesar 100.000 hektare (Arofah *et al*., 2023). Namun, saat ini terdapat masalah yang dialami petani umbi porang, yaitu terjadinya *over capacity* produksi umbi porang. *Over capacity* ini disebabkan oleh produksi atau budidaya umbi porang yang bertambah pesat, tetapi pendayagunaan terhadap umbi porang tidak meningkat sehingga menyebabkan harga umbi porang menurun drastis (Hatma *et al.,* 2022). Pemanfaatan umbi porang yang masih didominasi oleh produk pangan memerlukan adanya diversifikasi produk lain sebagai upaya untuk menaikkan nilai jual umbi porang. Umbi porang akan memiliki nilai tambah jika dibuat inovasi tabir surya bahan alam yang aman untuk kulit manusia. Oleh karena itu, pada penelitian ini dilakukan diversifikasi pemanfaatan umbi porang sebagai bahan baku kosmetika, yaitu tabir surya. Pemanfaatan umbi porang sebagai bahan tabir surya dapat meningkatkan pendayagunaannya serta menurunkan tingkat over produksi dari umbi porang.

2. BAHAN DAN METODE

2.1. Alat dan bahan

2.1.1. Alat

Spektrofotometer UV-Vis (Shimadzu UV-1900, Jepang), ultra turrax (IKA T25, Jerman), viskometer rheosys (Merlin VR2, USA), *hotplate*, mikroskop (miconos, Indonesia), pH meter (Ohaus, Indonesia), alat cukur, mikropipet, alat uji daya lekat, beban 1 kg, dan alat uji daya sebar, beban 50g; 100g.

2.1.2. Bahan

Umbi porang, aquadest (Brataco, Indonesia), aqua demineralisasi (Brataco, Indonesia), etanol 96% (Brataco, Indonesia), setostearil alkohol (Laurex, Jerman), gliserin (Brataco, Indonesia), vaselin (Brataco, Indonesia), metil paraben (Brataco, Indonesia), propil paraben

(Brataco, Indonesia), potassium hidroksida (Brataco, Indonesia), asam stearat (Brataco, Indonesia), asam sitrat (Brataco, Indonesia), garam, reagen IKI, perban non iritatif, kassa steril, kelinci (galur *new zealand*), sodium lauril sulfat (Brataco, Indonesia), dan produk tabir surya yang beredar dipasaran sebagai pembanding dengan nilai SPF 50.

2.2. Metode

2.2.1. Pengambilan sampel

Sampel yang digunakan dalam riset ini adalah umbi porang (*Amorphophallus onchophyllus*) yang diperoleh dari Kabupaten Madiun, Jawa Timur. Sampel yang diambil adalah umbi porang dengan usia 6-7 bulan dari masa tanam.

2.2.2. Pembuatan pati umbi porang

Pembuatan pati dari umbi porang dilakukan dengan cara membersihkan 2 kg umbi porang dan merendamnya dalam campuran air garam (konsentrasi 8% b/v) selama 3-4 jam dengan perbandingan umbi dan air 1:4. Berikutnya, 2 kg umbi porang dihaluskan menggunakan blender, disaring menggunakan saringan kain, dan diendapkan dalam waktu 6-24 jam. Air yang tersisa pada endapan pati diuapkan dengan *waterbath* suhu 90°C selama 6 jam. Pati kemudian dikeringkan dalam oven suhu 50°C selama 2-3 hari. Pati kemudian diayak menggunakan ayakan berukuran 100 mesh (Amalia *et al.,* 2020).

2.2.3. Karakterisasi pati umbi porang

Pemeriksaan karakteristik pati umbi porang antara lain organoleptis, identifikasi amilum, dan uji mikroskopis (Oktaviasari dan Zulkarnain, 2017). Pemeriksaan organoleptis dilakukan dengan mengamati bau, warna, dan tekstur dari pati menggunakan pancaindera (Nurbaiti *et al.,* 2023). Identifikasi amilum menggunakan metode tes iodin (IKI) dengan mengambil 2 mL sampel dan air sebagai kontrol, kemudian masing-masing ditambahkan 2-3 tetes reagen IKI dan diamati perubahan warnanya. Sampel positif mengandung pati jika berubah menjadi warna biru (Pooja *et al.*, 2022). Uji mikroskopis dilakukan dengan menimbang 100 mg sampel, diletakkan dalam gelas objek, lalu ditetesi 2 tetes air, kemudian ditutup gelas penutup (Ardana *et al*., 2015).

2.2.4. Formulasi tabir surya pati umbi porang

Formula tabir surya yang digunakan (Tabel 1) dimodifikasi dari penelitian Santhanam *et al.* (2017). Formulasi sediaan tabir surya menggunakan beberapa variasi konsentrasi pati umbi porang untuk melihat pengaruh penambahan pati umbi porang terhadap sifat fisik sediaan. Pembuatan sediaan tabir surya pati umbi porang dilakukan dengan membuat fase air terlebih dahulu. Fase air dibuat dengan memasukkan potassium hidroksida ke dalam air demineralisasi, kemudian ditambahkan gliserin dan metil paraben. Setelah itu, dipanaskan dan diaduk pada suhu 80°C. Fase minyak dibuat dengan mencampurkan propil paraben, asam stearat, setostearil alkohol, dan vaselin. Bahan-bahan tersebut diaduk dan dipanaskan pada suhu 80°C. Kedua fase dicampurkan dengan pati umbi porang dan dihomogenisasi pada kecepatan 8000 rpm (Santhanam *et al.,* 2017).

Tabel 1. Formula tabir surya pati porang dengan variasi konsentrasi pati umbi porang.

Bahan	Komposisi (%b/b)		
	F1	F2	F3
Pati Porang	5	10	15
Setostearil Alkohol	1	1	1
Gliserin	9	9	9
Vaselin	2	2	2
Metil Paraben	0,20	0,20	0,20
Propil Paraben	0,05	0,05	0,05
Potassium Hidroksida	1	1	1
Asam Stearat	2	2	2
Asam Sitrat	q.s.	q.s.	q.s.
Aquadest ad	100	100	100

2.2.5. Evaluasi sediaan tabir surya pati porang

2.2.5.1. Uji organoleptis

Uji organoleptis dilakukan dengan cara mengamati bau, warna, dan tekstur dari sediaan menggunakan panca indera.

2.2.5.2. Uji viskositas

Uji viskositas diakukan dengan instrumen Rheosys Merlin VR dengan sistem *cup* dan *bob*. Pengaturan yang digunakan adalah *start* RPM 1 dan *end* RPM 60.

2.2.5.3. Uji pH

Uji pH dilakukan dengan membuat konsentrasi sediaan 1% dalam air suling, kemudian elektroda dicelupkan dan ditunggu hingga nilai pH meter konstan. Nilai pH yang baik untuk sediaan tabir surya menurut SNI 16-4399-1996 berada pada rentang 4,5-8,0.

2.2.5.4. Uji daya sebar

Uji daya sebar dilakukan dengan meletakkan sediaan sebanyak 0,5 g di tengah cawan petri secara terbalik dan didiamkan selama 1 menit. Selanjutnya, diletakkan beban 50 g sampai 150 g setiap 1 menit. Pengukuran daya sebar sediaan yang baik adalah 5-7 cm (Nurbaiti *et al.,* 2023).

2.2.5.5. Uji daya lekat

Uji daya lekat dilakukan dengan meletakkan 1 g sediaan di atas gelas objek yang diketahui luasnya. Gelas objek yang lain diletakkan di atas sampel, kemudian beban 1 kg diletakkan selama 5 menit. Setelah itu, beban 80 g dilepaskan dan waktu yang diperlukan hingga kedua gelas objek terlepas dicatat. Daya lekat yang baik untuk sediaan topikal adalah tidak kurang dari 4 detik (Nurbaiti *et al.*, 2023).

2.2.6. Uji *Sun Protection Factor* (SPF)

Penentuan nilai SPF dilakukan secara *in vitro* dengan instrumen spektrofotometer UV-Vis. Sediaan tabir surya pati umbi porang diencerkan menjadi 1% menggunakan etanol 96%

dan dibaca absorbansinya pada panjang gelombang 290-320 nm. Pada penentuan SPF digunakan pembanding berupa salah satu produk *physical sunscreen* yang berada di pasaran dengan nilai SPF 50. Menurut SNI, nilai SPF yang baik minimal 4 (Listiani *et al*., 2023; Syoufian *et al*, 2014). Penentuan nilai SPF menggunakan Persamaan 1 (Pratiwi *et al*., 2016). Nilai efektivitas eritema (EE) dikali intensitas sinar UV (I) pada panjang gelombang 290-320 nm untuk perhitungan nilai SPF (Tabel 2).

$$\text{Nilai SPF} = \text{CF}. \sum_{290}^{320} \text{Abs}. \text{EE}. \text{I}$$

Persamaan 1. Penentuan nilai SPF secara *in vitro*. Keterangan: Faktor koreksi (10) (CF), Absorbansi sampel (Abs), Efektivitas eritema yang disebabkan sinar UV pada panjang gelombang (nm) (EE), Intensitas sinar UV pada panjang gelombang (nm) (I).

Tabel 2. Nilai EE x I pada panjang gelombang 290-320 nm untuk perhitungan nilai SPF.

Panjang gelombang (nm)	EE x 1
290	0,015
295	0,0817
300	0,2874
305	0,3278
310	0,1864
315	0,839
320	0,018

2.2.7. Uji iritasi

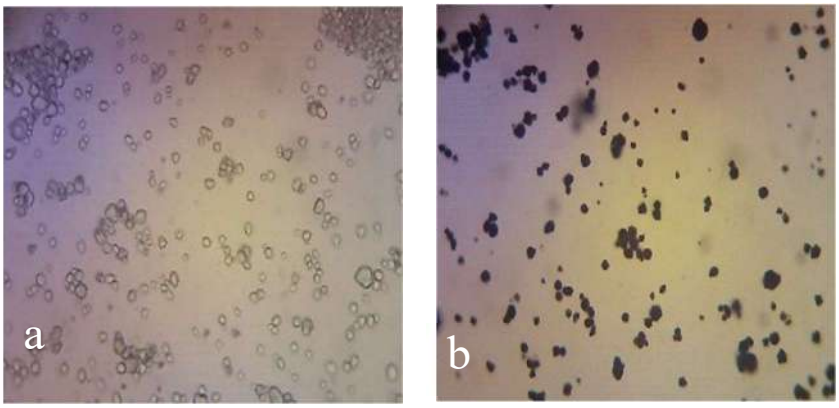
Riset ini telah mendapatkan persetujuan dari Komite Etik Penelitian Universitas Ahmad Dahlan untuk penelitian yang menggunakan hewan coba dengan nomor 012307153. Uji iritasi dilakukan dengan hewan uji kelinci menggunakan metode uji *patch test method.* Punggung kelinci dicukur dan dibagi menjadi empat bagian dengan area berukuran 2 x 3 cm. Uji dilakukan dengan mengoleskan 0,5 mL sampel formula terbaik, pembanding berupa produk tabir surya dari pasaran dengan SPF50 dan bersifat tabir surya fisik, kontrol positif berupa Sodium Lauril Sulfat, dan kontrol negatif berupa kulit hewan uji tanpa perlakuan. Area uji ditutup menggunakan perban noniritan dan didiamkan selama 4 jam. Kassa dibuka dan residu sampel dihilangkan. Pengamatan dilakukan pada jam ke-24, 48, dan 72 dan dilakukan skoring pada hewan uji (Pradana dan Nugroho, 2016).

3. HASIL DAN PEMBAHASAN

3.1. Pembuatan dan karakterisasi pati umbi porang

Pati yang dihasilkan dari proses pembuatan pati umbi porang adalah 405,04 g dari umbi porang 13,88 kg sehingga rendemen yang diperoleh sebesar 2,92%. Pati yang dihasilkan memiliki karakteristik membulat berwarna putih dengan permukaan tidak rata jika diamati secara mikroskopis (Gambar 1A). Menurut Brust *et al.* (2020), pati akan menghasilkan warna ungu gelap ketika direaksikan dengan reagen Iodida-Potasium Iodida (IKI). Hasil ungu gelap

ketika direaksikan dengan reagen IKI (Gambar 1B). Hal ini menandakan bahwa senyawa yang diperoleh merupakan pati.



Gambar 1. Pengamatan mikroskopis hasil karakterisasi pati umbi porang. Keterangan: Mikroskopis pati porang (400x) (a) dan Mikroskopis pati porang + reagen IKI (400x) (b).

3.2. Formulasi dan evaluasi sediaan

3.2.1. Formulasi, organoleptis, dan homogenitas

Pati umbi porang (A*morphophallus oncophyllus*) dapat diformulasikan menjadi sediaan tabir surya. Mekanisme pati umbi porang sebagai tabir surya mirip dengan mekanisme penghalangan cahaya dari zat aktif tabir surya fisik, seperti zink dioksida dan titanium dioksida yang bekerja dengan memantulkan dan menghamburkan cahaya (Gabros *et al.*, 2023). Hasil formulasi setelah diuji organoleptis menunjukkan bahwa semakin tinggi konsentrasi pati akan mempengaruhi warna dan bau dari tabir surya yang telah diformulasikan. Ketiga formula juga tergolong homogen ditunjukkan dengan tidak adanya gumpalan dan basis yang rata pada sediaan (Tabel 3).

Tabel 3. Hasil evaluasi sediaan tabir surya pati umbi porang dengan variasi konsentrasi pati umbi porang.

Formula	Organoleptis dan Homogenitas	Viskositas (cPs)	pH	Daya Lekat (detik)	Daya Sebar (cm)
I	Warna putih susu, bau khas pati lemah, homogen	2538,24 ± 213,97	7,24 ± 0,03	0,69 ± 0,23	6,38 ± 0,25
II	Warna putih tulang, bau khas pati sedang, homogen	3821,63 ± 22,49	7,45 ± 0,18	0,71 ± 0,23	6,35 ± 0,36
III	Warna putih tulang, bau khas pati kuat, homogen	14356 ± 4,30	7,74 ± 0,03	1,32 ± 0,09	6,25 ± 0,43
Pembanding	Warna putih susu, tidak berbau, homogen	9520,48 ± 202,65	6,09 ± 0,07	0,71 ± 0,03	6,98 ± 0,15

3.2.2. Uji daya lekat

Evaluasi daya lekat pada sediaan topikal bertujuan untuk mengukur seberapa lama waktu melekat sediaan pada alat uji daya lekat dan memprediksi waktu kontak sediaan topikal dengan

kulit. Nilai uji daya lekat yang baik secara teoritislebih dari 4 detik (Tungadi *et al.,* 2023). Formula I menunjukkan hasil daya lekat sebesar 0,69 detik, formula II sebesar 0,71 detik, dan formula III sebesar 1,32 detik (Tabel 3). SNI (Standar Nasional Indonesia) mengenai tabir surya tidak mempersyaratkan adanya evaluasi daya lekat sehingga daya lekat bukan merupakan hal yang krusial dalam formulasi tabir surya. Hasil analisis statistik uji *analysis of variant* (ANOVA) menunjukkan signifikansi 0,011 ($p<0,05$). Hal ini menunjukkan adanya perbedaan yang signifikan antarformula. Berdasarkan uji lanjutan post hoc Tukey HSD, formula III memiliki perbedaan yang signifikan antara formula I, formula II, dan pembanding.

3.2.3. Uji daya sebar

Pengujian daya sebar bertujuan mengevaluasi kecepatan penyebaran suatu sediaan pada kulit ketika sediaan tersebut dioleskan secara topikal. Daya sebar yang baik suatu sediaan topikal berada pada rentang 5-7 cm (Tungadi *et al.,* 2023). Daya sebar sediaan ketiga formula menunjukkan berada pada rentang 6-7 cm (Tabel 3). Dengan demikian, sediaan tersebut memiliki daya sebar yang baik. Uji analitik *analysis of variant* (ANOVA) menunjukkan bahwa antarformula tabir surya pati umbi porang tidak memiliki perbedaan daya sebar yang signifikan dengan *p value* 0,086 ($p>0,05$). Hasil tersebut mengindikasikan bahwa penambahan pati umbi porang tidak berpengaruh terhadap daya sebar sediaan tabir surya.

3.2.4. Uji pH

Uji pH untuk mengetahui apakah pH sediaan dapat diterima oleh kulit. Nilai pH yang baik untuk sediaan tabir surya menurut SNI 16-4399-1996 berada pada rentang 4,5-8,0. Sediaan tabir surya dengan pH yang terlalu rendah dapat menyebabkan kulit bersisik hingga iritasi. Sementara itu, pH yang terlalu tinggi dapat menyebabkan kulit terasa licin, kering, dan dapat mempengaruhi elastisitas kulit (Iskandar *et al.,* 2021; Listiani *et al*., 2023).

Peningkatan konsentrasi pati umbi porang berbanding lurus dengan peningkatan nilai pH (Tabel 3). Hasil uji statistik *analysis of variant* (ANOVA) menunjukkan nilai signifikansi $p<0,05$ (Tabel 3). Hal ini berarti terdapat perbedaan yang signifikan antarformula. Uji lanjutan post hoc Tukey HSD menunjukkan bahwa formula III memiliki perbedaan yang signifikan dengan formula I, formula II, dan pembanding.

3.2.5. Uji viskositas

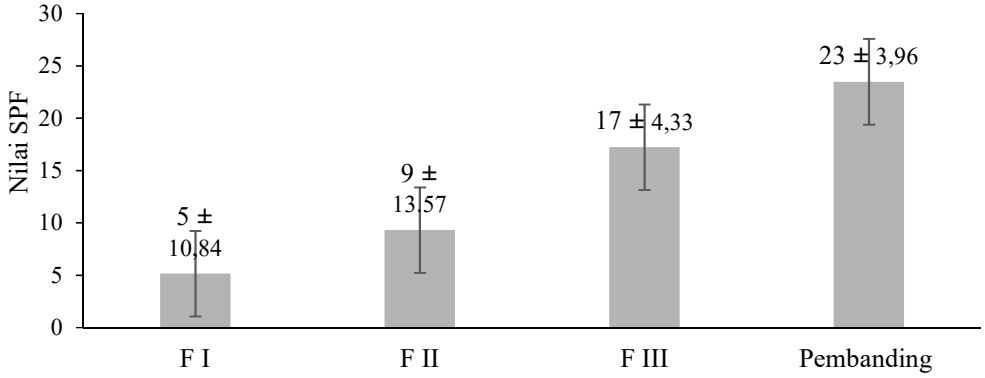
Uji viskositas terhadap sediaan dilakukan untuk mengukur tingkat kekentalan suatu sediaan. Menurut SNI 16-4399-1996, persyaratan viskositas untuk sediaan tabir surya yang baik berada pada rentang 2.000-50.000 cPs. Hasil pengujian viskositas masing-masing formula sediaan tabir surya pati umbi porang (Tabel 3) memiliki nilai viskositas dengan rentang 2538,24 – 14356 cPs. Hasil uji viskositas tabir surya pati umbi porang memenuhi rentang yang ditetapkan dalam SNI.

Hasil analisis statistik uji *analysis of variant* (ANOVA) menunjukkan signifikansi 0,000 ($p<0,05$) yang berarti terdapat perbedaan signifikan antarformula. Uji lanjutan post hoc Tukey HSD antarformula tabir surya memiliki *p value* <0,05. Hal ini menunjukkan bahwa formula I,

formula II, formula III, dan pembanding terdapat perbedaan viskositas yang cukup signifikan. Dengan demikian, setiap penambahan pati sebanyak 5% mampu mengubah nilai viskositas secara bermakna.

3.3. Penentuan nilai SPF

Penentuan SPF dilakukan dengan mengukur nilai *sun protection factor* (SPF) secara *in vitro* dengan metode spektrofotometri. Pengujian nilai SPF (*Sun Protection Factor*) digunakan untuk mengetahui seberapa lama sediaan dapat melindungi kulit dari sinar ultraviolet, terutama UV-B. Nilai SPF digolongkan menjadi beberapa kategori meliputi proteksi minimal (2-4), proteksi sedang (4-6), proteksi ekstra (8-10), proteksi maksimum (8-15), dan proteksi ultra (>15) (Halid *et al.,* 2023). Hasil penentuan SPF tabir surya pati umbi porang formula I, formula II, dan formula III (Gambar 2) secara berturut-turut masuk ke dalam proteksi sedang, maksimum, dan ultra. Pembanding yang digunakan adalah tabir surya fisik yang beredar di pasaran dengan SPF 50.



Gambar 2. Hasil uji *Sun Protection Factor* (SPF) tabir surya pati umbi porang.

Analisis statistik *post hoc tukey* HSD menunjukkan antar formula tabir surya pati umbi porang memiliki *p value* <0,05. Hasil tersebut dapat diartikan bahwa tiap penambahan konsentrasi pati sebanyak 5%, nilai SPF dari tabir surya pati umbi porang akan meningkat dan menghasilkan nilai SPF yang berbeda signifikan. Namun, semakin banyak pati umbi porang yang ditambahkan akan menyebabkan semakin tingginya viskositas sediaan. Oleh karena itu, tidak direkomendasikan untuk menaikkan konsentrasi pati umbi porang karena akan mempengaruhi sifat fisik sediaan yang dihasilkan.

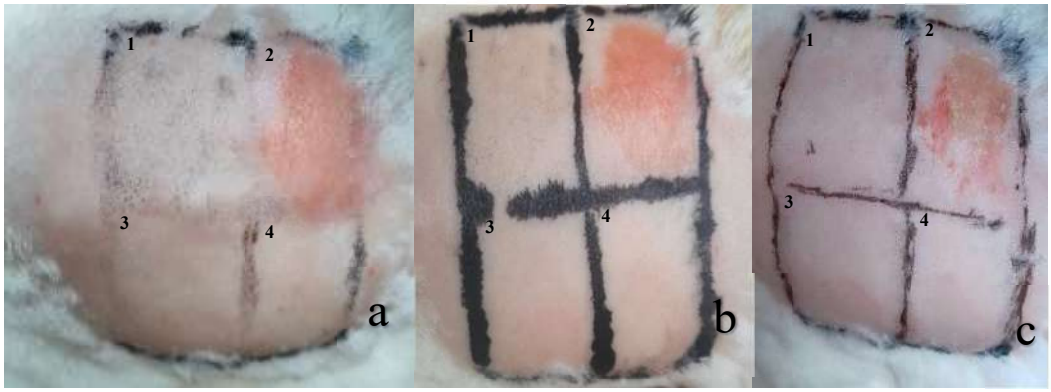
3.4. Uji iritasi

Uji iritasi dilakukan terhadap formula sediaan tabir surya terbaik. Seluruh formula telah memenuhi persyaratan sifat fisik yang tercantum pada SNI 16-4399-1996. Formula terbaik ditentukan dengan analisis statistik dan formula dengan nilai SPF paling tinggi. Berdasarkan analisis statistik dan karakterisasi fisik sediaan, formula III menunjukan hasil yang berbeda signifikan dengan formula lainnya dalam hal uji daya lekat, uji viskositas, dan penentuan SPF. Selain itu, formula III memiliki nilai SPF yang lebih baik dibandingkan formula I dan formula II. Pengujian iritasi dilakukan terhadap formula III sediaan tabir surya umbi porang (Tabel 4).

Tabel 4. Skor iritasi hewan coba kelinci yang diberi perlakuan kontrol positif, kontrol negatif, formula terbaik dan produk pembanding tabir surya.

No.	Perlakuan	Skor	Keterangan
1	Kontrol positif	0 ± 0	Tidak Iritasi
2	Kontrol negatif	2,25 ± 0,94	Iritasi sedang
3	Formula terbaik	0 ± 0	Tidak Iritasi
4	Kontrol normal	0 ± 0	Tidak Iritasi

Uji iritasi pada hewan uji penting dilakukan sebelum sediaan digunakan oleh masyarakat untuk menghindari efek samping yang tidak diinginkan, seperti iritasi (Ardhany *et al.*, 2019). Hasil pengamatan menunjukkan bahwa kelompok yang muncul eritema dan edema pada kulit adalah kelompok kontrol negatif. Kontrol negatif merupakan surfaktan *Sodium Lauryl Sulfate* (SLS) yang memiliki efek iritasi pada kulit dan telah digunakan secara luas sebagai model untuk menguji respons kulit terhadap iritan (Leskur *et al.*, 2019). Eritema dan edema yang muncul pada kelompok kontrol negatif memiliki skor iritasi 2,25 dengan kategori iritasi sedang pada pengamatan ke-72 jam. Kelompok dengan formula terbaik, kontrol positif, dan kontrol normal tidak menunjukkan adanya eritema dan edema atau tidak terjadi adanya iritasi (Tabel 4).



Gambar 3. Hasil uji iritasi terhadap kulit punggung kelinci. Keterangan: Kulit punggung kelinci setelah 24 jam pengamatan (a), Kulit punggung kelinci setelah 48 jam pengamatan (b) dan Kulit punggung kelinci setelah 72 jam pengamatan (c); Kontrol positif (1), kontrol negative (2), formula terbaik (3) dan kontrol normal (4).

Suatu sediaan tabir surya tidak menimbulkan iritasi apabila tidak menunjukan adanya edema dan eritrema pada kulit kelinci pada waktu 24 jam, 48 jam, dan 72 jam (Rahmadany *et al*., 2022). Berdasarkan hasil uji iritasi pada kulit punggung hewan coba kelinci (Gambar 3) dapat disimpulkan bahwa formula terbaik tabir surya pati umbi porang aman digunakan.

4. KESIMPULAN

Pati umbi porang (*Amorphophallus oncophyllus*) dapat diformulasikan menjadi sediaan tabir surya dengan hasil evaluasi yang memenuhi Standar Nasional Indonesia (SNI). Berdasarkan analisis statistik dan hasil karakterisasi sediaan, formula terbaik tabir surya pati

umbi porang adalah formula III dengan konsentrasi pati sebesar 15% karena tidak menunjukkan adanya iritasi pada hewan coba setelah dilakukan uji iritasi.

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Antidiabetic Potential and Metabolite Profile of Leaf and Stem Extract of *Castanopsis tungurrut* (Blume) A.DC.

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Abstract
Diabetes mellitus is a metabolic disorder caused by high blood sugar levels. One species in *the Castanopsis* genus is proven with a hypoglycemic effect. Therefore, the study aimed to discover the potential of *Castanopsis tungurrut* as an antidiabetic. Sample extraction, α-amylase inhibition, glucose diffusion analysis, GC-MS analysis, and molecular docking were applied in this study. Maceration of the leaf ethanol extract showed the highest yield value of 21.08%, while the stem extract was 14.04%. Leaf ethanol extract and stem ethyl acetate extract showed the highest inhibiting α-amylase activity with an inhibition value of 33.74%±1.54 and 34.45%±1.08 at 1 mg/mL concentration. The glucose entrapment assay showed that these two extracts could inhibit the diffusion of glucose in the dialysis bag. The final result was glucose concentration in dialysate for the two extracts of 0.114±0.001 mg/mL and 0.116±0.001 mg/mL which was lower than acarbose in 0.120±0.004 mg/mL. GC-MS analysis showed 6 metabolites in leaf ethanol extract and 22 metabolites in stems ethyl acetate extract from an alkane, salicylic, cinnamic, terpene, steroid, and fatty acid. Molecular docking resulting between the compounds with α-amylase enzymes complex showed γ-sitosterol and ß-bisabolene from *C. tungurrut* extract have the potential to be developed as an antidiabetic drug due to its good inhibitory activity with binding affinity values of -9.1 and -6.9 that considered better and quite close to acarbose as control of -7.7.

Keywords: Antidiabetic; *Castanopsis tungurrut*; Diabetes mellitus; Enzyme inhibition; Gass Chromatography-Mass Spectrometry (GC-MS)

1. INTRODUCTION

Diabetes mellitus is when blood sugar levels exceed normal limits, commonly called hyperglycemia (Deshpande et al., 2008). In 2021, The International Diabetes Federation (IDF) states that there are 537 million people with diabetes. Several factors, such as unhealthy lifestyles, limited access to healthy food, high rates of obesity, and genetic factors, lead to an increase in the number of people with diabetes (Betteng et al., 2014). There are two types of diabetes mellitus, namely type I diabetes mellitus, which is caused by damage to pancreatic β cells, and type II diabetes, which is caused by insulin resistance (Samocha-Bonet et al., 2021). Pharmacological treatments are now widely used to treat diabetes and have side effects in long-

term use. For example, the use of acarbose, which is an oral drug for diabetes with digestive enzyme inhibitory activity to prevent an increase in blood sugar, has side effects such as flatulence, diarrhea, abdominal distention, and borborygmus if used in the long term (Hilma et al., 2020). Alternative treatments, such as herbal plants, are now widely known to have hypoglycemic effects with minimal side effects, such as mild digestive discomfort. The hypoglycemic activity of this herbal plant is caused by phytochemicals such as alkaloids, terpenes, flavonoids, and saponins contained therein (Suvarna et al., 2021). Flavonoids isolated from *Tamarix gallica* (Hmidene et al., 2017) and kaempferol derivatives isolated from Sedum dendroidum leaf extract (Da Silva et al., 2014)have been reported to have in vitro and in vivo antidiabetic activity.

Plants from the *Castanopsis* genus, namely *Castanopsis costata*, have been shown to have antidiabetic activity in a study by Alkandahri et al (2016) on diabetes-induced rats. The diverse phytochemical content of the *Castanopsis* genus, such as terpenoids, galloyl acid, shikimic, glycosides, saponins, steroids, flavonoids, alkaloids, and a distinctive compound in the form of Castanopsinins is suspected to support the activity of this plant as an antidiabetic (Alkandahri et al., 2016; Kim et al., 2020; Prakash et al., 2020). *Castanopsis tungurrut* is a species of the genus *Castanopsis*, which is widely found in Indonesia. This plant is included in the endangered group according to the IUCN Red List, and research related to its use as a food and pharmacological ingredient has yet to be carried out. However, *C. tungurrut* has received limited attention regarding its bioactivity potential. Research on *C. tungurrut* has primarily focused on conservation initiatives, with less documentation on its ethnobotanical aspects. The recent publication by *C. tungurrut* demonstrated its potential as an antibacterial agent (Ilyas et al., 2023).

The study of the bioactive properties of a plant species now facing the threat of extinction is of utmost importance in facilitating ex-situ conservation initiatives that actively engage the public in conservation activities. The dissemination of information regarding the advantageous properties of *C. tungurrut* is anticipated to generate public attention toward cultivating the plant outside the confines of the national park. Therefore, this study aimed to explore the potential of *C. tungurrut* as an antidiabetic agent. This study included inhibition of α-amylase enzymes, glucose entrapment, and molecular docking to assess the antidiabetic activity of extracts from the leaves and stems of *C. tungurrut* in different solvents. Furthermore, a GC-MS analysis was conducted to determine the composition and relative quantities of secondary metabolites within the leaf and stem extracts of *C. tungurrut.*

2. MATERIALS AND METHODS

2.1. Materials

Simplicia from leaves and stems of *C. tungurrut* was provided by the Research Center for Plant Conservation, Botanic Garden and Forestry, National Research and Innovation

Agency (BRIN). For extraction, sterile distilled water, ethanol (Merck), and ethyl acetate (Merck). Acarbose (Sigma Aldrich, Merck), α-amylase enzymes (TCI), starch (Merck), DNS reagents consisting of 3,5-dinitrosalicylate (Sigma Aldrich, Merck), NaOH 2N (Merck), Na-K Tartrate (Merck), phosphate buffer pH 7 consisting of the compounds $NaH_2PO_4.2H_2O$ and $Na_2HPO_4.2H_2O$ (Merck), and DMSO for inhibition of the α-amylase enzyme. Dialysis bags (Sigma Aldrich, Merck) and D (+)-Glucose (Merck) for the glucose entrapment test.

2.2. Methods

2.2.1. Extraction

Extraction was done using sterile aquadest, 70% ethanol, and ethyl acetate for each leaf and stem simplicia. The simplicia was maceration (1:10) for 3×24 hours in ethanol and ethyl acetate solvents. The filtrate obtained was concentrated using a rotary evaporator with a temperature of 40°C. For the aqua dest solvent, maceration was carried out for 24 hours in the refrigerator for samples previously heated in a water bath at 50°C for 45 minutes. The filtrate obtained was then concentrated using a rotary evaporator at 50°C, followed by a water bath at 75°C until a paste-shaped extract was obtained. The pasta extract was transferred into dark vials and subsequently stored at 10°C to set up for the following study.

2.2.2. α-Amylase Enzyme Inhibition

The amylase inhibition test (Table 1) was carried out according to the protocol of Bahtiarsyah et al. (2023). The inhibition value was then calculated using the formula 1.

$$\% \text{ Inhibition} = \frac{C-S}{C} \times 100\%$$

Formula 1. Calculation of α-Amylase Enzyme inhibition value. Description: Absorbance Control (C) and S1-S0 (Apparent absorbance) (S).

Table 1. Protocol for α-Amylase inhibition test.

Volume (mL)	Blank	Control	Sample with enzyme	Sample without enzyme
	B	C	S1	S0
Extract	-	-	0.25	0.25
Incubation (5 min, 37°C)				
Phosphate Buffer pH 7	1	0.5	0.25	0.5
α-Amylase enzyme	-	0.25	0.25	-
Incubation (5 min, 37°C)				
Starch 1%	-	0.25	0.25	0.25
Incubation (5 min, 37°C)				
DNS	0.5	0.5	0.5	0.5
Heated in waterbath (5 min, 100°C), then cooled to room temperature				
Aquadest	5	5	5	5
Vortex, and measured the absorbance in a spectrophotometer (540 nm)				

2.2.3. Glucose entrapment

This experiment aims to assess the extract's capacity to effectively bind sugar, thereby simulating the conditions within the digestive system when glucose levels increase following a meal. Dialysis bags with a diameter of 10 cm were immersed for 5 minutes in distilled water. A combined volume of 6.25 mL of 0.02M glucose and 0.125 mL of extract or sample were placed in a dialysis bag with one end tied. Following the tying of the opposite end of the bag, the dialysis bag was inserted into an Erlenmeyer containing 50 mL of distilled water. The Erlenmeyer was subsequently shaken at 150 rpm for 120 minutes. At the end of incubation, 1 mL of glucose sample was collected and measured for the glucose diffused outside of the dialysis bag by adding 1 mL of DNS followed by 80°C to 100°C heating in a water bath for 5 minutes. Subsequently, 8 mL of distilled water was added to the mixture and mixed thoroughly. Next, the absorbance of the mixture was determined at 540 nanometers using a UV-Vis spectrometer. The negative control and positive control used distilled water and acarbose at a 0.1 mg/mL concentration, respectively. The glucose concentrations were determined by plotting the absorbance to the standard glucose curve in the series of 0.2, 0.4, 0.6, 0.8, and 1 mg/mL. This protocol refers to a study by Bahtiarsyah et al. (2023).

2.2.4. Gass Chromatography-Mass Spectrometry (GC-MS)

The metabolite profiles of *C. tungurrut* ethanol and ethyl acetate extracts were examined using GC-MS (Shimadzu QP2010SE). The machine's conditions were set as follows: the GC column used was the Rtx 5 MS with 30 m length, 0.25 mm diameter, and 0.25 µm thickness. Helium gas was the carrier gas with a 28 mL/min flow rate. An average of 3 µL of the sample was injected in the spitless mode. The temperature was set to 70–300 °C with 5 °C increments every 1 minute, with a total running time of 60 min. The mass spectrometry condition used was a 70-electron volt ionization electron detector (EI 70 Ev). The GC-MS condition followed the protocol of Calvaryni and Nuringtyas (2022).

2.2.5. Molecular docking

The protein receptor is an α-amylase pancreatic enzyme with PDB ID: 1B2Y obtained from the RCSB PDB website (rcsb.org). Seventeen compounds with the highest %area of the GC-MS profiles was chosen and were collected for their 3D structures on the Pubchem website (https://pubchem.ncbi.nlm.nih.gov). 3D structures not available in Pubchem were constructed manually using Novopro (https://www.novoprolabs.com/tools/smiles2pdb). Protein preparation was carried out using UCSF Chimera software and AutodockTools 1.5.7. Ligan preparation and running of molecular docking was carried out in PyRx 0.8 software. Docking results were visualized using Pymol and DS Visualizer software.

2.3. Data analysis

Data analysis was performed for data on % inhibition and glucose concentration in IBM SPSS Statistics 20 software for the Nested test, One-way ANOVA, and Duncan's MRT post hoc.

3. Results and Discussion

Extraction using three different solvents separately was intended to determine the most effective extract in its activity as an antidiabetic. Ethanol and aquadest are polar solvents, so compounds with the same polarity can be separated in the extraction process. Ethyl acetate, with its semipolar properties, can attract compounds with polar and non-polar properties. Extraction results of 25 g simplicial of *C. tungurrut* leaves and stems in 250 mL solvent (Table 2). The highest yield value was found in the ethanol extract of *C. tungurrut* leaves, indicating that ethanol extract could separate compounds better than other solvents. The findings indicate that the compounds present in the leaves and stems of *C. tungurrut* possess a polarity similar to that of ethanol. It aligns with the solvent's tendency to separate compounds according to their polarities (Savitri et al., 2017; Verdiana et al., 2018).

Table 2. Extraction result of *Castanopsis tungurrut* leaves and stems using aquadest, ethanol, and ethyl acetate solvents.

Sample	Solvent	Extract Mass (g)	Yield (%)
Leaves	Aquadest	3.63	14.52
	Ethanol 70%	5.27	21.08
	Ethyl Acetate	0.06	2.4
Stems	Aquadest	2.25	9
	Ethanol 70%	3.51	14.04
	Ethyl Acetate	0.02	0.8

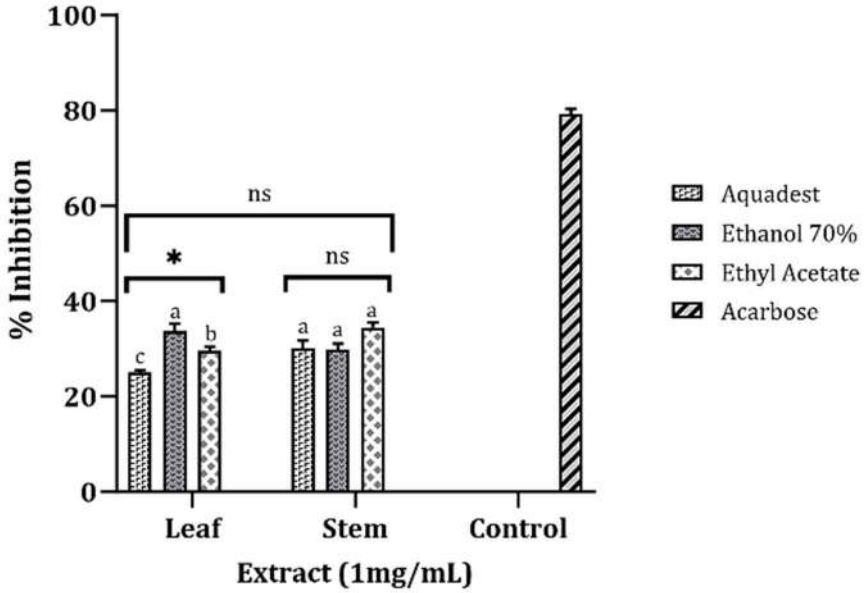


Figure 1. The α-Amylase inhibition activity of leaf and stem extract. The difference in letters is significant based on Duncan MRT (Multiple Range Test) at $p<0.05$.

In inhibiting the α-amylase enzyme, the percent inhibition in the leaves and stem samples of *C. tungurrut* was still lower than that of acarbose as the positive control. However, the best value was shown by the ethanol extract of leaves and ethyl acetate extract of *C. tungurrut* stems of 33.74 ± 1.54% and 34.45 ± 1.08%. Statistical test results using Nested ANOVA showed no significant difference in the % inhibition of the leaf and stem extract groups (p=0.536). However, the one-way ANOVA test for the three groups of solvents used had significantly different % inhibition values (p=0.003). The solvent groups that were significantly different were aquadest, ethanol, and ethyl acetate in the leaf extract, as indicated by the different subsets in Duncan's post hoc test (Figure 1). These results indicate that the inhibitory activity of α-amylase in both leaf and stem organs is not much different. However, the difference in the solvent used affects the inhibitory activity of the extract.

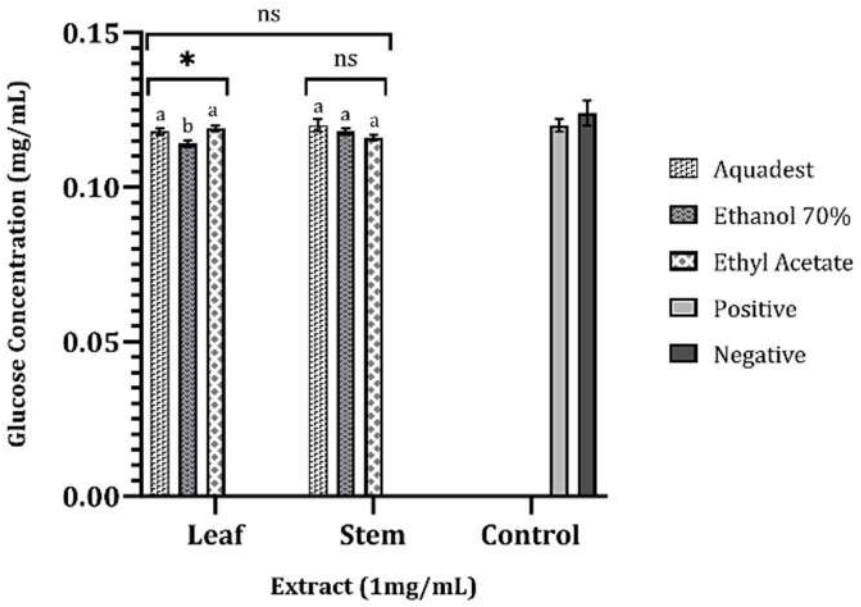


Figure 2. Inhibition of glucose diffusion from the dialysis bag in 120 min (mg/mL). The difference in letters is significant based on Duncan MRT (Multiple Range Test) at p<0.05.

The plant extract's limited inhibitory efficacy is attributed to several active chemicals involved in competitive binding with the enzyme. Furthermore, the higher potency of acarbose can be attributed to its competitive inhibition as a pure molecule, which has been demonstrated to possess significant inhibitory activity (Hilma et al., 2020; Wulandari et al., 2021). Hence, all plant extracts used in this study need to be concentrated 2-3 times to achieve a balance with acarbose's inhibitory effect. Fractionation may enhance the activity by separating the active molecules from the non-active ones through the division into multiple fractions. The inhibitory activity by plant extracts is due to the bonds that form between the hydroxy groups in the compound and amino acids on the active site of the enzyme, as a result of which enzymatic reactions can be blocked (Anugrahini and Wahyuni, 2021). In the α-amylase enzyme, inhibitor activity slows the digestion time of carbohydrates and reduces the rate of glucose absorption in the digestive tract. The hydrolysis reaction on the blocked enzyme causes disaccharides from

enzymatic reactions such as maltose, sucrose, and lactose not to be produced so postprandial blood glucose can be lowered (Torres-Naranjo et al., 2016; Wickramaratne et al., 2016).

The glucose entrapment test supported the results of α-amylase inhibition, which showed the best results in ethanol extract and ethyl acetate extract of *C. tungurrut* stems. Glucose entrapment aims to represent the process of glucose metabolism in the small intestine. The extract was tested for its ability to inhibit the transfer of glucose from the small intestine to the blood vessels, as represented by dialysis bags and dialysate (Qujeq and Babazadeh, 2013). The results obtained in this test are glucose concentration in the dialysate, indicating the possible glucose level in the blood (Figure 2).

The ethanol extract of the leaves showed the lowest glucose concentration of 0.114 ± 0.001 mg/mL, followed by the ethyl acetate extract of the stems of 0.116 ± 0.001 mg/mL. This result was lower than that of acarbose, the positive control, and aquadest, the negative control. The Nested ANOVA statistic test showed no significant difference in glucose concentration values between the leaf and stem groups (p=0,715). One-way ANOVA test showed that in the leaf group, there was a significant difference between the solvents used (p=0,008). Significant differences were shown in aquadest, significantly different from the other two solvents. The inhibitory activity by plant extracts in this glucose entrapment test was due to the concentration and molecular mass of soluble fiber in plants inhibiting the diffusion of glucose and delaying the absorption of carbohydrates. Plant fiber increases viscosity and performs binding activity, so glucose does not diffuse and glucose levels in the blood do not increase (Younas and Hussain, 2014).

Table 3. The secondary metabolite identified in the *Castanopsis tungurrut* leaf ethanol extract was analyzed using GC-MS.

No	R Time	Molecular ions (m/z)	Compounds	Area (%)	Group
1.	15.648	152,15	Methyl Salicylate	2.36	Salicylates
2.	17.927	152,24	Geranial	1.26	Monoterpenoid
3.	23.674	176,21	Ethyl Cinnamate	2.14	Cinnamates
4.	24.042	202	Curcumene	27.35	Sesquiterpenoid
5.	24.748	204,35	Beta-Bisabolene	12.52	Sesquiterpenoid
6.	38.027	296	Methyl petroselinate	1.88	Fatty acid

The GC-MS results showed that the ethanol extract of *C. tungurrut* leaves contained six types of secondary metabolites consisting of salicylic, terpenoid, cinnamic, and fatty acid groups (Table 3). The abundance of compounds indicated by % area showed that curcumene had the highest abundance of 27.35%, followed by beta-bisabolene with % area of 12.52%. Both of these compounds belong to the class of sesquiterpenoids. In the ethyl acetate extract of *C. tungurrut* stems, 22 types of compounds were found, dominated by alkane groups (Table 4). The highest abundance was shown by gamma-sitosterol, with a % an area of 16%, followed by palmitic acid, with 9.58%. Antidiabetic activity was found in ethyl cinnamate, curcumene, and

beta-bisabolene compounds in the ethanol extract of leaves (Dosoky and Setzer, 2018; Zhang et al., 2015). Meanwhile, in the ethyl acetate extract of the stems, neophyte diene, palmitic acid, oleic acid, linoleic acid, phytol, tetracosane, eicosane, 3-methyleicosane, and vitamin E compounds were reported to have antidiabetic activity in previous studies (Table 4). This activity includes inhibitory activity of related digestive enzymes and hypoglycemic activity by other assay methods (Rautela et al., 2018; Smorowska et al., 2021).

Compounds with antidiabetic activity on GC-MS identification and compounds with high abundance were subsequently subjected to molecular docking simulations to determine their inhibitory activity against α-amylase enzyme in docking simulation. This analysis was conducted to further explore the antidiabetic potential of the compound contained in the plant extract due to their weak inhibitory activity against the enzyme in previous tests. This may guide how the extract will be fractionated to obtain the active compounds.

Table 4. Secondary metabolite of *C. tungurrut* stems ethyl acetate extract analyzed using Gass Chromatogrpahy-Mass Spectrometry (GC-MS).

No	R Time	Molecular ions (m/z)	Compound	Area (%)	Group
1.	32.558	278	Neophytadiene	1.66	Sesquiterpenoids
2.	33.492	296,49	Phytol	0.67	Terpene
3.	35.283	256,42	Palmitic acid	9.58	Fatty acid
4.	38.692	280,45	Linoleic acid	5.42	Fatty acid
5.	39.208	282,47	cis-Oleic acid	1.37	Fatty acid
6.	39.942	338,69	Tetracosane	0.76	Alkane
7.	41.775	282,55	Eicosane	1.58	Alkane
8.	44.592	507	Hexatriacontane	0.79	Alkane
9.	45.217	338	Tetratetracontane	3.66	Alkane
10.	46.400	310,58	3-Methylheneicosane	0.61	Alkane
11.	47.625	296	Heneicosane	1.21	Alkane
12.	47.825	380	Pentatriacontane	1.10	Alkane
13.	48.408	394,83	Octacosane	4.61	Alkane
14.	48.867	605	Tritetracontane	4.37	Fatty acid
15.	49.350	380	Heptacosane	1.90	Alkane
16.	49.508	310	3-Methyleicosane	0.91	Alkane
17.	49.917	408	Nonacosane	4.00	Alkane
18.	50.367	801	1-Iodooctatetracontane	3.05	Alkane
19.	50.833	324	Tricosane	1.33	Alkane
20.	51.867	592	1-Hentetracontanol	1.50	Fatty acid
21.	55.433	430,71	Vitamin E	2.38	Terpenoid
22.	60.208	414,70	Gamma-Sitosterol	16.00	Sitosterols/steroid

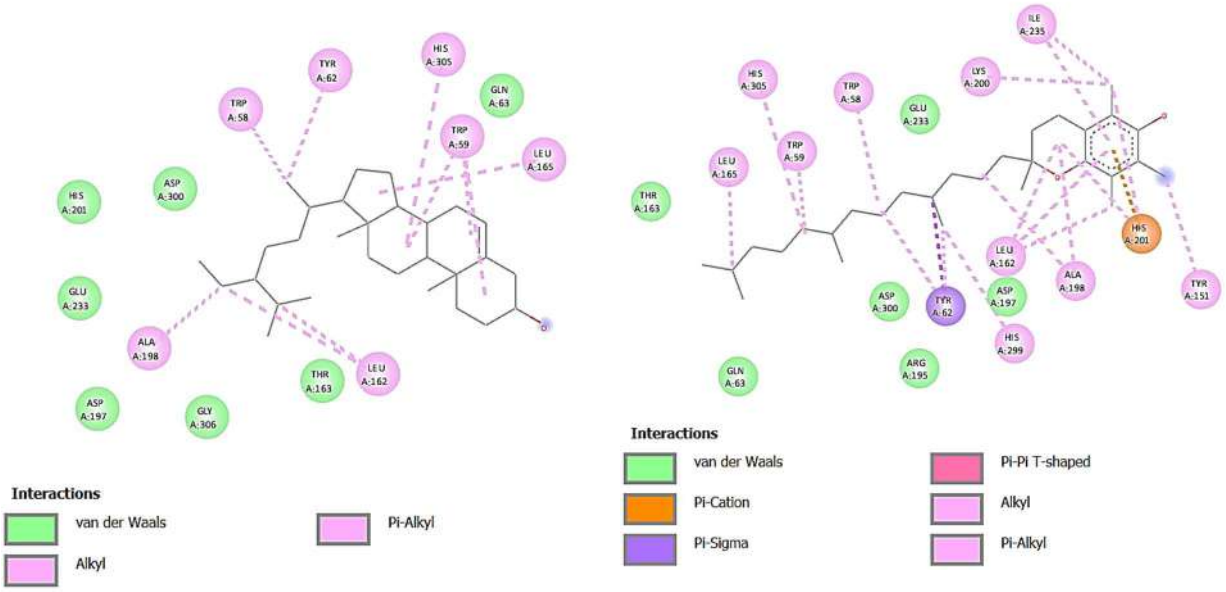
The results of molecular docking of the selected compounds with receptors in the form of α-amylase enzymes (PDB ID: 1B2Y) (Table 5). The gamma-sitosterol compound showed the best results, with the lowest binding affinity value of -9.1. This result was followed by a binding affinity value for vitamin E of -8.2 and beta-bisabolene of -6.9, close to the binding affinity of acarbose of -7.7. The lower the binding affinity value, the more likely the compounds

will bind to the protein target. The good results of these two compounds are supported by the many bonds formed with the amino acids on the receptors (Figure 3.). The gamma-sitosterol compound forms as many as 10 hydrophobic bonds in the form of Pi-Alkyl with the amino acid α-amylase enzyme.

Meanwhile, vitamin E forms as many as 17 hydrophobic bonds, and beta-bisabolene forms 9 bonds with amino acids in α-amylase consisting of Pi-Alkyl and Pi-Sigma bonds. These results indicate that these compounds have the potential to be diabetes drugs that can inhibit α-amylase enzyme activity. The activity of gamma sitosterol and vitamin E as enzyme inhibitors can be associated with their activity as antioxidants so that damage to the β cells responsible for producing insulin can be prevented and cell regeneration can be more effective. As a result, the secretion and sensitivity of insulin in controlling blood sugar levels can increase (Aly and Mantawy, 2012; Gaspersz et al., 2022). Another alternative compared to vitamin E that is commonly found is the beta-bisabolene compound, which also has an excellent binding energy during this docking. Previously, the beta-bisabolene compound had been reported to have antidiabetic activity, which was tested on the DPP-4 receptor in silico. The relatively good binding energy results indicate that this compound has potential as a type 2 diabetes mellitus drug that can handle DPP-4 protein. The results of this previous study support the activity of beta-bisabolene as an antidiabetic, which was tested against the α-amylase enzyme in this study (Sharma et al., 2022).

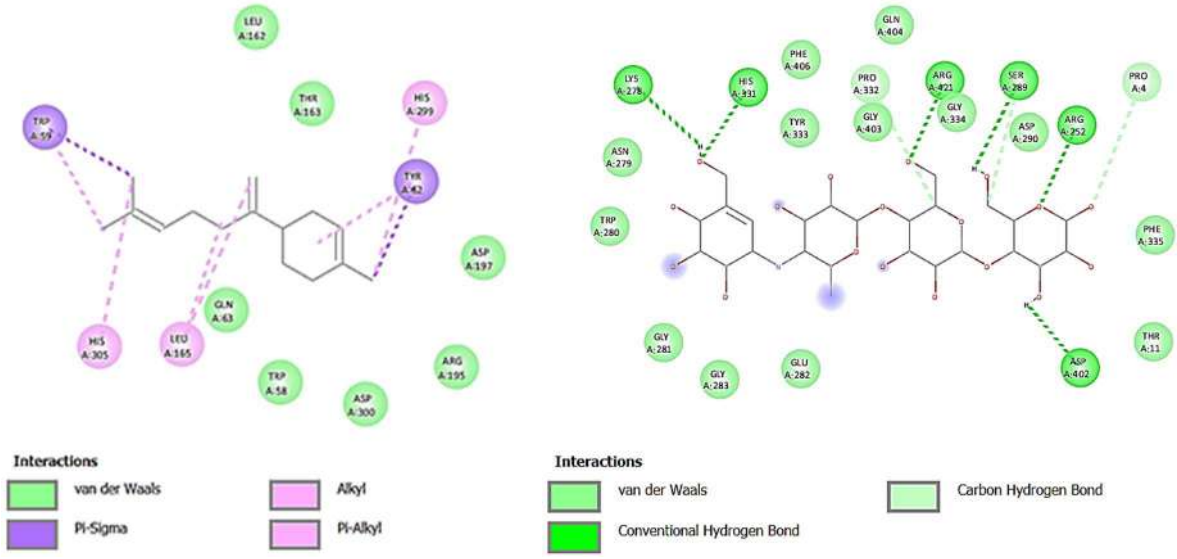
Table 5. Binding affinity of *C. tungurrut* secondary metabolite for 1B2Y Protein.

No	Ligan	Binding affinity (g/mol)
1.	Acarbose	-7,1
2.	Ethyl cinnamate	-6,1
3.	Curcumene	-6,5
4.	Beta-bisabolone	-6,9
5.	Neophytadiene	-5,5
6.	Phytol	-6,0
7.	Palmitic Acid	-5,6
8.	Linoleic Acid	-5,5
9.	Oleic Acid	-5,1
10.	Tetracosane	-5,1
11.	Eicosane	-5,0
12.	3-Methyleicosane	-4,9
13.	Nonacosane	-5,1
14.	Vitamin E	-8,2
15.	Octacosane	-4,8
16.	Heptacosane	-4,9
17.	Gamma Sitosterol	-9,1



(a) (b)

Figure 3. 2D Visualization of 1B2Y receptor docking results with gamma sitosterol (a), vitamin E (b), beta-bisabolene (c), and acarbose (d).



(c) (d)

Figure 4. 2D Visualization of 1B2Y receptor docking results with gamma sitosterol (a), vitamin E (b), beta-bisabolene (c), and acarbose (d) (*Continued*).

4. CONCLUSION

This study concludes that ethanol extract from leaves and ethyl acetate extract of *C. tungurrut* has good inhibitory activity for the α-amylase enzyme. However, a 2-3 times higher concentration is needed for maximum inhibitory activity. The glucose entrapment test showed that the activity of inhibiting glucose diffusion in the ethanol extract of leaves and ethyl acetate extract of *C. tungurrut* stems was better than acarbose. The in-silico antidiabetic potential of *C.*

tungurrut leaf and stem extracts also showed promising results, especially for the compounds gamma-sitosterol and beta-bisabolene, which are natural compounds in *C. tungurrut* plants. Gass Chromatography-Mass Spectrometry (GC-MS) analysis showed that the ethanol extract of leaves and ethyl acetate extract of *C. tungurrut* stems had secondary metabolites dominated by terpenoids, steroids, fatty acids, and alkanes.

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Caesalpinia sappan Reduce Fever with An Anti-inflammatory and Antioxidant Mechanism: A Review

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Abstract

Caesalpinia sappan L (Sappan wood) is an herbal plant that has long been trusted by the public as an herbal medicine for tuberculosis, diarrhea, dysentery, skin infections, anemia, and other diseases by utilizing the decoction of *C.sappan*. Sappan wood is an herbal plant widely used as a raw material for traditional medicinal products. Sappan wood has been reported to have substantial pharmacological effects in analgesic, antioxidant, antibacterial, anti-inflammatory, and anti-viral. Fever is a clinical manifestation of certain conditions or diseases characterized by increased body temperature above the normal range (36.5–37.5 °C). Many studies declare that antioxidant and anti-inflammatory activity in *C.sappan* reduces oxidative stress and pro-inflammatory cytokines. This literature review shows that the antioxidant and anti-inflammatory properties of *C.sappan* are linked to fever as a sign of illness. Literature review using the last ten years' Scopus, ScienceDirect, and Pubmed databases. There are as many as 20 journals regarding sappan wood's antioxidant and anti-inflammatory effects. Sappan wood has been shown to have an antioxidant effect by lowering reactive oxygen species levels via SOD, GPx, or CAT markers. It inhibits inflammation cytokines such as IL-1, IL-6, TNF-**α**, and INF produced during fever. Sappan wood also has an anti-inflammatory effect by inhibiting PGE2 production when someone has a fever. The findings of our review state that *C.sappan* can be used to treat fever for both of these reasons. The use of *C.sappan* as a component in producing traditional health beverages has potential.

Keywords: Anti-inflammatory; Antioxidant; *Caesalpinia sappan;* Fever; Herbs

1. INTRODUCTION

Fever is an adaptive body regulatory response to the activation of the immune system (biological and chemical). Fever is a condition where the body temperature rises above the usual range (36.5-37.5 °C) due to an abnormal reaction of the immune system to infection, injury, or tissue damage (Andrews et al., 2018; Prajitha et al., 2018a). Fever is generally associated with increased hypothalamic prostaglandin E2 (PGE2) levels, which causes an increase in the body temperature set point (Mosili et al., 2020a; Mota and Madden, 2022). Fever can be controlled by using antipyretic and anti-inflammatory drugs. The mechanism of anti-inflammatory activity is by inhibiting cyclooxygenase (COX-1 and COX-2 enzymes), thereby inhibiting the formation of prostaglandins (Drożdżal et al., 2021; Ur Rashid et al., 2019)—fever induced by pyrogen chemicals that can enter the body from the outside or inside. Pyrogens are thermostable

complex polysaccharide molecules containing free radicals. These antioxidants will fight free radicals that enter the human body, causing a chain reaction that can trigger inflammation, cancer, and other degenerative disorders. Some of these can cause fever (Nakamura et al., 2018; Sannasimuthu and Arockiaraj, 2019)

Caesalpinia sappan L (Sappan wood) is a Fabaceae family herbal plant found in Africa, America, and Asia, particularly India, China, and Southeast Asia, and it has various health advantages (Vardhani, 2019). Sappan wood has long been used as an herbal remedy for tuberculosis, diarrhea, dysentery, skin infections, and anemia using a sappan wood decoction. Furthermore, people are becoming more interested in drinking boiled sappan wood since it promotes blood circulation, increases energy, and reduces aging (Rajput et al., 2021). Sappan wood has analgesic, antioxidant, antibacterial, anti-inflammatory, anti-viral, anti-complementary, anti-convulsant, spasmolytic activity, hepatoprotective properties, anti-platelet concentration, antioxidative antioxidative stress, anticancer effects, and other activities (Wang et al., 2019). Brazilin ($C_{16}H_{14}O_5$), brazilein ($C_{16}H_{12}O_5$), resorcin ($C_6H_6O_2$), 3'-O-methylbrazilin, sappanon A ($C_{16}H_{12}O_5$), chalcone ($C_{15}H_{12}O$), and sappanchalcone ($C_{15}H_{12}O_5$) are among the bioactive chemicals (Vu et al., 2020). Fever treatment is still dominated by synthetic medications, which might induce adverse drug effects. The utilization of medicinal plants is one medication development technique.

No study has examined how antioxidants and anti-inflammatory affect sappan wood's fever process. Fever is a sign of almost all infections and immune system diseases, so this review is essential. Sappan wood makes health drinks and traditional medicines to give scientific knowledge.

2. MATERIAL AND METHODS

Various journals covered the period a little more than ten years ago. PubMed, Scopus, and ScienceDirect are the providers of the databases that we consult. “Sappan wood”, “*Caesalpinia sappan L*”, “fever”, “sappan wood components”, “*Caesalpinia sappan L* for human health”, and “antioxidant activity” were some keywords used in this study—*Caesalpinia sappan L*, anti-inflammatory activity *Caesalpinia sappan L*. The scope of all searches was limited to publications in the English language. Journal selection criteria include things like (a) clinical studies of *C. sappan L* extract and compounds of fever, (b) anti-inflammatory and antioxidant effects on *Caesalpinia sappan L*, and (c) the effect of sappan wood on MDA (malondialdehyde), SOD parameters (superoxide dismutase), CAT (catalase), GST (glutathione S-transferase), and GSH-P (glutathione peroxidase). Twenty articles were included in the analysis (Figure 1).

3. RESULTS AND DISCUSSION

3.1. Fever

Fever is an adaptive body regulatory reaction that occurs in response to the activation of the immune system (biological and chemical). Fever is when the body's internal temperature rises above its normal range (36.5-37.5°C) due to an impaired immune system response to

infection, injury, or tissue damage (Sasongko et al., 2019). Fever can be caused by several things, including infections, injuries, and tissue damage (Prajitha et al., 2018b). An increase in prostaglandin E2 (PGE2) levels, which then bind to prostaglandin EP3 and are produced in the hypothalamus, causing fever and an increase in body temperature set point, is what causes fever. Fever is induced by increased prostaglandin E2 (PGE2) levels (Mosili et al., 2020b). The three clinical phases that make up a fever are chills, fever, and a flush. This fever is a clinical symptom of at least one illness or disease, possibly more than one (Ohemeng et al., 2018). There are three distinct varieties of fever, each of which is designated by its underlying cause: infectious fever, non-infectious fever, and physiological fever. In 2019, the COVID-19 pandemic occurred, which was caused by the body being attacked by the coronavirus. The clinical manifestations of this illness are comparable to those of the flu and may be accompanied by a high temperature (Cann, 2021). According to the World Health Organization (WHO), a high temperature, which can induce shortness of breath, is one of the most prominent symptoms of COVID-19. Exogenous pyrogens encourage the production of pyrogenic cytokines or endogenous pyrogens, both of which work in the hypothalamus with the assistance of the cyclooxygenase 2 (COX-2) enzyme to produce prostaglandin E2, which raises the set point for the amount of fever (Wilhelms et al., 2014). As an anti-inflammatory agent, natural components or traditional medicine can be used. Sappan wood is an empirically utilized plant component to preserve health and medicine.

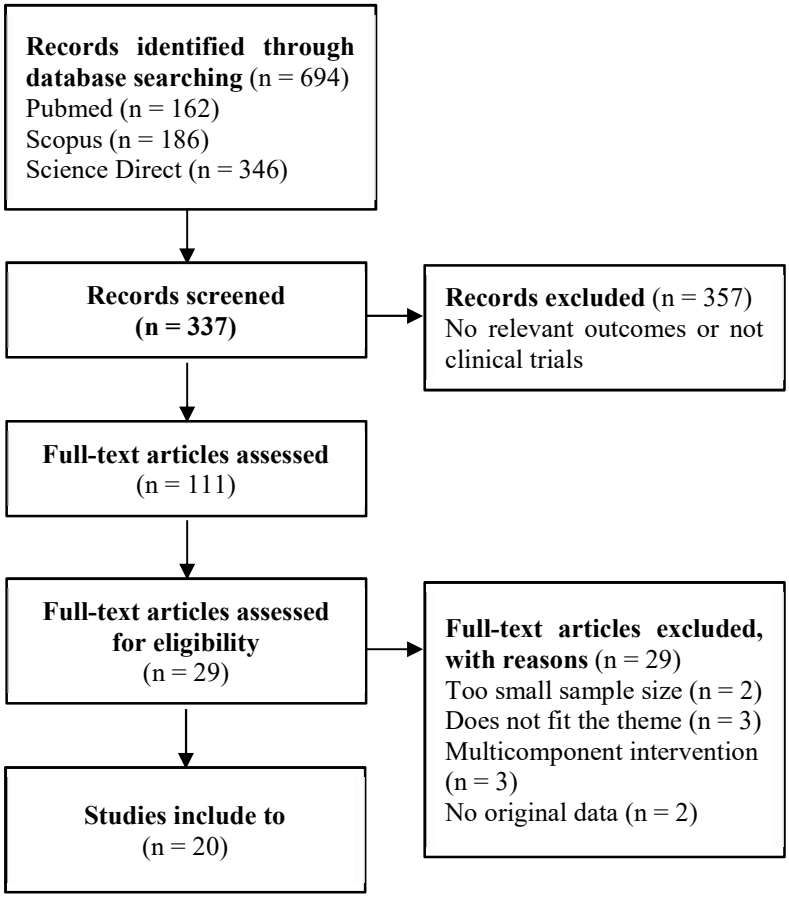


Figure 1. PRISMA flow diagram of the study selection process.

Fever can be a symptom of several conditions, including infections, inflammations, the effects of drugs, the effects of immunization, autoimmune diseases, and cancer (Figure 2). Temperature elevation, or fever, is one of the most typical signs of an infection or inflammation. Fever is caused by the binding of pro-inflammatory cytokines to receptors on brain endothelial cells, which then promotes the production of PGE2. It happens when an infection or inflammation takes place (Figure 3). The production of fever is caused by the binding of prostaglandin E2 in the preoptic hypothalamus (Pasikhova et al., 2017). Drug fever is defined as a fever that starts with administering a drug and fades after the drug is discontinued when no other cause of the fever can be determined after a thorough physical examination and adequate laboratory testing (Kurita et al., 2020).

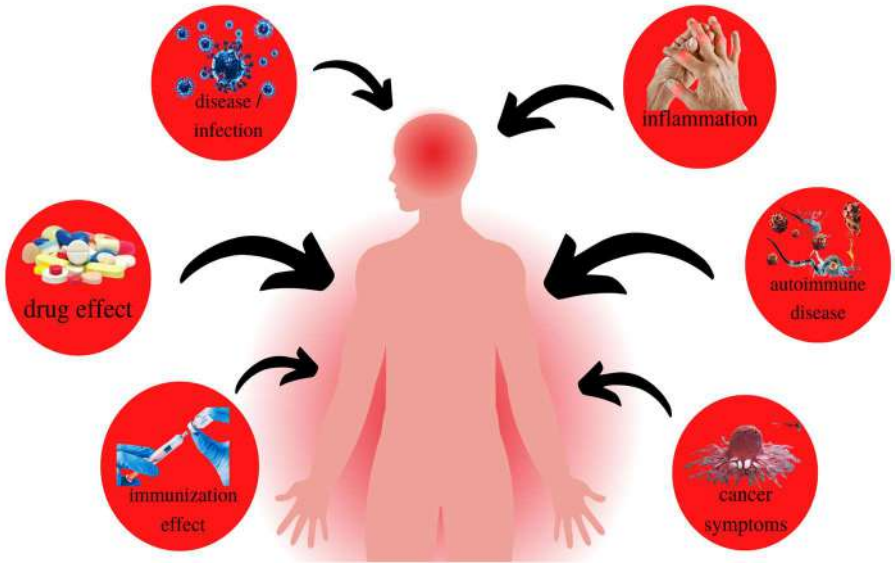


Figure 2. Several causes of fever in the body (Paquet et al., 2022; Peluso et al., 2021; Smid et al., 2018).

After receiving immunization, some people experience a well-recognized adverse effect known as fever. Some evidence suggests that infants receiving all three routine 2-month immunizations at once rather than in 2 or 3 doses have an increased risk of developing a fever after receiving the immunization (DeMeo et al., 2015). The term "non-infectious inflammatory diseases" refers to disorders that include various conditions, the most well-known being autoimmune diseases. Traditionally, it was believed that autoimmune disorders induced antigen-specific pro-inflammatory mechanisms, which could later lead to the development of fever (Kallinich et al., 2013). Fever in cancer patients can generally be connected with infectious or non-infectious, depending on the cause. Neoplastic, drug-induced, and venous thromboembolism are three significant factors that might contribute to fever in patients with hematological or solid-tumor malignancies (Pasikhova et al., 2017).

Sappan wood (*Caesalpinia sappan L*) is a herbal plant widely used and processed into traditional medicinal remedies. Existing reviews focus on the pharmacological activity of sappan wood's principal components, brazilin, and numerous additional chemicals. No studies have been published on the efficacy of sappan wood (*Caesalpinia sappan L*) as a fever therapy.

As a result, it is necessary to reread the article to learn about the benefits of sappan wood (*Caesalpinia sappan L*) in decreasing fever. Based on the research data, this article review aims to demonstrate that sappan wood has anti-inflammatory and antioxidant pharmacological activity as a fever reliever.

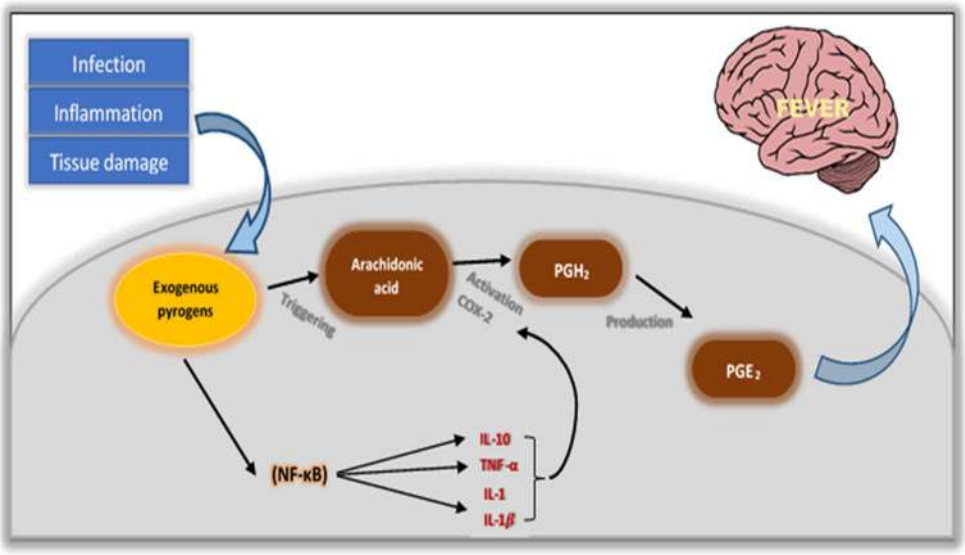


Figure 3. Mechanism of fever with expression of inflammatory markers (Prajitha et al., 2018a; Walter et al., 2016; Wrotek et al., 2020).

3.2. Sappan wood *(Caesalpinia sappan L.)*

Caesalpinia sappan is a member of the Fabaceae family and can be found in many different parts of the world, including India, China, Southeast Asia, Africa, and the Americas. Sappan can be found in various countries in the Southeast Asian region, including Indonesia, Malaysia, Thailand, Laos, and the Philippines (Vardhani, 2019). The sappan plant is harvested for the stem's heartwood, characterized by a round shape and a hue that falls between brown and green. This decoction of sappan wood shavings or pieces will create a natural red shade due to the presence of brazilin compounds; hence, sappan wood is frequently used as a natural dye (Sakti et al., 2019). By utilizing a decoction of this sappan wood, Southeast Asian people have relied on sappan wood for a very long time as a therapy for TB, diarrhea, dysentery, skin infections, and anemia. In addition, sappans have been known to treat anemia. Stomach ulcers, epilepsy, and diabetes were all treated with medicinal plants used by Indian society. In addition, the Chinese employ sappan wood to cure various illnesses, including obesity, syphilis, heart disease, ear and eye problems, and cancer (Nirmal et al., 2015).

3.3. Biochemical compounds in *Caesalpinia sappan L.* (Sappan wood)

Sappan wood has been shown to have analgesic, antioxidant, antibacterial, anti-inflammatory, anti-viral, anti-complementary, anti-convulsant, spasmolytic activity, hepatoprotective properties, anti-platelet concentration, antioxidative stress, anticancer effects, and other pharmacological effects (Wang et al., 2019).

Table 1. Bioactivity compounds, benefits, and structure of sappan wood.

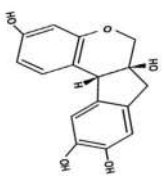
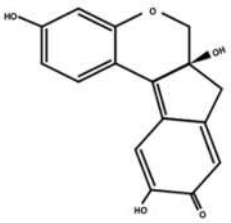
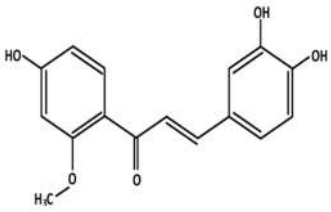
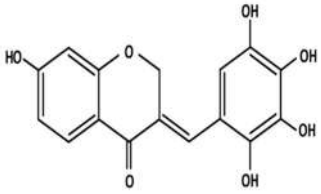
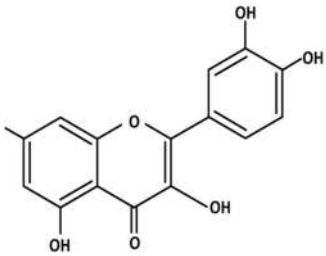
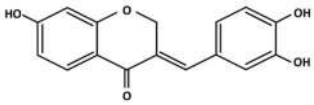
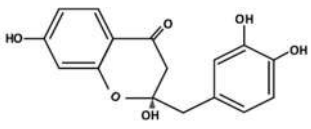
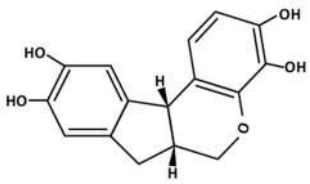
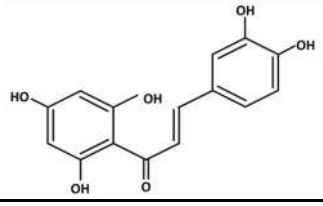
Compounds	Chemical Structure	Benefit	References
Brazilin ($C_{16}H_{14}O_5$)		Antioxidant, antibacterial, anti-inflammatory.	(Nirmal et al., 2015)
Brazilein ($C_{16}H_{12}O_5$)		Immunosuppressive agent in mice lymphocytes, cardiotonic effects in isolated rat hearts, and antioxidant properties, antitumor.	(Liang et al., 2013a; Zanin et al., 2012)
Sappanchalcone ($C_{15}H_{12}O_5$)		An anti-inflammatory, neuroprotective, inhibition of antigen-induced beta-hexosaminidase release, anti-influenza viral.	(Vu et al., 2020)
Caesalpiniaphenol		Inhibition and induction of apoptosis in human promyelocytic cancer cells.	(Vu et al., 2020)
Kuersetin ($C_{15}H_{10}O_7$)		Antioxidants, anti-inflammatory, antibacterial, anti-viral, radical scavenging, gastroprotective, and immune-modulatory activities.	(Vu et al., 2020)
Sappanone A		Anti-inflammatory, Antioxidants.	(Kang et al., 2016)
Sappanone B		Antitumor.	(Syamsunarno et al., 2021)
Hematoxylin		Antibacterial, antivirus, antitumor, inhibitor of Aβ42 fibrillogenesis.	(Tu et al., 2016)

Table 1. Bioactivity compounds, benefit, and structure of sappan wood (*Continued*).

Compounds	Chemical Structure	Benefit	References
Butein		Antioxidants, antitumor, anti-inflammatory, protein kinase inhibition	(Y.-N. Chen et al., 2012)

3.4. Antioxidants activity in *Caesalpinia sappan L.* (Sappan wood)

Including flavonoids and phenolics, have been shown to have high antioxidant activity. The antioxidant activity of timber is also regulated by age; stem extracts aged two years show higher antioxidant activity than extracts of fresh branches. A compound's antioxidant activity is mediated by numerous processes, including the prevention of chain initiation, the binding of transition metal ion catalysts, the prevention of hydrogen separation, the breakdown of peroxides, and the scavenging of free radicals that cause fever (Nirmal et al., 2015). Sappan wood contains many antioxidants, including Brazilin, Brazilein, Quercetin, Protasappanin, Sappanone A, and Butein.

3.4.1. Brazilin and brazilein

Brazilin is a homoisoflavonoid with a chemical structure that dissolves in water and causes it to turn red (Romruen et al., 2022a). When oxidized, the brazilin compound is in the form of yellow crystals and will produce the brazilein compound ($C_{16}H_{12}O_5$). Brazilein demonstrated the most potent free radical scavenging and iron reduction activities compared to other compounds such as sappanchalcone, protosappanin B, and protosappanin C. Furthermore, after the isolation of *Caesalpinia sappan L* from Indonesia, brazilin will suppress peroxide generation. The dibenzoxocin structure of this brazilin molecule allows it to effectively perform radical scavenging activities (Nirmal et al., 2015).

3.4.2. Quercetin

Quercetin is a flavonoid component that has the chemical structure of $C_{15}H_{10}O_7$. It is a compound commonly used as a supplement in food and beverages. Quercetin can be found in a variety of plants. Quercetin has been shown to have potent antioxidant activity and can efficiently scavenge free radicals and metal ions. Quercetin will produce GSH, and then superoxide dismutase will convert oxygen into H_2O_2. In addition, GSH and H_2O_2 will be catalyzed by GSH-Px, which will ultimately decrease peroxides' production. Quercetin's ability to regulate GSH levels will increase the antioxidants' therapeutic effects on the body (Kong et al., 2022).

3.4.3. Protasappanin

Oxidative stress is a condition that occurs when there is an imbalance between the number of oxidants (free radicals) and antioxidants in the body, causing damage to a chain beginning with cells and progressing to a higher level. Free radicals are highly reactive and unstable

entities that prefer to steal electrons from other compounds (oxidation). Nitrogen derivatives known as reactive nitrogen species (RNS) and oxygen derivatives known as reactive oxygen species (ROS) are two types of free radicals found in the body. ROS can be O2, hydroxyl radicals (OH), hypochlorous acid (HOCL), alkoxyl radicals, or peroxyl radicals. ROS can damage cells by causing lipid membrane damage through a sequence of chemical processes known as lipid peroxidation. Protosappanins have been demonstrated to have a considerable effect in suppressing the formation of ROS and NO by stimulating microglial cells in the CNS, allowing them to provide antioxidative effects on brain immunology and neuroinflammation affected by CD14 (Zeng et al., 2012).

3.4.4. Sappanone A

Sappanone A is a homoflavonoid found to have a substantial antioxidant impact in cases of ischemia or stroke, meaning it can protect brain function. This ability has been proven through scientific research. In addition, studies have shown that Sappanone A can exert antioxidant effects even in the presence of cardiomyocyte damage by inhibiting apoptosis in mitochondria. In addition, it has been proven that increased levels of the antioxidant enzyme SOD cause sappanone A to decrease the generation of ROS and MDA. Reducing ROS levels helps prevent the development of oxidative stress, which can cause cell damage (M. Wang et al., 2021).

3.4.5. Butein

Butein is a chemical that belongs to the hydroxychalcone family and can have an antioxidant impact. Butein, as an antioxidant agent, reduces lipid peroxidation and the production of superoxide anions, thereby protecting cells from free radical cytotoxicity. Additionally, this butein will suppress the generation of ROS, which is a factor that can lead to oxidative stress. Oxidative stress is the primary factor that can lead to several chronic diseases (Padmavathi et al., 2017).

3.5. Anti-inflammatory activity in *Caesalpinia sappan L.* (Sappan wood)

Flavonoids and phenolic compounds are two substances that work well as anti-inflammatory drugs (Sasongko et al., 2016); (Sasongko, 2017). Histamine, prostaglandins, kinins, and 5-HT receptors are a few of the inflammatory mediators this anti-inflammatory function inhibits. Due to excessive prostaglandin induction, the body has responded with a fever. *As demonstrated by various studies, Caesalpinia sappan* L. has a pharmacological role as an anti-inflammatory agent (Figure 4). Several substances, such as Bazilin, Sappanchalcone, Quercetin, Protosappanin, Sappanone A, and Butein, are known to have anti-inflammatory properties (Javed et al., 2020).

3.5.1. Brazilin

The anti-inflammatory impact of brazilin was the most potent, with a considerable suppression of IL-6 secretion down to 5 μg/ml-1 and a significant inhibition of TNF-α secretion

down to 10 µg/ml (Mueller et al., 2016). If brazilin is administered at a low dose, such as 0.1 µg/mL, it can potentially have an anti-inflammatory effect approximately equivalent to a 50% inhibition. Additionally, brazilin inhibits the process of iNOS and Heme Oxygenase-1 gene expression, which contributes to its anti-inflammatory properties. The production of prostaglandin E2, an inflammatory mediator, can also be inhibited by brazilin, which likewise inhibits the COX-2 enzyme (Nirmal et al., 2015).

3.5.2. Sappanchalcone

Sappanchalcone is recognized to have pharmacological effects as an anti-inflammatory drug and is one of the active flavonoid compounds derived from *Caesalpinia sappan* L. Sappanchalcone is one of the flavonoid compounds that *Caesalpinia sappan* L. produce In the same way that brazilin does, sappanchalcone acts as an anti-inflammatory agent by blocking the formation of prostaglandin E2. Prostaglandin E2 is one of the inflammatory mediators that contribute to fever. Sappanchalcone does this by suppressing the activity of the COX-2 enzyme (Jeong et al., 2010).

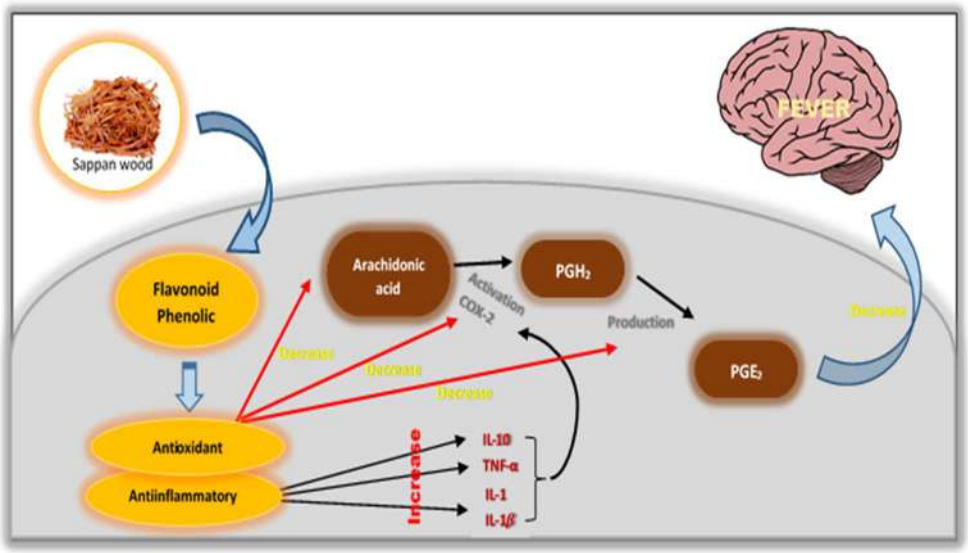


Figure 4. Mechanism of sappan wood in a fever (Azab et al., 2016; Kooti and Daraei, 2017; Xu et al., 2019).

3.5.3. Quercetin

Quercetin is a flavonoid compound found in many plants, one of which is *Caesalpinia sappan L*. Quercetin is a compound often used in the food and pharmaceutical industry because of its pharmacological effects. Quercetin is known to provide good progress when used as a treatment for COVID-19. *In vitro* testing stated that quercetin is anti-inflammatory by inhibiting iNOS and NO production. *In vivo* testing of quercetin showed an anti-inflammatory effect in diabetes-induced rats. Tests on human cells showed that quercetin provides an anti-inflammatory effect by inhibiting the activity of the COX-2 enzyme (Septembre-Malaterre et al., 2022). Quercetin can regulate inflammation and exert potent anti-inflammatory effects. It has been tested on several animals and human cell types (Chunlian Tian et al., 2021).

3.6. Antioxidant and anti-inflammatory parameters

Antioxidants have several parameters that can be used to determine their presence. Antioxidant parameters include Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Glutathione Reductase (GRx), Catalase (CAT), Malonyl Dialdehyde (MDA), Glutathione Peroxidase (GSH-Px), Myeloperoxidase (MPO), and also Xanthine Oxidase (XO) (Lu et al., 2021; Urfalioglu et al., 2021). Of these parameters, some of them are included in enzymatic systems such as GSH-Px, SOD, GRx, and CAT. In the enzymatic system, GSH-Px plays an essential role in antioxidants by protecting intracellular phagocytic cells. In addition to enzymatic systems, there are non-enzymatic systems such as GSH, melatonin, vitamin A, vitamin E, and ascorbic acid. GSH plays a vital role in this system by neutralizing harmful compounds and reducing oxidative stress (Derindağ et al., 2021).

The anti-inflammatory parameters used were lipooxygenase, cyclooxygenase, Interleukin-1β (IL-1β), Nitric Oxide (NO), Malondialdehyde (MDA), Tumor Necrosis Factor-α (TNF-α), Cyclooxygenase-2 (COX-2), and Nuclear Factor kappa B (NF-κB) (Xie et al., 2022). Lipooxygenase and cyclooxygenase are enzymatic pathways in arachidonic acid metabolism. In the lipooxygenase pathway, there are enzymes, including LOX-5, LOX-8, LOX-12, and LOX-15 enzymes and their products, leukotrienes (LTA4, an unstable intermediate, LTB4, LTC4, LTD4, and LTE4), lipoxins (LXA4 and LXB4 formed upon LXA4 degradation) and 8–12- 15- hydroperoxy-eicosatetraenoic acid (HPETE). In the cyclooxygenase pathway, there are several enzymes, such as COX-1 and COX-2, along with downstream enzymes that mediate the production of prostaglandins (PGH2, an unstable intermediate, PGE2, PGD2, and PGF2alpha, prostacyclins (PGI2), and thromboxanes (TXA2, TXB2) (Hanna and Hafez, 2018).

Sappan wood has pharmacological effects such as antioxidants and anti-inflammatories derived from its flavonoid compounds. Both of these effects have great potential to reduce fever. Fever occurs due to an increase in PGE2. Antioxidants in sappan wood can inhibit PGE2 production through the COX-2 pathway. These antioxidants play an essential role in the body, reducing oxidative processes and the effects of reactive oxygen species (ROS) (Gulcin, 2020). Determine the activity of this antioxidant can be done using several methods such as 2,2′-azinobis-(3- ethylbenzothiazoline- 6- sulphonate) radical (ABTS•+) scavenging, 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radical scavenging, $Fe^{3+}Fe^{2+}$transformation assay, ferric reducing antioxidant power (FRAP) assay, cupric ions (Cu^{2+}) reducing power assay (Cuprac), Folin–Ciocalteu reducing capacity (FCR assay), peroxyl radical (ROO•), superoxide radical anion (O2•−), hydrogen peroxide (H_2O_2) scavenging assay, hydroxyl radical (OH•) scavenging assay, singlet oxygen (O_2) quenching assay, nitric oxide radical (NO•) scavenging assay and chemiluminescence assay (Gulcin, 2020; Csepregi et al., 2016; Mendonça et al., 2022).

Table 2. Antioxidant and anti-inflammatory activity in *Caesalpinia sappan L.* Description: ↓ = *significant decrease*, ↑ = *significant increase.*

Pharmacological Activity	References	Animals/cells tested	Methods	Result
Antioxidant	(Masturi et al., 2021)	Comprises with phytochemical assessments	DPPH method	↑ The IC_{50} values in Extract Sappan Wood
	(Kadir et al., 2014)	Adult male healthy *Sprague Dawley* (SD) rats weighing 180–200 g	Western blot analysis and detection of endogenous antioxidant enzymes	↓ Catalase (CAT) ↓ Superoxide Dismutase (SOD) ↓ Glutathione Peroxidase (GPx)
	(Suwan et al., 2018)	Two aerobic bacterial strains, Staphylococcus aureus and Escherichia coli	Ferric Reducing Antioxidant Power (FRAP) assay, 2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH)	↑ The free radical scavenging in FRAP assay ↑ The free radical scavenging in ABTS ↑ The free radical scavenging in DPPH
	(Nirmal and Panichayupakaranant, 2015)	-	DPPH radical scavenging, Reducing power, and β-carotene bleaching assays	Reducing power ↑Brazilin-Rich Extract (BRE) and brazilin β-carotene bleaching assays ↑Brazilin-Rich Extract (BRE) and brazilin
	(F.-Z. Chen et al., 2014)	Vitamin C as control positif	DPPH assay	Brazilin > Neoprotosappanin > Vitamin C
	(Hwang and Shim, 2018)	Normal human epidermal keratinocytes	DPPH assay	↓ SOD1 ↓ SOD2 ↓ CAT ↑ GPX7
	(Khristian et al., 2022)	Male and female rats	Number of Kuffer cells	↓ Kupffer cells in the female group at a dose of 100 mg/kgBW and the male group at 200 mg/kgBW
	(Arsiningtyas, 2021)	Ascorbic acid, as the control positive	DPPH radical scavenging activity	↑ IC_{50} value from sapwood and heartwood on a branch, middle, and primary *C. sappan* wood

Table 2. Antioxidant and anti-inflammatory activity in *Caesalpinia sappan L.* Description: ↓ = *significant decrease*, ↑ = *significant increase* (*Continued*).

Pharmacological Activity	References	Animals/cells tested	Methods	Result
	(Liang et al., 2013b)	Human epidermoid carcinoma, human oral squamous cell carcinoma, human skin malignant melanoma, mouse leukemic monocyte macrophage, and mouse normal embryonic liver cells	DPPH and ABTS	↑ The EC_{50} values of brazilein for the scavenging of DPPH ↑ The EC_{50} values for the scavenging of ABTS
	(Romruen et al., 2022b)	-	Total Phenolic Content (TPC), DPPH radical scavenging activity, and Ferric Reducing Antioxidant Power (FRAP)	↑ The film's TPC values with ↑ sappan heartwood extract concentration ↑ The film's DPPH values with ↑ sappan heartwood extract concentration ↑ The film's FRAP values with ↑ sappan heartwood extract concentration
	(Khristian et al., 2022)	Male and female rats	Number of Kuffer cells	↓ Kupffer cells in the female group at a dose of 100 mg/kgBW and the male group at 200 mg/kgBW
	(Widodo et al., 2022)	Lung cancer cell	DPPH radical scavenging activity	↑ IC_{50} value from *Caesalpinia sappan* ethanol extract

Table 2. Antioxidant and anti-inflammatory activity in *Caesalpinia sappan L.* Description: ↓ = *significant decrease*, ↑ = *significant increase* (*Continued*).

Pharmacological Activity	References	Animals/cells tested	Methods	Result
Anti-inflammatory	(Pattananandecha et al., 2022)	Macrophage cells	Anti-inflammatory activity through an inhibition effect on nitric oxide (NO) and inducible nitric oxide synthase (iNOS) production	↓ NO production and Regulating Nuclear Factor kappa-B (NF-κB) and activator protein-1, ↓ COX-2, and iNOS expression.
	(Tewtrakul et al., 2015)	Male and female Swiss albino mice (30–40 g)	The inhibitory activity against NO, PGE2, and TNF-α.	↓ mRNA expressions of the iNOS, COX-2, and TNF-α ↓ PGE2 and TNF-α
	(K.-J. Kim et al., 2015)	Macrophage cells	Western blotting and cytokine assay	↓ iNOS and COX-2 protein significantly with the presence of brazilein.
	(Gao et al., 2015)	Mammary gland and mammary epithelial cells	Cytokine assays	Attenuated inflammatory cell infiltration ↓ The expressions of TNF-α, IL-1β and IL-6
	(C.-L. Chen and Zhang, 2014)	Macrophage cells	Cell viability by MTT assay	↓ over 90% NO production
	(Wu et al., 2011)	Primary cells, chondrocytes, and THP-1 macrophages	Griess assay and ELISA	↓ pro-inflammatory cytokines IL-1β and TNF-α suppressed the synthesis of NO blocking iNOS mRNA expression. The inhibition of COX-2 transcription
	(Jung, Han, Kwon, et al., 2015)	Male DBA/1J mice	A mouse immunoassay kit	↓ The blood serum levels of TNF-α, IL-1β, and IL-6
	(J.-H. Kim et al., 2014)	Macrophage cells	Measurement of NO and IL-6 and cell viability assay	↓ Production NO dan IL-6

Table 2. Antioxidant and anti-inflammatory activity in *Caesalpinia sappan L.* Description: ↓ = *significant decrease*, ↑ = *significant increase* (*Continued*).

Pharmacological Activity	References	Animals/cells tested	Methods	Result
	(Jung, Han, Hwang, et al., 2015)	Male DBA/1J mice	Serum biochemical analysis	Brazilin markedly attenuated mouse CIA and ↓ the serum levels of inflammatory cytokines, including TNF-α, IL-1β, and IL-6.

Most of the compounds in sappan are flavonoid compounds. The antioxidant action mechanisms of flavonoids include (a) direct scavenging of ROS, (b) inhibition of ROS formation through the chelation of trace elements or inhibition of the enzymes that participate in the generation of free radicals, and (c) activation of antioxidant defenses in addition to upregulation of antioxidant enzymes with radical scavenging ability. Glutathione S-transferase, microsomal monooxygenase, mitochondrial succinodidase, NADH oxidase, and xanthine oxidase are some of the enzymes that can be inhibited to prevent the generation of free radicals (Dias et al., 2021). Compounds derived from sappan wood can, as antioxidants, prevent the activation of COX-2 and NF-B activation, particularly in brazilein compounds (Aldini et al., 2018; Handayani et al., 2017).

A common trigger for inflammation is the presence of pathogenic microbes like bacteria, viruses, or fungi in the host body, localized tissue, and the bloodstream. Tissue damage, cell death, malignancy, ischemia, and degeneration can all trigger inflammatory responses (L. Chen et al., 2017). The B and T cells of the adaptive immune system are responsible for creating specific receptors and antibodies to destroy invading infections and cancer cells (Azab et al., 2016). Initiating an inflammatory response is dependent on several factors, including macrophage inflammatory protein-2 (MIP2), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), nitric oxide (NO), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) (K.-J. Kim et al., 2015)

Tumor necrosis factor (TNF)- α is a critical pro-inflammatory cytokine released by many different types of cells and has many effects on them (Abdulkhaleq et al., 2018; Zappavigna et al., 2020). IL-6 is usually a cytokine that worsens inflammation but can also help reduce inflammation. IL-10 is a strong anti-inflammatory cytokine that stops many pro-inflammatory mediators from doing their jobs. Attenuation and control of the inflammatory response: IL-10 helps keep tissue homeostasis and reduces damage from an overactive inflammatory response (Azab et al., 2016).

Prostaglandin E2 regulates body temperature, gastrointestinal mucosa integrity, renal blood flow, and female reproductive function—phospholipase A2 forms arachidonic acid from cell membrane phospholipids (PLA2). COX converts arachidonic acid to PG. The inducible COX-2 enzyme is the most active during inflammation (Azab et al., 2016). Nitric oxide

synthase (NOS) is another inflammatory enzyme (NO). Inducible NOS (iNOS) is pro-inflammatory like COX-2 (Zappavigna et al., 2020). The nuclear transcription factor kappa B (NFB) is crucial in immunological and inflammatory responses and cancer pathogenesis. When NFB is not being activated, it is found in the cytoplasm. The nuclear factor kappa B (NFB) protein relocates to the nucleus after being activated by various infectious/ inflammatory/ mitogenic stimuli. Then, it stimulates the transcription of inflammation-related genes (Azab et al., 2016).

Flavonoids can play multiple roles in the inflammatory response, including (a) antioxidants scavenging reactive oxygen species (ROS) or reducing free radical accumulation, (b) inhibitors of the activity of regulatory enzymes (e.g., protein kinases and phosphodiesterase) and transcription factors related to the control of mediators involved in the inflammatory response, and (c) modulators of the activity of the immune cells (e.g., inhibition of cell activation, maturation, signaling transduction, and secretory processes) (Dias et al., 2021; Maleki et al., 2019). Sappan wood contains compounds that have been shown to limit NO production and drastically decrease iNOS and COX-2 protein expression (K.-J. Kim et al., 2015). The production of interleukin-6 (IL-6) is suppressed by several biochemical compounds, including neoprotosappanin, protosappanin E, sappanone A, and two additional compounds whose identities (Chu et al., 2013).

The glucocorticoid system is particularly important in fever. Activator protein-1 and nuclear factor-kappa B are the mediators in this system (AP-1). Both have been shown to inhibit the production of pro-inflammatory cytokines. The body's response to fever involves regulating IL-1 receptor antagonists (IL-1RA), IL-10, and TNF-binding proteins, all of which reduce fever (Young and Saxena, 2014). Antioxidants and anti-inflammatories present in secang wood components have the same effect in lowering fever. Pro-inflammatory cytokines are decreased by antioxidants and anti-inflammatories, respectively.

COVID-19 is one of the infectious diseases that has rapidly spread over the planet and turned into a pandemic after 2019 came to a close (Cann, 2021). The symptoms of the disease produced by the SARS-CoV-2 virus are almost identical to those caused by the flu. These symptoms include fever, a cough, and asthenia (Shi et al., 2020). This viral infection has the potential to stimulate the local inflammatory response, which in turn has the potential to enhance the expression of cytokines such as transforming growth factor- β1 (TGF- β1), tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1β), IL-6, interferon (IFN)- γ, IL-1, and IL-2 (Pascarella et al., 2020; Shi et al., 2020).

An increase in TNF- α, IL-1, and IL-1 β (Figure 3) can activate COX-2, leading to an increase in the production of PGE-2 (Azab et al., 2016). This, in turn, will cause the set point to rise, resulting in fever. Sappan wood's anti-inflammatory properties can inhibit cytokine synthesis, preventing COX-2 activation and allowing for cooler internal temperatures. Although this anti-inflammatory impact cannot destroy the virus, it can lower the infection's symptoms. It is necessary to have an anti-viral agent that works efficiently in order to kill the virus.

4. CONCLUSION

Sappan wood can be an alternative for reducing fevers with its anti-inflammatory and antioxidant pharmacological effects. The flavonoid compounds produce this effect in sappan wood. Antioxidants in sappan wood inhibit oxidative stress and inflammatory mediators in the form of PGE2. Inhibition of these pathways will prevent further activation of the inflammatory response. In addition, sappan wood can be used as an ingredient in traditional health drinks. Using sappan wood to reduce fever can be done by drinking sappan wood boiled water. It is possible to complete the process of boiling sappan wood in at most forty-five minutes so that the properties it contains are maintained.

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CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

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Effect of Papaya Seeds *(Carica papaya)* and Gandarusa Leaves (*Justicia gendarussa)* on the Spermatozoa Quality of Male Mice

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Abstract

Papaya seeds (*Carica papaya* L.) and gandarusa leaves (*Justicia gendarussa* Burm.f.) are considered medicinal plants that can be used as natural male contraceptives. Papaya seeds contain alkaloids, triterpenoids, saponins, and papain. Meanwhile, the gandarusa leaves contain flavonoids. These compounds have the function of antifertility for men. The purpose of this study was to determine the effect of papaya seeds (*Carica papaya* L.) ethanol extracts, gandarusa leaves (*Justicia gendarussa* Burm.f.), and its combination on motility, viability, and morphology of male mice spermatozoa (*Mus musculus)* most effective decrease concentration on motility, viability, and morphology of male mice spermatozoa (*Mus musculus*). This research was an experimental study. The samples of this study were 18 male mice with a body weight of 20-30 grams, aged 3-5 months, and were divided into six groups. The treatments given were control, papaya seed ethanol extract, gandarusa leaves ethanol extract, and its combinations to concentrations of P1 Na CMC1%, P2 (papaya seed 100mg/kgW), P3 (gandarusa leaf 50mg/kgW), P4 (papaya seed : gandarusa leaf 50:50 mg/kgW), P5 (papaya seed : gandarusa leaf 50:100 mg/ kgW), P6 (papaya seed : gandarusa leaf 100:50 mg/kgW). The extract was given for 35 days. On the 36th day, the mice were dissected to take their epididymis to observe the spermatozoa's quality (motility, viability, morphology). Those observations used a light microscope. Data were analyzed using one-way ANOVA and LSD post hoc tests. This study resulted differences in P1, P2, P3, P4, P5, and P6 towards motility, viability, and morphology of spermatozoa ($p< 0.05$). The conclusion of this study showed that both single and combination ethanol extracts of papaya seeds and gandarusa leaves could reduce the motility, viability, and morphology of spermatozoa of male mice, and the effective dosage in reducing motility, viability, and morphology of spermatozoa is P3 gandarusa leaves 50mg/kgW.

Keywords: Ethanol extract; Gandarusa leaves; Papaya seeds; Spermatozoa

1. INTRODUCTION

The high urban population growth rate in developing countries has caused many problems. The most obvious problems are the uncontrolled growth rate and the increase in poverty rates in many cities. Therefore, the government has planned family planning programs (*KB*) to overcome this problem (Margono, 2013).

Family planning (*KB*) is an effort to control childbirth, ideal pregnancy interval and age pregnancy, natality, and control pregnancy through protection and assistance promotion according to reproductive rights to create a good quality family. Family planning allows couples of childbearing age to prevent pregnancy, plan the number of their children, and measure the interval of births (Dewi and Rahmawati, 2019)

Indonesia is implementing the family planning program (KB). It is used to achieve a thriving and healthy family. Data from the National Population and Family Planning Agency (*BKKBN*) shows that female contraceptive methods used (93.66%) are much higher than male contraceptive methods (6.43%). It shows that men's participation in contraceptive methods is minimal, and women still dominate contraception use (Walansendow *et al*., 2016).

Moreover, it has been found that 18 types of medicinal plants in Indonesia have the potential to be male antifertility (Febrianti and Sukarjati, 2015). It is a plant that contains alkaloids, which can affect spermatogenesis and suppress the secretion of reproductive hormones. Flavonoids can reduce the motility and viability of spermatozoa (Laili, 2018). Indian people use gossypol and papaya (Carica papaya) for male contraception (Lohiya et al. 2015). Giving papaya seed extract can cause infertility with reduced fertility, decreased sperm count, and motility (Pathak et al. 2018).

One medicinal plant with alkaloids and flavonoids that can affect sperm quality is papaya (*Carica papaya* L.). People in Indonesia have long used this plant as medicine. Based on prior research, one of the effective herbal contraceptives is papaya seeds (Satyarsa *et al*., 2017). Young papaya (*Carica papaya* L.) seeds of the California variety have a higher active compound content than old ones. Young papaya fruit can be seen from the fruit's skin, which is still green, and the seeds are yellowish white (Udoh et al., 2019). Therefore, young papaya seeds have a more significant effect on decreasing the quality of spermatozoa (Raji, 2015).

In addition to papaya seeds, one plant with an antifertility effect is the gandarusa plant (*Justicia gendarussa* Burm.f.). Some Papuan have used this plant as male antifertility, which is empirically used in Indonesia. Prior research shows that gandarusa leaves contain 12 flavonoid components (Bagia et al., 2011).

There are several mechanisms in the use of male contraception, namely as anti-spermatogenesis agents that suppress the production of spermatozoa, preventing sperm maturation and blocking the transport of sperm through the vas deferens (Sharma et al., 2019). The researchers also found that the use of male contraception containing hormones has a weakness. Namely, it can cause side effects when used for a long time even though there are conditions that must be met for ideal male contraception, including being safe, effective, reversible, and has no effect on libido (Kamal et al. 2020).

Based on the description above regarding the low participation of men in family planning programs, the researchers are interested in researching the effect of papaya seeds (*Carica Papaya L.*) ethanol extract, gandarusa leaves (*Justicia Gendarussa* Burm.F.) and its combinations on spermatozoa quality of male mouse (*Mus Musculus*)

2. MATERIALS AND METHODS

2.1. Materials

The materials to be used in this study were papaya seed extract (*Carica papaya* L.), gandarusa leaf extract (*Justicia gendarussa* Burm.f.), aquadest (Smart-Lab; Tangerang, Indonesia), ethanol 70% (Brataco; Tangerang, Indonesia), Cloroform (Merck; Darmstadt, Jerman), eosin-y solution (Merck; Darmstadt, Jerman), methanol (Brataco; Tangerang, Indonesia), safranin, buffer phosphate, gentian violet (Onemed; Tangerang, Indonesia), Na CMC 1% (Merck; Darmstadt, Jerman).

2.2. Methods

2.2.1. Preparation of papaya seeds and gandarusa leaf extract.

Samples of papaya seeds (*Carica papaya* L.) were obtained by separating the white, young papaya seeds and cleaning them from the dirt that sticks by washing them with running water (wet sorting). Then, it is dried in an oven at a temperature of 45-50^{o}C; after drying, the papaya seeds are powdered until they become like flour using a blender. Papaya seed powder (*Carica papaya* L.) was put into a jar and extracted by maceration method and 70% ethanol soluble was added. Ethanol is polar, so it is expected to be able to extract compounds that have polar properties. The solvent or filter will penetrate the cell wall of the simplicia. Maceration was carried out for three days, stirring three times a day. The maceration results were filtered to separate the filtrate and residue. Then, the filtrate is concentrated using a rotary evaporator and a water bath until the solvent evaporates and the extract becomes thicker but can still be poured. The thick extract was weighed according to the treatment dose.

The samples of gandarusa leaves (*Justicia gendarussa* Burm.f.) were cleaned of adhering dirt to separate impurities or other foreign materials that were not used after being cleaned of impurities and then dried by aerating to dryness. After that, the smooth leaves of gandarusa (*Justicia gandarusa* Burm.f.) were put into a jar and soaked using 70% ethanol solvent until all simplicia were submerged and soaked for three days. After three days, the extracted water was separated from the extract, and the liquid obtained was filtered with the help of a vacuum pump to produce filtrate, then evaporated using a rotary evaporator to form a thick extract. The thick extract was weighed according to the treatment dose.

2.2.2. Extract dosage

The dosage of the papaya seed ethanol extract P2 100mg/kgW is calculated so that the dose is weighed so that the extract is 0.3 grams, the ethanol extract of gandarusa leaves P3 50mg/kgW is weighed as much as 0.15 grams, the combined dose of P4 is 50;50mg/kgW each from the seed extract. Papaya and gandarusa leaves weighed 0.15 grams, P5 50:100mg/kgW papaya seed extract weighed 0.15 grams. Gandarusa leaves weighed 0.3 grams, P6 100:50mg/kgW seed extract Weighed 0.3 grams of papaya and 0.15 grams of gandarusa leaves, each dissolved with 1% Na CMC and then made up to 100 ml in volume, then administered orally using a probe/cannula (blunt tip needle) according to the weight of the mice.

2.2.3. The spermatozoa quality effect of male mice with papaya seeds and gandarusa leaves extract

Experimental animals first prepare a place for rearing experimental animals, which includes a drum, a place to eat and drink for mice, and feed for mice; then, the mice are acclimatized at laboratory temperature for seven days to adapt to new environmental conditions. General observations were made while adapting to standard food environmental conditions, and the body weight was weighed before the dose was determined. Types of extracts include Na CMC control (A), papaya seed extract (B), gendarusa leaf extract (C), papaya seed extract, and gendarussa leaf extract (D). The doses were 1% Na CMC, 100 mg/kgW, 50mg/kg W, and 50:50 mg/kgW. 100:50 mg/kgW, 50:100 mg/kgW. The extract was given to mice once a day for 35 days with a calculated dose according to the dose of mg/kg BW of mice; on day 36, all mice were anesthetized with ether or chloroform, dissected, and both testes were taken. Next, cut the tip of the cauda epididymis, accommodated in a watch glass containing two doses of 0.9% NaCl solution, and then stirred homogeneously to observe sperm quality. The parameters observed in this study were the calculation of motility, viability, and morphology of spermatozoa.

2.2.4. Data analysis

Data analysis used the one-way ANOVA test. If the test results showed a significant difference, the test would be followed by the LSD (Last Significant Different) test. The first step of using LSD is sorting the average treatment from the largest to the smallest. The second compares the average difference of a pair of treatments with the calculated value. The last, if the difference value is smaller than before, the average treatment is in one line.

3. RESULT AND DISCUSSION

In this study, samples of young papaya seeds (*Carica papaya L*) of California type were taken from Mangki Village, Cempa Regency, Pinrang City and Gandarusa Leaves (*Justicia gendarussa Burm.f.*) were taken from Duriasi Village, Konawe Regency, Kendari City. The experimental animals used in this study were mice (*Mus musculus*).

From the results of sperm motility observations in male mice (*Mus musculus*), the average values produced are P1 88.67%, P2 14.67%, P3 54.67%, P4 84.00%, P5 84.33% and P6 62,67%. The effective dose influencing the decrease in sperm motility of male mice (*Mus musculus*) is at a P2 of 14.67% (Table 1). At this dose, the progressive spermatozoa are less than the progressive spermatozoa in P1 (Control). It is in line with research (Wijayanti, 2016), which stated that spermatozoa's motility is abnormal if the calculation result is < 32% of progressively moving spermatozoa (Figure 1). This decrease is due to the presence of compounds in papaya seeds, namely saponins, and triterpenoids, which provide hormonal effects by affecting cell membranes so that they can interfere with the function of cell membranes in transporting food or nutrients.

Table 1. The average percentage value of spermatozoa motility in male mice**.**

No	Treatment Group	Percentage of result % ± SD
1	Na CMC 1% control	86.66 ± 9.45
2	Papaya seed extract 100 mg/kgW	14.67 ± 4.16
3	Gandarusa leaves extract 50 mg/kg	72.33 ± 4.04
4	Combination of papaya seeds 50 mg/kgW and gandarusa leaves 50 mg/kgW	84.00±12.12
5	Combination of papaya seeds 50 mg/kgW and gandarusa leaves 100 mg/kgW	84.33±11.01
6	Combination of papaya seeds 100 mg/kgW and gandarusa leaves 50 mg/kgW	62.67±15.66

The prior research by Hasanah and Sukarjati (2016) showed that 100 mg/kg BW papaya seed extract effectively reduced spermatozoa motility, and a mixture of papaya seed extract and neem leaves with a concentration of 100:100 mg/kg BW has the most effect on decreasing motility. It also can reduce the concentration of spermatozoa due to the presence of compounds from both extracts that can interfere with the transport process of spermatozoa, namely agglutinating sperm, and affect reducing motility and vitality. Therefore, the spermatozoa cannot reach the ovarium. (Hasanah and Sukarjati, 2016).

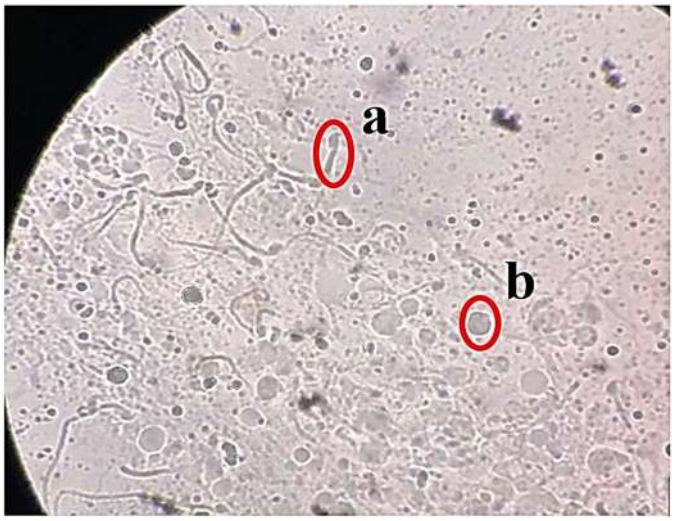

Figure 1. Representative microscopic appearance of spermatozoa motility in male mice. Description: Moving sperm(a) and Immobile sperm (b).

The percentage of spermatozoa motility differs in value (Table 1). It is because the weight and volume of spermatozoa fluid produced from each experimental animal significantly affect sperm quality. Meanwhile, in the treatment group, the value was changed considerably. It was because, apart from the compound content in the plant, the length of observation also greatly affected the quality of spermatozoa. In this observation, the various times were used according to the accuracy of observing sperm cells under a microscope, so the difference in values was significant.

From the percentage of the average value of spermatozoa motility in male mice, the data normality test was carried out using Shapiro Wilk, which showed the values of P1, P2, P3, P4, P5, and P6 where p>0.05. It means the data was normally distributed. Then, the homogeneity test was followed, which obtained p>0,05, meaning the information was homogeneously distributed. Next, a one-way ANOVA test is carried out, and the data obtained is 0.001 p<0.05, which means a significant (significant) difference in each treatment group. Last, in the post hoc test using the LSD (Least Significant Difference) method, there was a significant difference p<0.05.

Table 2. The average value of spermatozoa viability in male mice.

No	Treatment Group	Percentage of result % ± SD
1	Na CMC 1% control	75,67±30.92
2	Papaya seed extract 100 mg/kgW	35,67±21.00
3	Gandarusa leaves extract 50 mg/kgW	4,67±3.20
4	Combination of papaya seeds 50 mg/kgW and gandarusa leaves 50 mg/kgW	69,33±2.08
5	Combination of papaya seeds 50 mg/kgW and gandarusa leaves 100 mg/kgW	88,33±8.32
6	Combination of papaya seeds 100 mg/kgW and gandarusa leaves 50 mg/kgW	53,33±12.22

In this observation, the diluted spermatozoa were dripped with 2% Eosin-y solution to determine the difference between dead spermatozoa (sucking many colors/having a pink color) and live spermatozoa (slightly sucking color/transparent). So, in observing the viability of the spermatozoa of male mice (Mus musculus), the average values produced are P1 75.67%, P2 35.67%, P3 4.67%, P4 69.33%, P5 88.33%, and P6 53.33%. It can be seen in the graph of 2 effective doses influencing the decrease in viability, namely at P3 of 4.67%, where at this dose, the viability of spermatozoa was less than the viability of spermatozoa in P1 (control). It follows the theory (Wijayanti, 2016), which states that viability is abnormal if the value is < 58% and the ability of spermatozoa to survive is tiny. The decrease in spermatozoa viability is thought to be affected by the decreasing percentage of motile/moving spermatozoa (Bagia et al., 2011). In addition, the active compounds in gandarusa leaves, namely flavonoids, which act as antifertility, are estrogenic compounds capable of stimulating estrogen formation in the body, increasing estrogen levels. This increase provides negative feedback to the anterior pituitary that does not release FSH and LH, disrupting testosterone hormone secretion. The presence of disturbance in these hormones will affect the quality of spermatozoa, such as the movement of spermatozoa (Laili, 2018).

The percentage of spermatozoa viability had significantly different values, especially in the control group or without extract (Table 2). In that group, there was a decrease in viability in the mice 1 group. It is because after staining, the spermatozoa were left for approximately 2 minutes to see the different colors in the head (*corpus*) of the spermatozoa. It turns out that the

stagnation causes the viability of the spermatozoa to decrease so that the following observation after staining is no longer resisted (Figure 2).

From the percentage of the average value of spermatozoa viability in male mice, the data normality test was carried out using Shapiro Wilk, which showed the values of P1, P2, P3, P4, P5, and P6 where $p>0.05$. It means that the data was normally distributed. Then, the homogeneity test was followed, and a $p>0,05$ was obtained. The data is homogeneously distributed, a one-way ANOVA test is carried out, and the data obtained is 0.001 $p<0.05$. It means that there is a significant difference between the treatment groups. The post hoc test will continue using the LSD (Least Significant Difference) method. Therefore, it resulted in a significant difference of $p<0.05$.

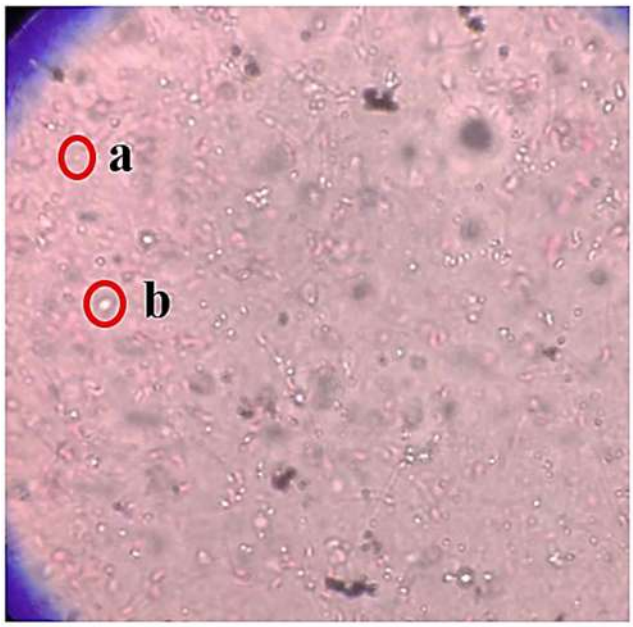

Figure 2. Representative microscopic appearance of spermatozoa viability in male mice. Description: Pink sperm/ dead sperm (a) and Colorless sperm/live sperm (b).

In this morphological observation, the spermatozoa taken are first fixed in the air, and the cells can be attached to the slides. If the cells are not fixed on the slide, the cell layers to be stained can be washed off during the staining procedure, then dipped in methanol to maintain the cell structure remains the same as the original, then washed with safranin and dyed with phosphate buffer. It is a substance needed to maintain pH when a small amount of acid or base is added to the solution. Then, it is stained with crystal violet, the main stain that will give color to microorganisms that will be observed, which can ease this process. Then, it was washed under running water to reduce the excessive color during observation, followed by observation under a microscope. It obtained the result of P1 51.00%, P2 87.00%, P3 6.67%, P4 39.00%, P5 42.67%, and P6 68.33%. It can be seen in the graph that three effective doses most affect the decrease in normal morphology of spermatozoa, namely at P3 6.67%. In that dose, there are fewer normal spermatozoa than normal spermatozoa in the control group. These results follow the study (Wijayanti, 2016), which states that spermatozoa morphology is abnormal if the total

percentage is <40%. The increased abnormality of spermatozoa was due to the presence of Gandarusa leaf extract, which can reduce the weight of the testes, epididymis, and vas deferens by interfering with the function of these organs in the production and maturation of spermatozoa in the epididymis. It causes the mature spermatozoa to decrease in quality due to a spermatozoa defect, which causes the sperm to be abnormal and unable to move forward. The greater the number of abnormal spermatozoa, the ability to move (motility) of the spermatozoa will decrease so that abnormal spermatozoa will have difficulty in movement and cannot move forward quickly towards the ovarium.

Based on the observations, the most common abnormalities found were primary ones. Those are abnormalities in the neck, such as without a head and a tail. Meanwhile, secondary abnormalities include entangled tails and fractures in the tail. It also found that the primary abnormalities are due to disruption of spermatogenesis in the phase of spermatogenesis. It occurs when spermatozoa are formed from spermatids, while secondary abnormalities are thought to occur during spermatozoa maturation disorders in the epididymis. Then, it results in the discovery of abnormal spermatozoa (Figure 3). In prior research, ethanol extract of gandarusa leaves at 0.195 mg/kg W can reduce motility and viability and increase spermatozoa abnormalities in mice (Bagia et al., 2011). The previous researchers stated that the extract of gandarusa leaves LD_{50} was 180 mg/kg BW of mice, which was included in the practical and non-toxic category (Bagia et al., 2011).

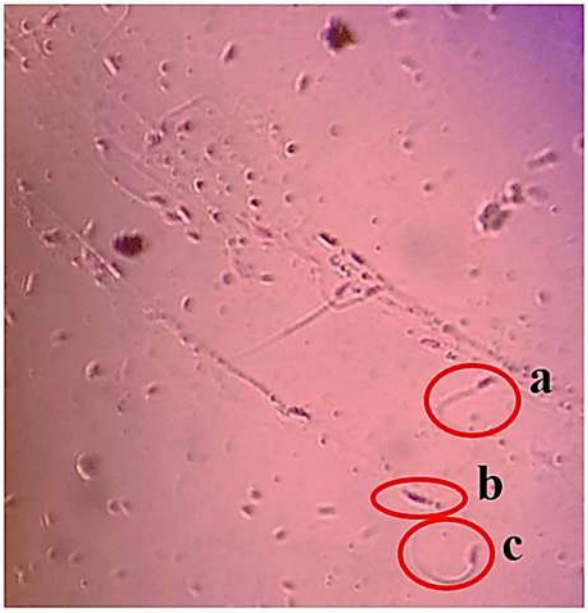

Figure 3. Representative microscopic appearance spermatozoa morphology of male mice. Description: Entangled sperm tail (a), sperm without tail (b) and abnormal movement (c).

Male mice's most decreased spermatozoa morphology (Table 3) shows that the most significant decrease in normal spermatozoa occurred at P3. It was due to the compound content in the Gandarusa leaves extract, which was more potent than the state after being combined. In addition, it was caused by one of the error factors during administering the liquid extract that

was administered orally, not entirely drunk by the experimental animals according to the specified volume. The volume of spermatozoa fluid obtained during the observation process differed, so the decrease was more significant than after combined.

From the percentage of the average value of spermatozoa morphology in male mice, the normality test of the data was carried out using Shapiro-Wilk. It showed the values of P1, P2, P3, P4, P5, and P6 where $p>0.05$, which means the data was normally distributed. Then, the homogeneity test obtained the $p>0,05$ value. It means the information is homogeneously distributed, then a one-way ANOVA test is performed, and the data obtained is 0.003 $p<0.05$, meaning there is a significant difference in each treatment group. Then, the post hoc test using the LSD (Least Significant Difference) method revealed a significant difference $p<0$.

Table 3. The average value of spermatozoa morphology in male mice.

No	Treatment Group	Percentage of result % ± SD
1	Na CMC 1% control	51,00±36.71
2	Papaya seed extract 100 mg/kgW	87,00±4.35
3	Gandarusa leaves extract 50 mg/kgW	6,67±1.52
4	Combination of papaya seeds 50 mg/kgW and gandarusa leaves 50 mg/kgW	39,00±15.00
5	Combination of papaya seeds 50 mg/kgW and gandarusa leaves 100 mg/kgW	42,67±7.50
6	Combination of papaya seeds 100 mg/kgW and gandarusa leaves 50 mg/kgW	68,33±16.50

Previous studies have shown that abnormal spermatozoa morphology increases because papaya seed extract causes abnormal cell organelles in the neck of spermatozoa, namely vacuolization in mitochondria. It causes the function of mitochondria in producing energy to be not optimal and thus affects the motility of spermatozoa (Lohiya *et al*., 2017). The content of papain enzymes in papaya seed extract can also reduce total lipids in testicular and epididymal tissue. Papaya seed extract reduces the activity of the lipoprotein lipase enzyme. It inhibits the absorption of nutrients from the gastrointestinal system. In contrast, the energy produced from these nutrients is needed for spermatogenesis in the testes and the maturation of spermatozoa in the epididymis (Basha and Cangamma, 2018).

4. CONCLUSION

Based on the research that has been done, it can be concluded that the administration of extracts of papaya seeds (*Carica papaya L.*) and gandarusa leaves (*Justicia gandarusa Burm.f.*) and their combination toward the quality of spermatozoa of male mice (*Mus musculus*) can reduce motility, viability, the morphology of good spermatozoa both in single or combination. The test results p value <0.005. The effective dose in reducing spermatozoa's motility, viability, and morphology is P3 ethanol extract of gandarusa leaves 50 mg/kgW.

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Potensi Daun Keji beling (*Strobilanthus crispus*) sebagai Antibakteri dan Antibiofilm terhadap Bakteri *Cutibacterium acnes*

The Potential of Keji Beling Leaves (Strobilanthus crispus) as Antibacterial and Antibiofilm against Cutibacterium acnes

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Abstrak

Cutibacterium acnes, merupakan salah satu mikroorganisme permanen pada permukaan stratum korneum, dimana populasinya mewakili 20-70% dari mikroorganisme yang banyak ditemukan pada kondisi muka berjerawat. Pembentukan biofilm dari mikroorganisme ini merupakan salah satu faktor yang mengakibatkan obat anti jerawat tidak efektif dalam bekerja. *Strobilanthus crispus* (keji beling) merupakan salah satu tanaman yang memiliki potensi cukup tinggi dalam pengobatan tradisional, khususnya sebagai antibakteri. Penelitian ini akan menguji kemampuan ekstrak etanol daun keji beling sebagai antibakteri dan antibiofilm terhadap bakteri *Cutibacterium acnes*. Ekstrak etanol daun keji beling di dapatkan dengan menggunakan metode maserasi dengan pelarut etanol 96%. Metode Difusi Sumuran dengan Diameter Hambat Pertumbuhan (DHP) sebagai parameter pengukuran digunakan sebagai metode pengujian untuk aktivitas antibakteri. Ekstrak etanol daun keji beling pada konsentrasi 50% memberikan DHP 7,21 ± 0,103 mm. Aktivitas antibiofilm diuji dengan menggunakan metode mikrodilusi pada rentang konsentrasi ekstrak 0,06 % - 30%. Hasil menunjukkan 1,88% ekstrak etanol daun keji beling telah mampu memberikan persentase penghambatan pembentukan biofilm sebesar 98,23 ± 0,230%, dimana hasil ini menunjukkan hasil yang tidak berbeda dengan klindamisin 10µg/100µL. Skrinning fitokimia yang dilakukan pada ekstrak etanol daun keji beling menunjukkan adanya flavonoid, alkaloid dan tanin, yang di duga menunjukkan potensi sebagai antibiofilm.

Kata kunci: Antibakteri; Antibiofilm; *Cutibacterium acnes*; *Strobilanthus crispus*

Abstract

Cutibacterium acnes is one of the commensal bacteria on the surface of the stratum corneum, where it is population represents 20-70% of the microorganisms commonly found in acne. Forming biofilms from these microorganisms is one of the causes of anti-acne drugs being ineffective. Strobilanthus crispus (keji beling) is a potential plant in traditional medicine, especially as an antibacterial. This study aimed to determine the ability of the ethanol extract of keji beling leaves as an antibacterial and anti-biofilm against Cutibacterium acnes. The ethanol extract of keji beling leaves was obtained using the maceration method with 96% ethanol as a solvent. Well Diffusion method was used for antibacterial testing using Inhibition Zone Diameter (IZD) as a parameter. The ethanol extract of keji beling leaves was at a

concentration of 50% giving an IZD of 7.21 ± 0.103 mm. Anti-biofilm activity was tested using the microdilution method at an extract concentration range of 0.06% - 30%. The results showed that the ethanol extract of keji beling leaves inhibited the biofilm formation as much as 98.23 ± 0.230% at the concentration of 1.88%, which was not significantly different from clindamycin 10µg/100µL. Phytochemical screening performed on the ethanol extract of keji beling leaves showed the presence of flavonoids, alkaloids, and tannins, which can potentially be anti-biofilms.

Keywords: *Antibacterial; Antibiofilm; Cutibacterium acnes; Strobilanthus crispus*

1. PENDAHULUAN

Jerawat atau yang biasa disebut sebagai acne vulgaris merupakan suatu gangguan pada kulit yang umumnya ditandai dengan adanya nodul, papul, pustule dan komedo pada beberapa area tubuh manusia (Taleb *et al*., 2018). Penyakit ini merupakan penyakit paling umum ke -8 yang dijumpai dan merupakan jenis penyakit kulit terbanyak ke -2, dimana penyakit ini kerap terjadi pada remaja pada usia 16-19 tahun hingga dewasa usia 30 tahun (Wardani, 2020; Taleb *et al*., 2018). Prevalensi tertinggi umumnya dijumpai lebih tinggi pada pria (95%-100%), bila dibandingkan dengan wanita (83%-85%) (Yurlina *et al*., 2019). Faktor penyebab jerawat diantaranya adalah keturunan atau gen, keadaan psikis, ras, hormonal maupun karena infeksi bakteri. Terjadinya jerawat pada manusia, secara umum terbagi menjadi tiga tahapan yaitu (1) meningkatnya produksi sebum, (2) terjadinya hiperproliferasi keratinosit dan (3) kolonisasi bakteri penyebab jerawat (Wardani, 2020). *Cutibacterium acnes*, *Staphylococcus aureus* dan *Staphylococcus epidermidis* merupakan beberapa bakteri yang memiliki peran dalam penyakit ini (Fissy *et al*., 2014).

Cutibacterium acnes merupakan flora normal yang umumnya dapat dijumpai pada kulit manusia, dimana dengan adanya peningkatan sebum dapat mengakibatkan jumlah bakteri ini ikut meningkat. Peningkatan jumlah bakteri ini akan menjadi patogen dan menimbulkan lesi inflamasi pada kulit (Feuillolay *et al*., 2016; Wardani, 2020). *Cutibacterium acnes* telah dibuktikan dapat membentuk biofilm, dimana pembentukan biofilm oleh mikroorganisme anaerob fakultatif Gram-positif ini menunjukkan indikasi keterlibatan dalam kegagalan pengobatan antibiotik (Coenye *et al*., 2022). Biofilm sendiri merupakan kumpulan mikroorganisme yang melekat satu sama lain pada suatu permukaan. Lapisan biofilm ini bertindak sebagai pelindung bagi mikroba dari dunia luar, termasuk menghalangi penetrasi antibiotik ke dalam mikroba (Linfante *et al*., 2017). Studi yang dilakukan menunjukkan *Cutibacterium acnes* memiliki empat karakterisasi pola biofilm yaitu (1) perlekatan bakteri pada dinding folikel; (2) perlekatan pada batang rambut; (3) penyebaran ke lumen folikel rambut; dan (4) terbentuknya biofilm di tengah-tengah folikel rambut, tanpa keterikatan yang terlihat pada dinding folikel (Jahns dan Alexeyev, 2014).

Tanaman herbal sejak jaman dulu secara turun temurun telah banyak digunakan oleh nenek moyang kita sebagai alternatif pengobatan dalam mengatasi berbagai macam jenis penyakit. Salah satunya adalah daun keji beling yang dalam masyarakat digunakan sebagai salah satu obat tradisional untuk mengatasi jerawat. Daun keji beling mempunyai beragam

kandungan diantaranya alkaloid, flavonoid, saponin, terpenoid dan tanin (Fardiyah *et al*., 2020). Keji beling telah banyak digunakan masyarakat dalam pengobatan, antara lain sebagai antibakteri, antioksidan, antilithiasis, antidiabetes, antiangiogenesis, antikanker serta aktivitas penyembuhan luka (Ng *et al.*, 2021). Aktivitasnya sebagai antibakteri ditunjukkan pada beberapa penelitian sebagai berikut: (1) ekstrak etanol daun keji beling 25% memberikan Daerah Hambat pertumbuhan (DHP) sebesar 10 mm pada bakteri *Pseudomonas aeruginosa* (Suproborini *et al*., 2022); (2) ekstrak etanol daun keji beling pada *Staphylococcus epidermidis* konsentrasi 10% (DHP 15,10 mm), 25% (DHP 16,40 mm), dan 50% (DHP 18,86 mm) (Adriana *et al*., 2023); (3) ekstrak etanol daun keji beling memberikan Kadar Hambat Minimum (KHM) 200 mg/mL pada bakteri *Staphylococcus aureus* and *Streptococcus pneumoniae* (Lim *et al*., 2015); (4) ekstrak etanol daun keji beling pada konsentrasi 25% memberikan DHP sebesar 10 mm pada bakteri *Aggregatibacter actinomycetemcomitans* (Suproborini *et al*., 2023); (5) nano partikel ekstrak air daun keji beling 2 % memberikan DHP 18 mm pada bakteri *Escherichia coli* (Samsulkahar *et al*., 2023). Penelitian tentang potensi daun keji beling terhadap *Cutibacterium acnes* belum pernah dilakukan.

Oleh sebab itu, penelitian ini bertujuan untuk menentukan uji aktivitas antibakteri dan antibiofilm ekstrak etanol daun keji beling (*Strobilanthus crispus*) dengan menggunakan metode difusi sumuran dan mikrodilusi terhadap bakteri *Cutibacterium acnes.* Serbuk simplisia kering dan ekstrak etanol daun keji beling yang akan digunakan terlebih dahulu akan distandarisasi untuk menjamin identitas, kualitas dan keamanan dari bahan baku yang digunakan. Diharapkan melalui hasil penelitian ini, daun keji beling dapat dimanfaatkan dan dikembangkan sebagai salah satu bahan aktif sediaan topikal dalam pengobatan jerawat.

2. BAHAN DAN METODE

2.1. Bahan penelitian

Sampel yang digunakan dalam penelitian adalah simplisia daun keji beling (*Strobilanthus crispus*) kering yang diperoleh dari Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional Tawangmangu, Karanganyar, Jawa Tengah. Sampel telah dideterminasi dan specimen kering telah di dokumentasikan oleh Laboratorium Botani Farmasi Universitas Katolik Widya Mandala Surabaya. Mikroba uji yang digunakan dalam penelitian ini adalah isolat *Cutibacterium acnes* ATCC 11827 yang diperoleh dari Balai Besar Laboratorium Kesehatan, Surabaya, Jawa Timur. Media bakteri yang digunakan dalam penelitian ini adalah *Brain Heart Infusion Agar* (BHIA) (Merck, Germany) dan *Brain Heart Infusion Broth* (BHIB) (Merck, Germany). Pembanding yang digunakan adalah Klindamycin (Clyndamycin 150, Dexa Medica, Indonesia) dengan konsentrasi 2µg/20µL. Bahan-bahan kimia (etanol, etil asetat, asam format, tween, kristal violet) merupakan bahan kimia dengan grade pharmaceutical.

2.2. Alat penelitian

Alat yang digunakan dalam penelitian ini antara lain alat-alat gelas laboratorium, timbangan analitis (Sartorius TE 214 S, Germany), waterbath (Thermostatic waterbath DHH-6 XMTD 294), Laminar Air Flow (LAF) (Type V-130, Indonesia), autoklaf (All America Model

25x, USA), inkubator (Memmert and Binder, Germany), oven (Memmert, Germany), mikroskop (Olympus, Germany), microplate reader (Thermofisher Scientific OY, Finlandia).

2.3. Metode penelitian

2.3.1. Pembuatan simplisia kering daun keji beling

Daun keji beling yang digunakan diperoleh dari Balai Materia Medika Indonesia Batu Jawa Timur. Daun yang diperoleh di sortasi basah, lalu diangin-anginkan dan dikeringkan sampai kering. Pemeriksaan makroskopis dilakukan pada daun segar untuk memastikan daun yang digunakan adalah daun keji beling.

2.3.2. Pembuatan ekstrak etanol daun keji beling

Simplisia kering yang diperoleh dihaluskan dengan menggunakan blender dan diayak dengan menggunakan pengayak berukuran mesh 40. Pengamatan organoleptis, mikroskopis, susut pengeringan dan penetapan kadar abu akan dilakukan pada serbuk simplisia kering sebelum dilakukan proses ekstraksi. Serbuk simplisia yang telah diperoleh di timbang sebanyak 500 gram dan direndam dalam pelarut etanol 96% sebanyak 1 L hingga menjadi massa basah selama 4 jam. Selanjutnya massa basah dipindahkan ke tabung perkolator dan ditambahkan etanol 96% sehingga semua simplisia terendam, lalu didiamkan selama 24 jam. Tahapan selanjutnya adalah penetesan filtrat dengan kecepatan aliran 40 tetes tiap menit dengan secara teratur ditambahkan pelarut baru. Total seluruh pelarut yang digunakan dalam tahapan ekstraksi adalah 2,8 L (perbandingan 1: 5,6). Ekstrak cair yang diperoleh dipekatkan dengan menggunakan *waterbath* suhu 60°C hingga didapatkan ekstrak kental. Ekstrak kental yang di peroleh ditimbang untuk menghitung rendemen hasil. Ekstrak kental kemudian disimpan dalam wadah tertutup sebelum digunakan pada pengujian selanjutnya.

2.3.3. Standarisasi ekstrak

Standarisasi ekstrak yang dilakukan meliputi pengamatan organoleptis susut pengeringan dan kadar abu total. Metode pengerjaan standarisasi dilakukan sesuai dengan prosedur yang terdapat pada standarisasi parameter ekstrak (Kementerian Kesehatan RI, 2017).

2.3.4. Skrining fitokimia

Skrining fitokimia dilakukan dengan menggunakan metode Kromatografi Lapis Tipis (KLT). Kondisi KLT yang digunakan: fase diam silika gel F254, fase gerak etil asetat:asam format:air (perbandingan 8:1:1), konsentrasi ekstrak 10% dan volume penotolan 10µL. Plat KLT yang sdah dieluasi, dikeringkan, kemudian diamati dan didokumentasikan pada pengamatan visible, UV 254 nm dan UV 366 nm. Penampak noda *dragendorf* digunakan untuk alkaloid, $FeCl_3$ untuk tanin, $AlCl_3$ untuk flavonoid dan *Liebermann burchard* untuk golongan terpenoid.

2.3.5. Pemeriksaan bakteri uji

Pemeriksaan makroskopis, mikroskopis dan uji biokimia katalase dilakukan pada bakteri uji yang digunakan pada penelitian ini. Pemeriksaan mikroskopis yang dilakukan meliputi

pengamatan terhadap bentuk, tepi, kenaikan permukaan, warna, ukuran dan tekstur koloni yang terbentuk pada media BHIA yang digunakan. Pemeriksaan mikroskopis dilakukan dengan menggunakan pewarnaan Gram untuk melihat bentuk sel, susunan sel dan warna sel. Uji biokimia katalase dilakukan dengan menambahkan H_2O_2 3% pada kaca obyek yang telah berisi 1 ose koloni bakteri, kemudian diamati ada atau tidak nya gelembung oksigen.

2.3.6. Uji aktivitas antibakteri metode difusi

Metode difusi sumuran dipilih untuk pengujian aktivitas antibakteri. Suspensi bakteri *Cutibacterium acnes* yang telah setara dengan ½ Mc Farland I (1,5 x 10^8 CFU/mL) dipipet sebanyak 0,1 mL lalu ditambah dengan 10 mL media BHIA 50°C lalu dituang ke dalam cawan petri steril dan dirotasi. Pra-pengeraman dilakukan selama 1,5 – 2 jam pada suhu 37°C. Cawan petri dibagi menjadi 5 lubang sumuran dengan diameter 6,0 mm. Lima lubang sumuran tersebut berisi 20 µL ekstrak etanol daun keji beling 30%, 40% dan 50%; klindamisin 2µg/20µL dan akuades steril. Cawan petri yang telah berisi sampel diinkubasi pada suhu 37°C selama 24 jam. Pengamatan daerah hambatan pertumbuhan dilakukan dengan mengukur daerah jernih disekitar sumuran dengan menggunakan jangka sorong. Diameter zona bening diukur dalam satuan milimeter (mm) dengan cara dibagi menjadi dua garis yaitu diameter zona vertikal dan diameter zona horizontal, kemudian diukur dan dirata – rata (Kusuma *et al*., 2020).

2.3.7. Uji aktivitas antibiofilm metode mikrodilusi

Pengujian aktivitas antibiofilm dilakukan dengan metode mikrodilusi menggunakan *microplate U-bottom 96 well*. Kelompok sampel yang diujikan yaitu kelompok ekstrak (0,06 % - 30 %), kelompok pembanding dan kelompok kontrol negatif. Microplate yang telah berisi sampel diinkubasi selama 48 jam pada suhu 37°C. Setelah inkubasi, isi sumuran dibuang dan dibilas 3 kali dengan air mengalir. Masing – masing sumuran diberi kristal violet 0,1% sebanyak 200 µL dan didiamkan selama 15 menit pada suhu ruang. Microplate dicuci atau dibilas dengan air mengalir sebanyak 2 kali. Masing-masing sumuran diberi Tween 2% sebanyak 200 µL dan didiamkan selama 15 menit. Microplate dimasukkan dalam *microplate reader* dan dibaca densitas optik pada λ 599 nm. Hasil absorbansi yang didapatkan akan digunakan dalam perhitungan untuk mendapatkan persentase penghambatan biofilm (Kim *et al*., 2021).

3. Hasil dan pembahasan

Daun keji beling (*Strobilanthus crispus*) yang digunakan dalam penelitian ini diamati karakteristik daunnya yang meliputi ukuran, bentuk, ujung bawah dan atas, tepi dan permukaan daun (Tabel 1). Pengamatan makroskopis (Tabel 1) dan mikroskopis (Gambar 1) juga dilakukan terhadap serbuk kering daun keji beling, dimana hasil pengamatan yang berupa fragmen-fragmen pengenal dibandingkan dengan Farmakope Herbal Indonesia (2017), untuk memastikan serbuk daun yang diperoleh adalah serbuk daun keji beling (Gambar 1).

Hasil pengamatan mikroskopis serbuk simplisia daun keji beling menunjukkan adanya fragmen-fragmen pengenal yang sesuai dengan yang terdapat pada Farmakope Herbal

Indonesia Edisi 2 (Kementerian Kesehatan RI, 2017). Pengamatan terhadap susut pengeringan dan kadar abu total (Tabel 2) dan hasil yang didapatkan telah sesuai dengan pustaka yang diacu.

Tabel 1. Hasil pengamatan makroskopis daun keji beling dan hasil pengamatan makroskopis simplisia kering daun keji beling. Keterangan: *= Sumber pustaka dari Kementerian Kesehatan RI, 2017).

Parameter	Hasil Pengamatan	Pustaka*	Keterangan
Simplisia Kering			
Ukuran	Panjang daun: 7 – 8,5 cm Lebar daun: 4 – 5,4 cm	Panjang daun: 5 – 8 cm (tanpa tangkai) Lebar daun: 2 – 5 cm	Sesuai
Bentuk daun	Lonjong	Lonjong	Sesuai
Pangkal daun	Meruncing	Meruncing	Sesuai
Ujung daun	Meruncing	Meruncing	Sesuai
Tepi daun	Bergerigi	Bergerigi	Sesuai
Permukaan daun	Kasar ditumbuhi rambut halus	Kasar ditumbuhi rambut halus	Sesuai
Serbuk Simplisia			
Warna	Hijau tua	Hijau tua sampai hitam kelabu	Sesuai
Bau	Aromatis lemah	Lemah	Sesuai

Gambar 1. Hasil pengamatan mikroskopis dalam media kloralhidrat-Floroglusin HCl pada perbesaran 10x40. Keterangan: a. sistolit (kristal kalsium karbonat); b. trikoma multiseluler glanduler; c. stomata bidiasitik; d. berkas pembuluh; e. epidermis atas dengan sistolit.

Berat ekstrak kental yang diperoleh adalah 49,6 gram dengan rendemen sebesar 9,92%. Hasil pengamatan menyatakan bahwa ekstrak daun keji beling berbentuk ekstrak kental, berwarna hitam kehijauan, memiliki rasa pahit dan berbau aromatis lemah. Pengujian kadar air yang dilakukan didapatkan hasil sebesar 13,478 ± 0,319%, hasil ini sesuai monografi ekstrak

daun keji beling pada Farmakope Herbal Indonesia edisi II (2017) yaitu tidak lebih dari 30%. Hasil kadar air ekstrak etanol daun keji beling menunjukkan hasil yang >10%, hal ini diduga karena keberadaan sistolit yang banyak terdapat pada tanaman keluarga Acanthaceae. Kristal kalsium karbonat yang merupakan komponen penyusun sistolit adalah garam yang pada dasarnya memiliki karakteristik menyerap kelembapan dari udara.

Hasil uji KLT ekstrak etanol daun keji beling menunjukkan positif mengandung golongan senyawa flavonoid, alkaloid dan tanin. Pengujian flavonoid dilakukan menggunakan penampak noda $AlCl_3$ dan menunjukkan hasil positif berupa noda berfluoresensi hijau kekuningan pada pengamatan UV 366 nm. Uji alkaloid menunjukkan hasil positif setelah disemprot penampak noda *dragendrof* dan memberikan warna noda merah kecoklatan pada pengamatan visual. Uji tanin dilakukan menggunakan penampak noda $FeCl_3$ dan menghasilkan noda hitam pada pengamatan visual. Hasil uji KLT ekstrak etanol daun Keji beling telah sesuai dengan penelitian terdahulu (Fardiyah *et al*., 2020).

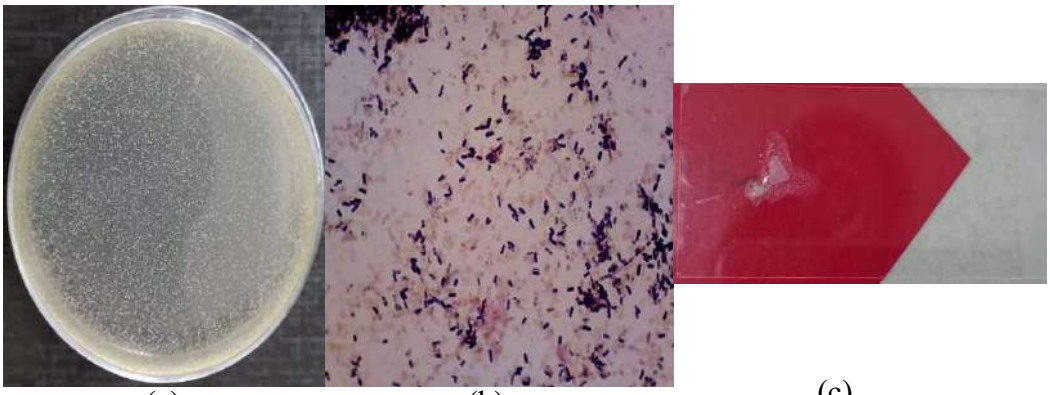
Tabel 2. Hasil standarisasi simplisia daun keji beling (*Strobilanthus crispus*). Keterangan: *= Sumber pustaka dari Kementerian Kesehatan RI, 2017).

Parameter	Hasil Pengamatan (%)	Pustaka*	Keterangan
Susut Pengeringan	9,124 ± 0,106	Tidak lebih dari 10%	Sesuai
Kadar Abu Total	19,711 ± 0,313	Tidak lebih dari 20,4%	Sesuai

Hasil pemeriksaan makroskopis dan mikroskopis *Cutibacterium acnes* (Gambar 2 dan Tabel 3). Pada uji biokimia katalase menunjukkan adanya gelembung atau busa, sehingga mengindikasikan bahwa *Cutibacterium acnes* memiliki enzim katalase yang dapat mengubah hidrogen peroksida (H_2O_2) menjadi air (H_2O) dan oksigen (O_2). Hasil pengamatan makroskopis, mikroskopis dan uji biokimia katalase sesuai dengan pustaka yang diacu (Ahmed *et al*., 2020; Mustarichie *et al*., 2020; Stefan *et al.*, 2019).

Hasil pengujian antibakteri terhadap *Cutibacterium acnes* (Gambar 3 dan Tabel 4) diperoleh bahwa ekstrak etanol daun keji beling pada konsentrasi 30% dan 40% tidak memiliki aktivitas antibakteri yang ditunjukkan tidak terdapat Daerah Hambat Pertumbuhan (DHP) terhadap bakteri *Cutibacterium acnes*. Pada konsentrasi 50% memberikan DHP rata – rata 7,21 ± 0,103 mm terhadap bakteri *Cutibacterium acnes*. Tidak terbentuknya daerah jernih di sekitar lubang sumuran pada konsentrasi 30% dan 40% diduga karena ekstrak etanol daun keji beling mempunyai kadar metabolit sekunder yang lebih rendah dibandingkan kandungan pada konsentrasi 50%, sehingga tidak mampu menghambat pertumbuhan bakteri *Cutibacterium acnes*. Hal ini sejalan dengan penelitian Suproborini *et al.* (2022), semakin besar konsentrasi ekstrak maka jumlah metabolit sekunder juga akan semakin besar. Berdasarkan hasil DHP dari ekstrak etanol daun keji beling yang diperoleh termasuk dalam kategori sedang, dimana *Cutibacterium acnes* merupakan bakteri gram positif yang memiliki dinding sel yang terdiri dari lapisan peptidoglikan yang tebal sehingga dapat memberikan stabilitas stuktural yang tinggi dan lebih mudah mengalami resistensi terhadap tekanan osmotik dan mekanik (Sangani

et al., 2015). Pembanding klindamisin memberikan DHP rata – rata 25,52 ± 0,965 mm terhadap *Cutibacterium acnes*, hal ini dikarenakan klindamisin memiliki sifat bakteriostatik melalui penghambatan sintesis protein mikroorganisme dengan mengikat RNA pada subunit 50s ribosom bakteri, sehingga klindamisin mampu memberikan efek *post*-antibiotik yang panjang, menurunkan produksi toksin, mengikat opsonisasi mikroba dan fagositosis (Hong *et al*., 2014).



(a) (b) (c)

Gambar 2. (a) Makroskopis *Cutibacterium acnes*, (b) Mikroskopis *Cutibacterium acnes*, (c) Uji biokimia katalase *Cutibacterium acnes*.

Tabel 3. Hasil pemeriksaan makroskopis dan mikroskopis *Cutibacterium acnes*. Keterangan: *= Sumber pustaka dari Ahmed *et al*., 2020; Mustarichie *et al*., 2020; Stefan *et al*., 2019.

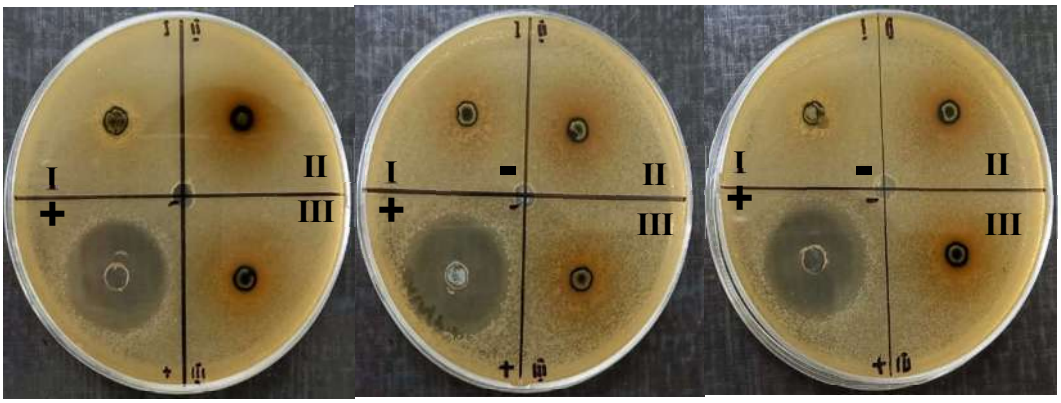
	Pemeriksaan	**Hasil Pengamatan**	**Pustaka***	**Keterangan**
Makroskopis	Bentuk koloni	Bulat	Bulat	Sesuai
	Warna koloni	Putih agak kuning	Putih agak kuning	Sesuai
	Tepi koloni	Bergerigi	Bergerigi	Sesuai
	Kenaikan permukaan	Cembung	Cembung	Sesuai
	Tekstur koloni	Basah, *semi-opaque*, halus	Basah, *semi-opaque*, halus	Sesuai
	Ukuran koloni	1 – 2 mm	1 – 2 mm	Sesuai
Mikroskopis	Bentuk sel	Batang pendek	Bulat, batang pendek	Sesuai
	Susunan sel	Tunggal, berpasangan, membentuk huruf V dan Y	Tunggal, berpasangan atau rantai pendek membentuk huruf V atau Y	Sesuai
	Warna sel	Biru ungu	Biru ungu	Sesuai
	Reaksi Gram	Gram positif	Gram positif	Sesuai
	Ukuran sel	0,5 – 0,8 mm	0,5 – 0,8 mm	Sesuai

Ekstrak etanol daun keji beling (*Strobilanthus crispus*) memiliki kemampuan menghambat pembentukan biofilm *Cutibacterium acnes*. Pada konsentrasi 0,06% menunjukkan hasil penghambatan biofilm sebesar 85,81 ± 0,137 % dan terus mengalami peningkatan hingga konsentrasi 30%. Teori yang dikemukakan oleh Sangani *et al*., (2015) yang

menyatakan bahwa mekanisme penghambatan pertumbuhan biofilm dapat dengan cara menembus dinding sel bakteri sehingga dapat mengganggu sinyal – sinyal komunikasi (*quorum sensing*) antar bakteri yang berperan dalam pembentukan biofilm atau menginaktivasi gen – gen pada bakteri yang memicu sistesis EPS (*Extracelluler Polymeric Substance*). Pada pengujian antibakteri konsentrasi 30% tidak terdapat Daerah Hambat Pertumbuhan (DHP), namun pada pengujian antibiofilm terdapat persen penghambatan biofilm, hal ini disebabkan karena antibiofilm hanya dapat menghambat atau mengurangi pembentukan biofilm bakteri dan tidak untuk membunuh bakteri *Cutibacterium acnes*.

Tabel 4. Hasil pengukuran diameter Daerah Hambatan Pertumbuhan (DHP) ekstrak etanol daun keji beling (EEDKB), pembanding klindamisin dan akuades steril terhadap *Cutibacterium acnes.*

Keterangan	Replikasi 1 (mm)	Replikasi 2 (mm)	Replikasi 3 (mm)	Rata-rata DHP (mm) ± SD
EEDKB 30%	-	-	-	-
EEDKB 40%	-	-	-	-
EEDKB 50%	7,10	7,30	7,24	7,21 ± 0,103
Klindamisin 2µg/20µl	26,6	24,75	25,2	25,52 ± 0,965
Akuades steril	-	-	-	-



(a) (b) (c)

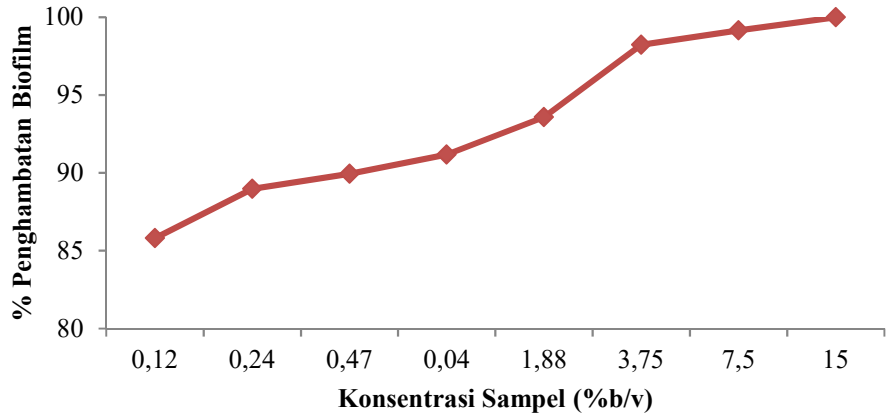
Gambar 3. Hasil uji daya antibakteri ekstrak etanol daun keji beling, pembanding klindamisin dan akuades steril terhadap *Cutibacterium acnes.* Keterangan: Replikasi 1 (a), Replikasi 2 (b), Replikasi 3 (c); Ekstrak etanol daun keji beling 30% (I), Ekstrak etanol daun keji beling 40% (II), Ekstrak etanol daun keji beling 50% (III); Klindamisin 2µg/20µL (+) sebagai kontrol positif; Akuades steril (-) sebagai kontrol negatif.

Hasil pengujian antibiofilm menggunakan pembanding klindamisin dilakukan pada konsentrasi 10µg/100µl dan menghasilkan persentase penghambatan sebesar 97,00 ± 0,624 %. Ekstrak etanol daun keji beling mulai konsentrasi 1,88% hingga konsentrasi 30% memberikan persen penghambatan biofilm lebih besar dibandingkan persen penghambatan biofilm oleh klindamisin pada konsentrasi 10µg/100µl (Tabel 5 dan Gambar 4). Klindamisin memiliki kemampuan meningkatkan opsonisasi dan fagositosis bakteri melalui penghambatan sintesa protein bakteri dan menyebabkan kerusakan dinding sel sehingga menurunkan *adhesi* bakteri ke sel host dan menghambat pembentukan biofilm (Luchian *et al*., 2021).

Mekanisme flavonoid sebagai antibakteri melalui tiga hal yaitu, menghambat sintesis asam nukleat, menghambat fungsi membran sel, dan menghambat metabolisme energi sehingga menyebabkan kerusakan permeabilitas dinding sel dan mengakibatkan keluarnya senyawa intraseluler bakteri (Górniak *et al*., 2019). Alkaloid bekerja sebagai antibakteri membentuk ikatan hidrogen dengan komponen penyusun peptidoglikan sehingga dinding sel tidak terbentuk dan sel akan mati (Alibi *et al*., 2021). Tanin dapat menginaktivasi adhesin sel mikroba dan mentarget pada polipeptida dinding sel, akibatnya pembentukan dinding sel bakteri menjadi tidak sempurna dan mengalami lisis akibat tekanan osmotik (Kaczmarek, 2020).

Tabel 5. Persentase penghambatan biofilm *Cutibacterium acnes* oleh ekstrak daun keji beling (*Strobilanthus crispus*) dan antibiotik klindamisin.

Sampel	% Penghambatan Biofilm
Ekstrak etanol daun keji beling (% b/v)	
30	100 ± 0,378
15	100 ± 0,338
7,5	100 ± 0,029
3,75	99,15 ± 0,203
1,88	98,23 ± 0,230
0,94	93,58 ± 0,149
0,47	91,17 ± 0,451
0,24	89,94 ± 0,740
0,12	88,98 ± 0,526
0,06	85,81 ± 0,137
Klindamisin	
10 µg/100µl	97,00 ± 0,624



Gambar 4. Grafik persentase penghambatan biofilm *Cutibacterium acnes* oleh ekstrak etanol daun keji beling (*Strobilanthus crispus*) pada beberapa konsentrasi.

Melalui beberapa penelitian tidak terlihat jelas bagaimana flavonoid dapat mencegah terbentuknya biofilm, namun teerdapat dugaan bahwa flavonoid dapat berinteraksi dengan permukaan dinding sel bakteri dan mencegah terbentuknya formasi biofilm *(*Górniak *et al*., 2019). Penelitian dengan menggunakan bakteri Gram positif lain yaitu *Stapylococcus aureus*

menunjukkan flavonoid dapat menghambat transkripsi AgrA dan RNAIII sehingga pembentukan biofilm dapat digagalkan (Guzzo *et al*., 2020). Alkaloid dapat mengganggu proses pelekatan satu sel dengan sel lain, yang mengakibatkan proses pembentukan biofilm terhambat (Lahiri *et al*., 2019). Tanin merusak integritas dinding sel dengan merusak ikatan peptidoglikan, sehingga dapat menghambat pembentukan biofilm (Dong *et al*., 2018).

4. KESIMPULAN

Ekstrak etanol daun keji beling (*Strobilanthus crispus*) terbukti menunjukkan potensi sebagai antibakteri dan antibiofilm terhadap *Cutibacterium acnes*. Aktivitas antibakteri ditunjukkan melalui besaran daerah hambatan pertumbuhan sebesar 7,21 ± 0,103 mm pada konsentrasi ekstrak etanol 50%. Ekstrak etanol daun keji beling memberikan persen penghambatan biofilm yang lebih besar dibandingkan persen penghambatan biofilm oleh klindamisin pada konsentrasi 1,88%. Golongan senyawa yang terkandung pada ekstrak etanol daun keji beling (*Strobilanthus crispus*) adalah flavonoid, alkaloid dan tanin. Untuk penelitian selanjutnya, perlu dilakukan penelitian lebih lanjut mengenai perlakuan pra-ekstraksi dan perbedaan metode ekstraksi terhadap kemampuan dalam menghambat pertumbuhan biofilm bakteri *Cutibacterium acnes*, serta perlu dilakukan penelitian lebih lanjut mengenai metode uji bioautografi untuk mengetahui senyawa mana yang dapat menghambat pertumbuhan bakteri *Cutibacterium acnes*.

DEKLARASI KONFLIK KEPENTINGAN

Semua penulis menyatakan tidak ada konflik kepentingan terhadap naskah ini.

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Overview of Indonesian Community Pharmacy: Understanding Practice Changes

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Abstract
Community pharmacy practice in Indonesia has shifted to a patient-centered model, offering a range of services that include treatment advice, chronic disease management, and public health promotion. This shift benefits consumers who visit community pharmacies as their initial healthcare point. The Indonesian healthcare system, a mix of public and private providers, is governed by a decentralized structure, fostering significant investment in private healthcare despite access limitations due to financial capacity. Medicines distribution, managed by the District Health Office, ensures supply to primary healthcare facilities, with community pharmacies regulated by the Ministry of Health and the Indonesian National Food and Drug Agency. Despite stringent regulations mandating comprehensive services, most pharmacists are not remunerated for their services. Pharmacy staff, including formally qualified pharmacists and pharmacy technicians, are registered professionals, with recent trends indicating a shift towards employing pharmacy technicians to enable pharmacists to focus on clinical roles. Economic factors and innovative service delivery modes, such as telepharmacy and online purchasing, are expected to influence future practices, enhancing the pharmacist's role in chronic disease management and other health conditions. The evolving community pharmacy practice in Indonesia reflects broader changes in the healthcare system and professional roles, with continued progression anticipated.

Keywords: Community pharmacy; Community pharmacy services; Developing country; Healthcare system; Indonesia; Pharmacist

INTRODUCTION

In recent years, the practice of community pharmacy in Indonesia has undergone substantial changes, reflecting a broader shift towards a more integrated and patient-centered healthcare model (Hermansyah et al., 2018a; Hermansyah et al., 2018b; Wulandari et al., 2022). This transformation is not merely a superficial expansion of services but a fundamental realignment of the roles and responsibilities of pharmacists within the healthcare system. As Indonesia's population grows and the healthcare landscape evolves, community pharmacies have become initial access points for a variety of health services, ranging from chronic disease management to public health care (Hermansyah et al., 2020). This evolving role is driven by

both the increasing demand for accessible healthcare and the strategic efforts of the Indonesian government to improve health outcomes across the nation. Through a comprehensive review of these changes, we can gain a deeper understanding of how community pharmacy practices are adapting to meet contemporary health challenges and the impact these adaptations have on patient care and public health.

Extensive research has been conducted on community pharmacies in Indonesia, providing valuable insights into the current state of practice and the impacts of recent changes (Wiryanto et al., 2014; Mizranita and Pratisto, 2015; Hermansyah et al., 2018a; Hermansyah et al., 2020). These studies have documented the benefits of expanded pharmacy services, such as improved patient outcomes and increased access to healthcare. For example, research has shown that community pharmacies are effectively managing chronic diseases, providing essential health education, and promoting public health initiatives. These findings underscore the importance of community pharmacies in delivering comprehensive healthcare services (Supardi et al., 2011; Wiryanto et al., 2014; Herman and Handayani, 2015; Mizranita and Pratisto, 2015; Wulandari et al., 2022).

However, these studies also reveal significant challenges in implementing changes in community pharmacy practice. Issues such as regulatory problems, lack of adequate remuneration for pharmacists, and varying levels of access to resources highlight the complexities of evolving healthcare practices. Understanding these challenges is important for developing strategies to support the ongoing transformation of community pharmacy services (Hermansyah et al., 2018b; Hermansyah et al., 2020).

The aim of this review is to address several key questions: How effective are the expanded services in improving patient outcomes? What regulatory and operational challenges do community pharmacies face? How do economic factors and healthcare system dynamics shape the future of community pharmacies in Indonesia? By examining these aspects, the review seeks to provide a comprehensive understanding of the evolving landscape of community pharmacy practice, highlighting its contributions to patient-centered care, health promotion, and the overall healthcare system.

This manuscript aims to inform healthcare policymakers, practitioners, and the public about the vital role of community pharmacies in Indonesia. It explores their expanded functions, regulatory frameworks, and the potential for future developments, underscoring the importance of these pharmacies in enhancing healthcare delivery and addressing the needs of the Indonesian population. This review offered a well-rounded perspective on the current and future state of community pharmacy practice in Indonesia.

Community pharmacy practice in Indonesia has encompassed patient-centred care by involving a wide range of professional services and public health initiatives (Hermansyah et al., 2018a; Miller and Goodman, 2016; Makhlouf et al., 2021; Mizranita et al., 2021; Wulandari et al., 2022; Mizranita et al., 2023). Many healthcare services have increased a diversity range from the provision of advice about lifestyle, pharmacological and non-pharmacological treatments, advice on symptoms and product sales for managing minor ailments (Hermansyah

et al., 2018b; Wulandari et al., 2022). These extensions and diversification of community pharmacy services benefit to consumers who frequently visit a community pharmacy as their first destination for their health problems (Andayani and Satibi, 2016; Ferdiana et al., 2021; Mizranita et al., 2023). Indonesian pharmacists have delivered their professional services beyond the traditional roles such as the provision of medicines information, chronic disease management services, more formalized management of minor ailments, public health promotion, medication management services, and providing therapeutic decisions (e.g. smoking cessation, blood glucose and blood pressure measurements) (Kristina et al., 2015; Hermansyah et al., 2018a). Through these expanded services, Indonesian community pharmacy makes a major contribution to patient-centered care, health promotion and relief of symptoms for patients who seek advice for their health-related problems (Hermansyah et al., 2018a; Mizranita et al., 2021).

The Indonesian health care system

The Indonesian health care system has a mixture of public (state) health care and private providers that respect patient's health decisions and choices (the people have the freedom to choose). The public system is administered in accordance with Indonesia's decentralized government system, with responsibilities divided among the central, provincial, and district/municipal governments. The Indonesian Central Ministry of Health is responsible for providing standard and strategic directions, regulation and assuring financial and human resources availability. The provincial government has responsibilities of managing provincial-level healthcare facilities and monitoring district health services. District/municipal government manages the operation of district community health centres (Pusat Kesehatan Masyarakat/Puskesmas) and associated subdistrict health services facilities.

The rapid population growth will have a major effect on the health care system. Therefore, the Indonesian government has encouraged investment in the healthcare sector, resulting in an increasing number of private profit-driven providers. Private health providers are mostly self-funded. The private sector encompasses a wide range of private hospitals, individual practices (e.g. physicians and midwifery clinics), community pharmacies and clinical laboratories. However, the Indonesian government regulates the private sector through mandatory accreditation, registration, and licensing (Mahendradhata et al., 2017a). Access to the private health sector is only limited by people's ability and willingness to pay. Indonesians are free to select any private insurance policy based on their personal needs and ability to pay.

Medicines distribution in Indonesia

Since decentralization occurred in 2000, the medicine supply in the public sector has been regulated and managed by the local district government (Holloway, 2011). The District Health Office (DHO) purchases and distributes supplies and medicines to the following primary healthcare facilities: community health centres (Puskesmas), maternal child health clinics (Pondok Bersalin Desa/Polindes), community health clinics (Pos Pelayanan Terpadu/Posyandu), and mobile health clinics (Pusat Kesehatan Masyarakat

Keliling/Puskesling). A budget is provided from the central government to the DHO to manage medicines supply. District hospitals manage their procurement using the local government budget and either by charging for medicines directly to non-insured patients or the government or private insurance providers (Holloway, 2011).

All medicines are provided free of charge to patients who visit community health centres. A patient pays an Indonesian Rupiah (Rp) registration fee of 15,000 (approximately AUD 1.5) at the community health centre. A community health centre reports the physical stock on hand and the expected stock of medicines to the district health office every month (Holloway, 2011). Usually, a pharmacist is assigned to a community health centre pharmacy to manage the supply and dispense medicines. However, it is common in many districts to have no pharmacists in community health centres. Therefore, community health centre pharmacies are supervised by pharmacy technicians or other staff in the DHO (Holloway, 2011).

In Indonesia, community pharmacies and drugstores are licensed by the local government through the Ministry of Health (MoH) office and the Indonesian National Food and Drug Agency (locally known as Badan Pengawasan Obat dan Makanan/BPOM) will monitor their compliance with the regulations (President of the Republic of Indonesia, 2017). Once the license is obtained, the licensed retail (merchant) submits to the MoH and BPOM, including the provincial level. The BPOM monitors ongoing compliance with the safety regulations. In addition, the BPOM supervises foods and medicines in Indonesia (Mahendradhata et al., 2017b; President of the Republic of Indonesia, 2017).

Indonesian community pharmacy structure

There are more than 24,000 community pharmacies in Indonesia, where half of the medicines available (including prescription-only medicines) are sold through general stores, private practices, supermarkets, and street vendors. The private providers (including community pharmacies) dominate, with around 80% of the market supplying over 17,000 types of medicines, of which only 15% are generic medicines(Mahendradhata et al., 2017b; Marlina and Sardjono, 2018).

Community pharmacies in Indonesia range from small to large chain pharmacies. The Indonesian MoH stated that in 2019, Indonesia had 26,658 community pharmacies, of which approximately 60% were located on Java Island (Ministry of Health Indonesia, 2017; Ministry of Health Indonesia, 2019). An independent pharmacy is owned individually, chain pharmacies can be owned by individual or a group of owners who use the same retail branding in various locations, and state-owned enterprise community pharmacies are owned by the government. Community pharmacy ownership is not restricted to pharmacists. However, by legislation, a non-pharmacist owner must employ a pharmacist as a mandatory requirement for opening a pharmacy, and legally, a pharmacist must provide professional responsibilities during the pharmacy business hours (Indonesian Health Minister, 2004).

Community pharmacies in Indonesia generate their income from general sales of medicines and other pharmaceutical products (Andayani and Satibi, 2016). Medicines in Indonesia can be classified as follows:

1.1.1. Over-the-counter (OTC) medicines (a green circle logo) are legally allowed to be sold by pharmacists without needing a prescription to the community. OTC medicines are easily purchased in the community pharmacies and drugstores. These medicines are also available off the shelf in non-pharmacy outlets without prescription (e.g., paracetamol, antacids, vitamins, etc.).

1.1.2. Cautionary labelled medicines (a dark blue circle logo) are limited OTC medicines that can be sold to the public using a warning label (e.g. antihistamines, chloroquine, etc.).

1.1.3. Pharmacist-only medicines are known as Obat Wajib Apotek/OWA (Indonesian Health Minister, 2021) (a term similar to "pharmacy medicines" in the United Kingdom (UK) (Tullett et al., 2003) and pharmacist-only medicines in Australia) (Benrimoj and Roberts, 2005). Pharmacist-only medicines in Indonesia are available to the public without a prescription and must be supplied by a pharmacist on the premises or under the supervision of a pharmacist. Some antibiotics are listed as pharmacist-only medicines or OWA in Indonesia, such as tetracycline skin salve, chloramphenicol eye drops, and anti-tuberculosis medicines (can be sold with pharmacist's recommendations for repeat medicines after being used as initial therapy with an initial prescription).

1.1.4. Prescription-only medicines (a red circle logo and must be supplied with a valid prescription).

1.1.5. Narcotics and psychotropic substances (must be supplied with a valid prescription from a licensed prescriber by law).

By law, a community pharmacy is authorized to stock all classifications of medicines. A non-pharmacy outlet (e.g. drugstore) is only allowed to supply OTC and limited OTC medicines when qualified pharmacy technicians are in charge on the premises. However, non-pharmacy outlets such as kiosks, hawkers, and markets can be operated by anyone and are not permitted to sell OTC.

Community pharmacy regulations

Community pharmacy practice and services in Indonesia are highly regulated by the Pharmacy Practice Act and Community Pharmacy Decree (Indonesian Health Minister, 2017). Regulation covers the scope of pharmacy practice by authorizing pharmacists as the main provider of pharmacy services, community pharmacy operations, including ownership and the range of services required to be provided (e.g. medication counselling, self-medication, pharmacy home care services, and medication monitoring services). While the government requires community pharmacies to provide such services, no remuneration is allocated for pharmacists and pharmacy services in these acts. Further, the Indonesian MoH established the

Indonesian Pharmacy Service (IPS) standard practice guidelines, which incorporate a set of standard operating procedures (SOPs) that require implementation in all community pharmacies in Indonesia (Indonesian Health Minister, 2017).

Community pharmacy workforce

Pharmacy staff providing community pharmacy services are qualified pharmacists and pharmacy technicians who have gained formal qualifications. The qualified pharmacy staff are registered pharmacists and pharmacy technicians who have formal training and educational qualifications. To become a pharmacist in Indonesia, a student undertakes a minimum of five years of pharmacy education. This five-year program is divided into two parts: a four-year B.Pharm and a one-year pharmacist degree (apothecary degree) (Andayani and Satibi, 2016). On the other hand, in 2020, the President issued a government regulation that a pharmacy technician who completed either a three-year Diploma course in pharmacy or a Dip.Pharm in pharmaceutical and food analysis (pharmacy analyst) or BPharm without the one-year pre-registration pharmacist program. Pharmacists and pharmacy technicians practicing in clinical settings must be registered by the National Pharmacist Board (KFN), the Indonesian Pharmacists Association (IAI) for pharmacists, and the Indonesian Pharmacy Technicians Association (PAFI) for pharmacy technicians (Indonesia Health Minister, 2011). A pharmacy student must pass a test before being registered. The President may issue Government regulations as required to implement laws.

An individual pharmacist working within their scope of practice may depend on their training, experience, expertise, workplace, and competency, which matures in response to healthcare needs (Pharmaceutical Society of Australia (PSA), 2016). Currently, pharmacists' scope of practice in Indonesia varies from managing the preparation and supply of medicines to performing interprofessional practice with doctors and patients (Hermansyah et al., 2018a; Athiyah et al., 2019; Ferdiana et al., 2021; Mizranita et al., 2022).

Over the past decade, there has been an increasing interest in utilizing pharmacy technicians to deliver pharmacy services, mainly to provide the opportunity for pharmacist to perform their clinical roles in developing countries (Kadia and Schroeder, 2015). While pharmacists concentrate on providing patient care, pharmacy technicians are managers of medicine distribution, where they are capable. Interestingly, Indonesia's cases are quite different from those in other developing countries, where non-pharmacists and non-qualified staff often deliver many pharmacy services due to a lack of available pharmacists and pharmacists' absence.

Future practice in Indonesian community pharmacy

Future practices for community pharmacy in Indonesia are mainly influenced by economic aspects such as health-related product sales and professional services and encompass not only the product aspect but also the many modes of service delivery, including telepharmacy and online purchasing. Pharmacy product sales can be included as supplies of non-prescription medicines, prescription medicines and other goods ranging from wound dressings, diagnostic

tools (e.g. blood pressure kits), and other medical devices. Supply and sales of prescription medicines (particularly pharmacist-only medicines) involve the pharmacist or patient in managing minor ailments. For over a decade, there has been a shifting trend for pharmacy products from prescription to pharmacist-only or non-prescription medicines status. Professional services, including clinical interventions and medication reviews, are expected to strengthen the pharmacist's role in managing chronic diseases and other health conditions.

In addition, professional services such as clinical intervention and medication reviews are likely to influence future practice for community pharmacies and strengthen the pharmacist's role in the healthcare system. These clinical interventions and reviews include involvement in the management of chronic diseases such as diabetes mellitus and hypertension.

CONCLUSION

Community pharmacy practice in Indonesia has significantly evolved over recent years, transitioning from a traditional, product-focused model to a more integrated, patient-centered approach. This shift has been driven by both the changing healthcare needs of the population and strategic government policies aimed at improving healthcare delivery and outcomes. The evolution of community pharmacy practice in Indonesia is a result of both professional developments within the pharmacy sector and proactive government policies. The integration of new services and the strategic expansion of roles have positioned community pharmacies as essential components of the healthcare system. However, to fully realize their potential, it is imperative to address the regulatory and economic challenges identified in this review. By doing so, community pharmacies can continue to enhance healthcare delivery and meet the evolving needs of the Indonesian population.

CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

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The Influence of Release Modifier Differences in Formulations on the Pharmacokinetic Profile of Ketoprofen in Rats: A Scoping Review

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Abstract
The pharmacokinetic profile of drugs can be changed by genetic, environmental, and physiological variables such as age, sex, pregnancy, and different preparations and formulations. Ketoprofen is widely used in many different preparations and formulations. The various formulations can made by adding solubilizing and extending release agents. This study aimed to determine the influence of formulation differences on the pharmacokinetic profile of ketoprofen in rats. This research was a Literature Review. Articles were retrieved from the ScienceDirect and PubMed databases from 2011 to 2020. The inclusion criteria were the research article, the presence of ketoprofen was formulated with the addition of a solubilizing or extended-release agent, given orally and available in open access. The study resulted in differences in formulation, notably the addition of various dissolving agents or extended-release agents, which caused changes in the pharmacokinetic profile of ketoprofen. The highest increase in the pharmacokinetic parameters C_{max} and AUC of ketoprofen was observed with poloxamer-188 as a release modifier agent. Therefore, the use of release modifier agents could have a significant effect on the drug's pharmacokinetic profile.

Keywords: Ketoprofen; Pharmacokinetics Profile; Systematic Review; Formulation

1. INTRODUCTION

Ketoprofen is a non-steroidal anti-inflammatory inhibition drug (NSAID) derived from propionic acid derivatives, which have anti-inflammatory, analgesic, and antipyretic properties in acute and chronic rheumatoid arthritis therapy and for the treatment of osteoarthritis (Kuczynska and Nieradko-Iwanicka, 2022; Mariniello et al., 2022; Sarzi-Puttini et al., 2010). Ketoprofen is classified as a BCS class II (Biopharmaceutical Classification System) drug with low solubility and high permeability. Oral ketoprofen is very effective because of its relatively short onset, about 30 minutes, with a duration of action of 6 hours. However, ketoprofen has several side effects across various systems: cardiovascular reactions (peripheral edema), central nervous system effects (headache, drowsiness), dermatological issues (skin sensitization and

photosensitization after topical use), hematological effects (edema, platelet dysfunction), hepatic effects (increased liver enzymes), ocular effects, renal issues, respiratory effects (asthma), systemic reactions (sweating, itching), and gastrointestinal problems (vomiting, diarrhea, ulcers, and stomach bleeding) (Carbone et al., 2013; Thibault et al., 2019). Additionally, when administered orally, ketoprofen undergoes first-pass metabolism, which affects its bioavailability in the human body (Pandey et al., 2023; Zhang et al., 2021).

Ketoprofen works by inhibiting the cyclooxygenase (COX) enzyme, thus inhibiting the formation of prostaglandins, and it can have anti-inflammatory, analgesic, and antipyretic effects (Fox et al., 2016). Ketoprofen is more effective in managing pain than paracetamol (Gazal and Al-Samadani, 2017; Velásquez et al., 2014). Ketoprofen is widely used as a postoperative analgesic and is effective for moderate to severe pain (Gaskell et al., 2017). In a previous study, ketoprofen demonstrated superior efficacy to diclofenac in alleviating post-surgical pain, as evidenced by a longer duration of analgesia and a lower incidence of patients requiring initial rescue analgesia following surgery (Velásquez et al., 2014). Furthermore, it reported that administering dexketoprofen trometamol 30 minutes before the conclusion of surgery resulted in adequate analgesia, diminished opioid consumption, and a reduced necessity for rescue analgesics compared to diclofenac sodium in patients undergoing laparoscopic cholecystectomy (Anil et al., 2016).

Ketoprofen is widely chosen to treat moderate to severe pain because it can reduce the risk of bleeding, nausea, and vomiting. It indicates ketoprofen has many advantages as a postoperative analgesic. Another advantage of ketoprofen is having a short onset, and it can quickly deal with pain in patients, especially in postoperative patients (Gaskell et al., 2017). Ketoprofen is widely used in different preparations and formulations and can change the drug's pharmacokinetic profile.

Drug pharmacokinetics describes changes in drug concentration per unit of time after administration of a particular drug dose (Fan and De Lannoy, 2014). It is used to determine the dosage setting of a drug, and it is necessary to know its pharmacokinetic profile. The pharmacokinetic profile has three parameters: primary, secondary, and derivative. Primary pharmacokinetic parameters include absorption rate, volume distribution (Vd), and clearance (Cl). Secondary pharmacokinetic parameters include elimination half-time (t½ elimination) and elimination rate constant (Ke). Meanwhile, the derived pharmacokinetic parameters include peak levels (C_{max}), peak time ($_{Tmax}$), and Area Under Curve (AUC).

The pharmacokinetic profile of a drug can change. It can happen because of the many factors that play a role, including genetic, environmental, and physiological variables such as age, sex, and pregnancy, as well as using different preparations and formulations. Other

formulations can be made by adding a new active compound or excipient, such as solubilizing and extending release agents.

The impact of changing the pharmacokinetic profile of a drug is the achievement of the pharmacokinetic target of the drug (Adepu and Ramakrishna, 2021; Van der Merwe et al., 2020). This study aimed to determine the effect of solubilizing addition and extended-release agents on the pharmacokinetic parameters of ketoprofen through a literature review of existing research.

2. MATERIALS AND METHODS

2.1. Materials and tools

The search strategy for identifying papers in this study was to make questions by formulating them in the form of PICO (Population, Intervention, Comparison, and Outcome), then matching the keywords obtained and limiting the paper according to inclusion and exclusion criteria (see 2.3). The articles were searched using MeSH (Medical Subject Headings) terms, a controlled vocabulary of biomedical and health-related terms used to describe the subject of a journal article, making searches more specific. The data sources used were published papers from PubMed and ScienceDirect with the following terms and keywords: "Ketoprofen" and "Pharmacokinetics," adjusted according to the search instructions of each respective database. The databases and search engines used focused on medical and herbal medicine research.

2.2. Search strategy and selection of articles

The selection of articles was based on the formulation of research questions. The formulation of these questions focused on predetermined research topics. Determination of the formulation of the problem in this study referred to the PICO instrument, which aimed to make the search method more selective and sensitive so that the literature obtained was genuinely related to the research topic (Methley et al., 2014). The PICO instrument consists of four elements, namely: Population (P): Rats, Intervention (I): Formulated ketoprofen without the addition of solubilizing and extended-release agents, Comparison (C): Formulated ketoprofen with the addition of release modifier agents, Outcome (O): Pharmacokinetics parameter

2.3. Inclusion and exclusion criteria

All candidate articles were considered eligible for the literature review to inclusion criteria: 1) original research paper; 2) published from 2011−2020; 3) available through open access; 4) utilized rat test animals as research subjects; 5) involved ketoprofen formulated with the addition of release modifier agents and administered orally; 6) available study of the pharmacokinetic profile of ketoprofen. All articles, non-original papers (e.g., review articles or

editorial texts), and multiple publications (duplicate entries within the database used) were excluded.

2.4. Quality assessment

Quality assessments were evaluated using instruments from the Center for Laboratory Animal Experimentation SYRCLE's Risk of Bias Tool for Animal Studies (Hooijmans et al., 2014). This instrument was one of the recommended instruments for evaluating papers in research with animals (Zeng et al., 2015). This analysis with the RoB SYRCLE instrument had ten critical points related to the bias of a study. Points 1 to 3 explained selection bias, points 4 and 5 explained performance bias, points 6 and 7 explained detection bias, point 8 explained attrition bias, point 9 explained reporting bias, and point 10 explained other biases.

2.5. Data extraction

Data extraction in this research discussed data on this study that have significant changes on the pharmacokinetics profile of ketoprofen and have the same parameters as other selected studies.

2.6. Data Analysis

In synthesizing data, a qualitative approach in systematic review was used to synthesize (summarize) the results of descriptive qualitative research. This method of synthesizing the results of research is called "meta-synthesis." Data analysis processing was carried out descriptively by summarizing and extracting the data obtained to determine the influence of formulation differences on the pharmacokinetic profile of ketoprofen in rats.

3. RESULTS AND DISCUSSION

3.1. Article selection

The article was selected by reading the articles regarding ketoprofen's pharmacokinetic profile and their addition with or without solubilizing and extended-release agents. This study sample selection was based on the PRISMA Guidelines (Moher et al., 2009). The PRISMA guidelines consist of a flow chart with four phases describing the criteria: identification, screening, eligibility, and inclusion of paper reports included in the scope of review (Liberati et al., 2009). Outlines of the procedure for including or excluding potential studies in a flowchart based on PRISMA guidelines are depicted in Figure 1.

The search results using the appropriate keywords in the PubMed and ScienceDirect databases found 695 identified reference articles (128 from PubMed and 567 from ScienceDirect). Articles related to the category of duplicate papers were eliminated, so the total number of articles that could be screened in the full text was 685. Observations based on titles and abstracts found 87 articles with criteria and relationships to the scope of the review. An assessment of these 87 articles was conducted based on the predetermined inclusion and

exclusion criteria. A total of 69 articles were excluded for not studying the pharmacokinetic profile of ketoprofen (reason 1), 1 article was excluded for not being original research (reason 2), 19 articles were excluded for not using rat test animals or ketoprofen formulations with the addition of release modifier agents (reason 3), and two articles were excluded because the full text was not accessible (reason 4).

Rats were chosen as subjects because, in formulation development research, most pharmacokinetic profile studies use rats before progressing to other test subjects. This approach facilitates easier comparison of pharmacokinetic parameters based solely on the effects of formulation differences. The papers excluded are in the form of article reviews and articles irrelevant to the related topic based on the criteria applied. Three articles met the inclusion criteria after screening, identification, and eligibility processes.

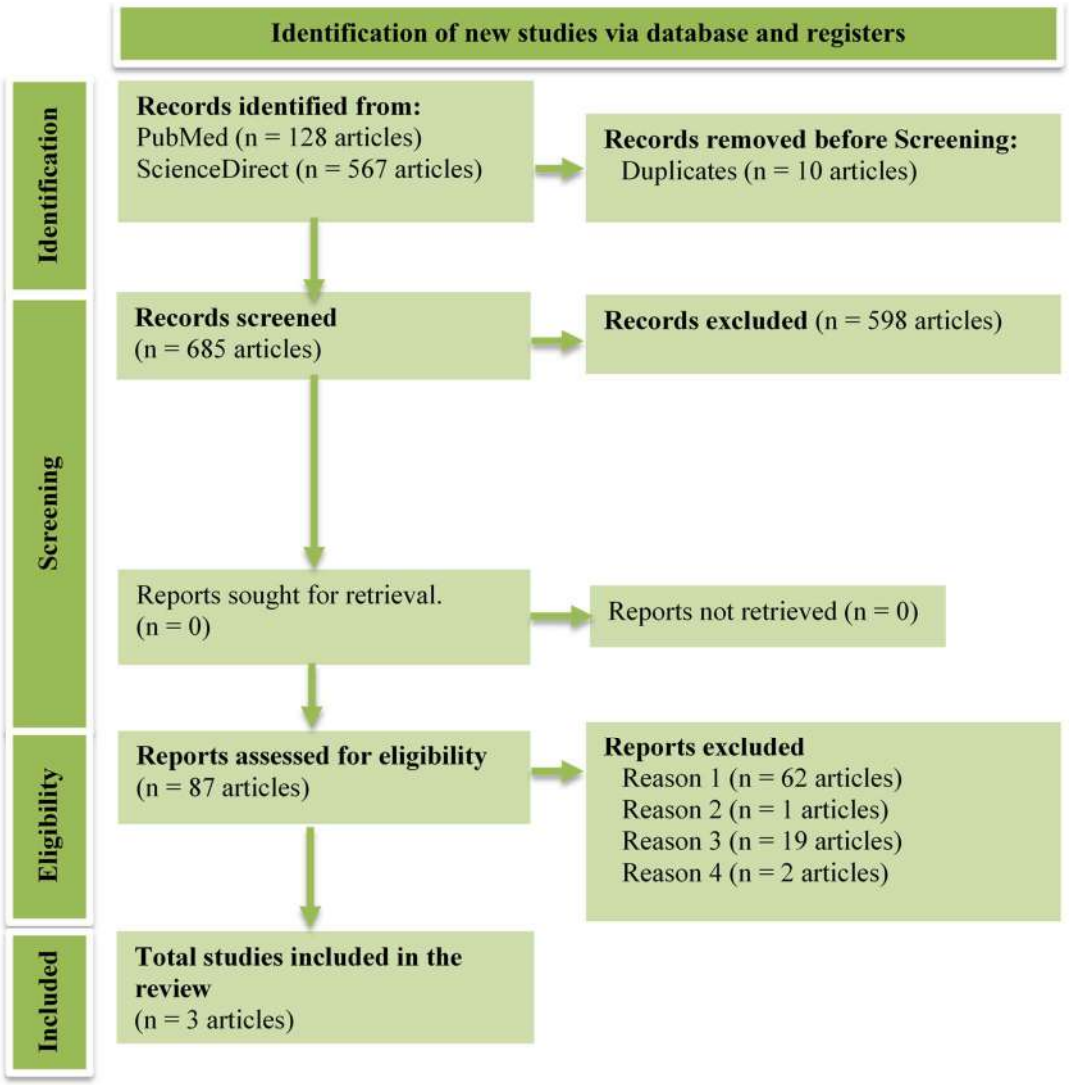

Figure 1. Flow PRISMA diagram of the article selection process.

3.2. Quality assessment of the article

A reporting study on paper quality and risk of bias from papers is carried out using the SYRCLE risk of bias tool where there are ten items related to 6 types of bias (selection, performance, detection, attrition, reporting, and other preferences) (Hooijmans et al., 2014).

The results of the evaluation based on these instruments each point present information that researchers can assess as positive if they choose "Yes," which indicates a low risk of bias; negative when selecting "No," which indicates a high risk of bias; and is not clear when selecting "Unclear" which means if the detail is not sufficient at that point. The included studies reported an average low risk of bias of 9 out of 10 points. The lowest score was 6 out of 10 points, and the highest-scoring study reported 10 points out of 10. The percentage of bias risk assessment on the paper is presented in Table 1.

From the existing domains, it can be determined which one most influences the study results. In this case, the baseline characteristics (selection bias) are the most important because the pharmacokinetic profile's value significantly affects the type and test animal used in presenting the drug profile. The bias assessment for this domain is stated to have a low-risk bias with the result "✓" for each paper used. Furthermore, this paper can be used as data in this study.

Table 1. The study quality assessment includes the percentage of risk bias from the paper. Abbreviations: n.a.: not available.

Assessment parameter	Article		
	Pai *et al.*, 2011	**Fischer *et al.*, 2012**	**Mazzoni *et al.*, 2017**
Sequence generation	✓	✓	✓
Baseline characteristic	✓	✓	✓
Allocation concealment	✓	✓	✓
Random housing	✓	✓	✓
Blinding (performance bias caregiver)	n.a.	n.a.	n.a.
Random outcome assessment	n.a.	n.a.	n.a.
Blinding (detection bias assessors)	✓	✓	✓
Incomplete outcome data	✓	✓	✓
Selective outcome reporting	✓	✓	✓
Other sources of bias	✓	✓	✓

3.3. Data synthesis and discussion

The research results on the pharmacokinetic profile of ketoprofen in rats are summarized in Table 2. Three articles describe the pharmacokinetic profile values of ketoprofen with different results for each pharmacokinetic profile parameter.

Poloxamer is a nonionic polyoxyethylene-polypropylene copolymer used in the preparation of pharmaceuticals as emulsifiers or solubilizers and is used in drug delivery systems. Polyoxyethylene is hydrophilic, increasing ketoprofen's solubility (Chiappisi, 2017). The bioavailability of the drug can be affected by the solubility of the drug (Diaz-Reval et al.,

2001). Increased solubility of ketoprofen with poloxamer-188 will increase the amount of ketoprofen absorbed and absorption of maximum ketoprofen. Poloxamer-188 is also used to increase the dissolution rate of ketoprofen by reducing surface or interfacial tension. Besides, Poloxamer-188 is capable of aggregating to form micelle structures. Forming this structure is one of the advantages of solubilizing drugs that are difficult to dissolve in water, such as ketoprofen (Newa et al., 2007). This mechanism contributes to the increased AUC and C_{max} values. The increase in the Cmax value in ketoprofen suspension with 5 mg/mL poloxamer-188 was 18.252 µg/mL (613.5%).

Table 2. Summary of pharmacokinetic parameters from selected articles. Abbreviations: mg/kg BW (milligram per kilogram of body weight); C_{max} (maximum concentration); t_{max} (maximum time to peak); AUC (area under the curve); µg/mL (micrograms per milliliter); µg h/mL (micrograms hours per milliliter); $scCO_2$ (Supercritical fluid CO_2).

Dosage form and dose (mg/kg BW)	Addition solubilizing or extended-release agent	Pharmacokinetic parameter			Article
		C_{max} (µg/mL)	T_{max} (h)	AUC (µg h/mL)	
Tablet (4)	-	0.0063	1.50	0.0137	(Pai et al., 2011)
SR tablet (4)	Etil selulosa and *Shellac*	0.0045	5.00	0.0402	
Suspension (5)	-	2.975 ± 0.288	-	185 ± 43	(Fischer et al., 2012)
	Poloxamer-188	21.227 ± 2.146	-	796 ± 272	
Solution (1.27)	-	5.171 ± 1.210	-	665 ± 104	
	Poloxamer-188	6.816 ± 0.652	-	720 ± 145	
Capsule (14.2)	-	0.488 ± 0.105	3.53 ± 1.00	19.2 ± 2.94	(Mazzoni et al., 2017)
	$scCO_2$	0.657 ± 0.078	1.55 ± 0.28	24.3 ± 2.4	

Meanwhile, the increase in the AUC value with adding 5 mg/mL poloxamer-188 was 611 µg·h/mL (330.2%). This improvement in the pharmacokinetic profile was caused by the presence of Poloxamer-188 in the formulation, which increased the solubility of ketoprofen by approximately 2.5 times (Fischer et al., 2012). This result was consistent with other research, which showed that Poloxamer 188 could enhance the solubility of drugs and the speed of their release from the dosage form, thereby increasing their bioavailability (De Stefani et al., 2022; Munir et al., 2022).

The Ethyl Cellulose-Shellac Coating (ECS) utilizes ethyl cellulose and shellac as binders and polymers to modulate drug release for sustained release (SR) applications. Shellac restricts drug release in the gastric environment, while ethyl cellulose governs drug release within the intestinal region (Pearnchob et al., 2003; Wakamatsu et al., 2021). The increase in ECS t_{max} values compared to immediate release (IR) was 3.5 hours, representing a percentage increase of 233.3%. This significant increase in t_{max} value is due to the slow release of the drug, causing

a more prolonged absorption period for the released ketoprofen to reach maximum levels (Pai et al., 2011).

On the other hand, there was a decrease in the C_{max} value for ECS compared to IR by 28.5%. The C_{max} value decreases because ECS releases the drug slowly and gradually in small quantities, resulting in a smaller amount of drug being absorbed and a lower maximum level in plasma. The use of an inert ethyl cellulose matrix, which is insoluble in water, slightly inhibits the dissolution of ketoprofen, causing limited drug absorption and a decrease in maximum plasma concentration (van der Merwe et al., 2020; Vueba et al., 2013). Furthermore, the AUC parameter for ECS is three times greater than the AUC for IR. This difference is caused by the ECS extended-release mechanism, which results in the drug remaining in the body longer, thus increasing the AUC value compared to IR (Pai et al., 2011).

Supercritical fluid CO_2 ($scCO_2$) is used as a polymer impregnation to increase the solubility of ketoprofen, resulting in more excellent absorption of the drug. Consequently, the C_{max} value is significantly higher than the control (Sabegh et al., 2012; Verano Naranjo et al., 2021). During the impregnation process using $scCO_2$, ketoprofen is obtained from the form the crystals become amorphous. Ketoprofen is a BCS drug class II, which means it has poor solubility in water; therefore, its solubility can be increased by taking advantage of the amorphous form. Increased solubility of ketoprofen causes the drug to be easily absorbed so that the maximal value is much higher than controls (Abou-Taleb et al., 2023; Das et al., 2021). The difference is an increase in the C_{max} value of 0.169 µg/mL with a percentage of 34.6%, and the difference in the decrease in the t_{max} value is 1.98 h with a rate of 127.7%. The results of the analysis of the literature study from the three papers used above used a significance level of 5% or p-value <0.05. Level significance indicates the probability of error of a quantity statistic to be used to estimate parameters or apply to its population. It shows that the three papers above have a tolerable likelihood of error in the study by 5%. Based on the percentage change, the highest increase and lowest values of C_{max} were found in poloxamer-188 (613.5%) and $scCO_2$ (34.6%), respectively. The highest percentage increase and lowest AUC values were found in poloxamer-188 (330.2%) and ethyl cellulose + shellac (193.4%).

The highest percentage increase in C_{max} was in poloxamer-188 (613.5%), while the lowest was in $scCO_2$ (34.6%). It is significant because Poloxamer 188 utilizes a dosage form suspension integrated with a solid dispersion system, whereas ScCO2 is administered as a capsule containing a powder with a low dissolution rate. The reliable dispersion technique has the following advantages: particle size reduction, barriers to crystallization occurring in medicinal ingredients, and solubility enhancement (Malkawi et al., 2022; Patel et al., 2022). Poloxamer-188 can increase lipid packing density through direct incorporation into phospholipid bilayers, modulated by lipid membrane surface tension, as demonstrated by in vitro lipid monolayer experiments. Additionally, Poloxamer 188 can repair damaged cell membranes and was approved by the FDA over 50 years ago as a copolymer for pharmaceutical and cosmetic applications (Moloughney and Weisleder, 2012).

Mixing hydrophobic drugs with hydrophilic carriers like poloxamer-188 can increase the drug release surface area and decrease the interfacial tension between hydrophobic drugs and the dissolution medium, leading to enhanced drug release (Munir et al., 2022). This mechanism enables medicines difficult to dissolve in water, such as ketoprofen, to be absorbed more effectively than when using ScCO2 in capsule form (Newa et al., 2007).

The highest percentage increase in AUC was found with Poloxamer 188 (330.2%), while the lowest was with ECS (193.4%). This result is because ethyl cellulose, used in ECS, is a matrix with inert and water-insoluble properties, inhibiting the drug dissolution rate (Wasilewska & Winnicka, 2019). Using ECS (2 – 7% w/w) in tablet preparations can slow down the dissolution rate of ketoprofen, resulting in the drug being released in small amounts and a smaller amount being absorbed. The study indicates that higher ECS concentrations correlate with ketoprofen's lower in vitro release profile. In contrast, poloxamer-188 at a concentration of 5 mg/mL can increase the solubility and formation of micelle structures, thereby enhancing the dissolution rate of ketoprofen. The higher the concentration of poloxamer-188, the better the drug solubility, leading to an improved drug release profile (Newa et al., 2007). Therefore, in the formulation of drug preparations, it is necessary to consider the additional ingredients used according to the desired drug release profile. Using poloxamer-188 can enhance the dissolution profile and bioavailability of the drug. At the same time, ECS can slow down the dissolution rate, making it suitable for preparations with slow-release purposes. Additionally, attention must be paid to the concentration of the release modifier agent used, as it will affect the resulting dissolution profile and bioavailability.

4. CONCLUSION

The study concluded that poloxamer-188, as a release modifier agent, causes a significant increase in the C_{max} and AUC of ketoprofen. Meanwhile, the use of ethyl cellulose-shellac caused a rise in t_{max} and AUC but resulted in a decrease in C_{max}. Additionally, the use of $ScCO_2$ significantly increased both C_{max} and t_{max}. Overall, the most significant changes in C_{max} and AUC were observed with the addition of poloxamer-188.

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CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

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