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Kementerian Riset, Teknologi, dan Pendidikan Tinggi
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LAPORAN AKHIR PENELITIAN MULTI TAHUN

ID Proposal: 86700e82-de7e-47d3-8039-e6d3aea0357d
laporan akhir Penelitian: tahun ke-1 dari 2 tahun

1. IDENTITAS PENELITIAN

A. JUDUL PENELITIAN

Ekplorasi Bioaktivitas dan Analisis Metabolomik Tanaman Pucuk Merah (Syzygium myrtifolium) sebagai Sumber Senyawa Antimikroba dan Antikanker

B. BIDANG, TEMA, TOPIK, DAN RUMPUN BIDANG ILMU

Bidang Fokus RIRN / Bidang Unggulan Perguruan Tinggi	Tema	Topik (jika ada)	Rumpun Bidang Ilmu
Kesehatan	-		Biologi Farmasi

C. KATEGORI, SKEMA, SBK, TARGET TKT DAN LAMA PENELITIAN

Kategori (Kompetitif Nasional/ Desentralisasi/ Penugasan)	Skema Penelitian	Strata (Dasar/ Terapan/ Pengembangan)	SBK (Dasar, Terapan, Pengembangan)	Target Akhir TKT	Lama Penelitian (Tahun)
Penelitian Kompetitif Nasional			SBK Riset Dasar	3	2

2. IDENTITAS PENGUSUL

Nama (Peran)	Perguruan Tinggi/ Institusi	Program Studi/ Bagian	Bidang Tugas	ID Sinta	H-Index
MUNAWAROHTHUS SHOLIKHA - Ketua Pengusul	Institut Sains Dan Teknologi Nasional	Farmasi	Analisis Fitokimia, Uji antioksidan, Uji toksisitas, Uji Antikanker, Analisis Metabolomik	6127058	1
VILYA SYAFRIANA -	Institut Sains	Farmasi	Metode Ekstraksi,	6075403	2

Anggota Pengusul	Dan Teknologi Nasional		Standardisasi Simplisia, Uji Antimikroba, Analisis Metabolomik		
YASMAN - Ketua TPM	Universitas Indonesia	Ilmu Kelautan	membina dalam bidang analisis metabolomik dan bioaktivitas tanaman	5985605	6
RATNA YUNIATI - Anggota TPM	Universitas Indonesia	Biologi	membina dalam bidang taksonomi dan fisiologi-kimia tumbuhan	5986313	4
ROSARIO TRIJULIAMOS MANALU - Anggota Pengusul	Institut Sains Dan Teknologi Nasional	Farmasi	uji antimikroba dan uji in silico	6074519	0

3. MITRA KERJASAMA PENELITIAN (JIKA ADA)

Pelaksanaan penelitian dapat melibatkan mitra kerjasama, yaitu mitra kerjasama dalam melaksanakan penelitian, mitra sebagai calon pengguna hasil penelitian, atau mitra investor

Mitra	Nama Mitra
Mitra Pelaksana Penelitian	Dr. rer. nat. Yasman, S.Si., M.Sc.

4. LUARAN DAN TARGET CAPAIAN

Luaran Wajib

Tahun Luaran	Jenis Luaran	*Status target capaian (accepted, published, terdaftar atau granted, atau status lainnya)*	*Keterangan (url dan nama jurnal, penerbit, url paten, keterangan sejenis lainnya)*
1	Artikel di Jurnal Internasional Terindeks di Pengindeks Bereputasi	Submited	HAYATI Journal of Biosciences
2	Artikel di Jurnal Internasional Terindeks di Pengindeks Bereputasi		Indonesian Journal of Pharmacy

Luaran Tambahan

Tahun Luaran	Jenis Luaran	*Status target capaian (accepted, published, terdaftar atau granted, atau status lainnya)*	*Keterangan (url dan nama jurnal, penerbit, url paten, keterangan sejenis lainnya)*

5. ANGGARAN

Rencana anggaran biaya penelitian mengacu pada PMK yang berlaku dengan besaran minimum dan maksimum sebagaimana diatur pada buku Panduan Penelitian dan Pengabdian kepada Masyarakat

Total RAB 2 Tahun Rp. 0

Tahun 1 Total Rp. 0

Jenis Pembelanjaan	Komponen	Item	Satuan	Vol.	Biaya Satuan	Total

Tahun 2 Total Rp. 0

Jenis Pembelanjaan	Komponen	Item	Satuan	Vol.	Biaya Satuan	Total

Tahun 3 Total Rp. 0

Jenis Pembelanjaan	Komponen	Item	Satuan	Vol.	Biaya Satuan	Total

6. KEMAJUAN PENELITIAN

A. RINGKASAN

Genus Syzygium memiliki beberapa spesies tumbuhan dengan nilai historis tinggi dalam sistem pengobatan tradisiona, seperti Syzygium aromaticum (cengkih) dan Syzygium polyanthum (salam). Salah satu spesies lain dari genus ini yang juga berpotensi sebagai tanaman obat adalah Syzygium myrtifolium atau dikenal dengan tanaman pucuk merah. Pucuk merah selama ini dimanfaatkan sebagai tanaman hias atau pembatas jalan. Berdasarkan studi analisis fitokimia daun dari tanaman ini diketahui mengandung flavonoid, tanin, dan saponin yang dapat berperan sebagai antimikroba Informasi terkait bioaktivitas dari tanaman ini sebagai agen antimikroba belum banyak dilaporkan. Penelitian ini bertujuan untuk mengeksplorasi bioaktivitas tanaman pucuk merah sebagai agen antimikroba. Eksplorasi dilakukan pada daun muda (berwarna merah) dan daun dewasa (berwarna hijau). Ekstraksi dilakukan dengan metode maserasi menggunakan tiga macam pelarut berdasarkan kepolarannya, yaitu n- heksan (nonpolar), etil asetat (semi polar), dan etanol 96% (polar). Ekstrak kasar selanjutnya dilakukan fraksinasi menggunakan pelarut organik n-heksan dan etil asetat. Ekstrak yang diperoleh dianalisis kandungan fitokimianya secara kualitatif, lalu diuji bioaktivitasnya terhadap berbagai bakteri patogen. Hasil penelitian menunjukkan bahwa ekstrak daun dewasa memiliki kemampuan menghambat pertumbuhan beberapa bakteri uji. Hasil daya hambat diketahui lebih besar pada ekstrak etanol daun dewasa dibandingkan ekstrak lainnya. Luaran yang ditargetkan dari penelitian ini adalah diterima pada jurnal internasional bereputasi. Teknologi (TKT) dari penelitian ini adalah tingkat 2 karena pada penelitian ini dilakukan penulusuran kandungan aktivitas dari bahan uji serta uji aktivitas secara in vitro.

B. KATA KUNCI

antibakteri;ekstraksi;maserasi;pucuk merah

Pengisian poin C sampai dengan poin H mengikuti template berikut dan tidak dibatasi jumlah kata atau halaman namun disarankan seringkas mungkin. Dilarang menghapus/memodifikasi template ataupun menghapus penjelasan di setiap poin.

C. **HASIL PELAKSANAAN PENELITIAN:** Tuliskan secara ringkas hasil pelaksanaan penelitian yang telah dicapai sesuai tahun pelaksanaan penelitian. Penyajian meliputi data, hasil analisis, dan capaian luaran (wajib dan atau tambahan). Seluruh hasil atau capaian yang dilaporkan harus berkaitan dengan tahapan pelaksanaan penelitian sebagaimana direncanakan pada proposal. Penyajian data dapat berupa gambar, tabel, grafik, dan sejenisnya, serta analisis didukung dengan sumber pustaka primer yang relevan dan terkini.

1. Pengumpulan Bahan Uji

Bahan uji yang digunakan berupa daun muda yang berwarna merah dan daun dewasa yang berwarna hijau. Daun segar diperoleh dari Badan Penelitian Tanaman Rempah dan Obat (Balittro), Bogor sebanyak masing-masing 3 kg. Daun yang diperoleh dicuci, sortasi basah, dan dikeringkan dengan cara kering-angin. Daun yang telah kering kemudian disortasi kembali. Daun kering kemudian dihaluskan dengan menggunakan blender dan diayak dengan mesh 60 dan 44 [1]. Proses pengayakan dilakukan untuk memperoleh ukuran partikel serbuk simplisia yang homogen. Ukuran yang homogen pada serbuk akan mengoptimalkan proses penyarian karena interaksi antar partikel dan pelarut menjadi rata [1,2]. Hasil pengayakan serbuk simplisia yang diperoleh dapat dilihat pada Tabel 1.

Tabel 1. Hasil pengeringan daun muda dan daun dewasa tanaman pucuk merah

Sampel	Berat daun segar (g)	Pengayakan mesh 44 (g)	Pengayakan mesh 60 (g)
Daun muda (merah)	3.000	270	165
Daun dewasa (hijau)	3.000	300	180

2. Hasil Ekstraksi

Proses ekstraksi pada penelitian ini menggunakan tiga macam pelarut berdasarkan kepolarannya, yaitu n-heksan (nonpolar), etil asetat (semi polar), dan etanol 96% (polar). Metode ekstraksi yang digunakan adalah maserasi secara bertingkat. Maserasi dipilih karena merupakan cara penyarian yang sederhana, mudah, dan menjaga kualitas senyawa bioaktif yang tidak tahan panas [3].

Maserasi menggunakan perbandingan pelarut dan simplisia sebesaar 1:10 [1,4]. Maserasi dilakukan selama 1 x 24 jam yang disertai dengan pengadukan dan dilakukan proses remaserasi sebanyak satu kali. Pengadukan dilakukan setiap 8 jam, proses pengadukan bertujuan untuk mempermudah kontak pelarut pada rongga sel tumbuhan sehingga senyawa yang terkandung di dalamnya dapat tertarik keluar oleh pelarut, pengadukan dapat menimbulkan sirkulasi pelarut sehingga ekstraksi dapat berlangsung dengan optimal [5]. Berikut hasil organoleptik dan rendemen ekstrak yang diperoleh dari tiap sampel (Tabel 2 dan Tabel 3).

Tabel 2. Hasil organoleptik ekstrak daun muda dan daun dewasa tanaman pucuk merah

Simplisia	Pelarut	Warna	Bau	Tekstur
Daun muda (merah)	n-heksan	Hijau tua	Khas daun	padat
	Etil asetat	hijau	Khas daun	cair
	Etanol 96%	Cekoelat kemerahn	Khas daun	kental
Daun dewasa (hijau)	n-heksan	Cokelat	Khas daun	kental
	Etil asetat	Hijau tua	Khas daun	padat
	Etanol 96%	Hijau kemerahan	Khas daun	kental

Tabel 3. Persentase rendemen ekstrak daun muda dan daun dewasa tanaman pucuk merah

Simplisia	Pelarut	Berat Serbuk (g)	Berat Ekstrak (g)	Rendemen (%)
Daun muda (dm)	n-heksan	250	13,65	5,46
	Etil asetat	250	44,28	17,7
	Etanol 96%	250	199,65	79,86
Daun dewasa (dh)	n-heksan	300	76	25,33
	Etil asetat	300	12	4
	Etanol 96%	100	46,5	46,5

Berdasarkan hasil ekstraksi (Tabel 3) terdapat perbedaan besarnya hasil rendemen antara ekstrak daun muda dan daun dewasa terhadap pelarut n-heksan dan etil asetat. Pada daun muda tampak bahwa rendemen dengan pelarut semi polar etil asetat lebih besar (17,7%) dibandingkan dengan pelarut nonpolar n-heksan (5,46%), sedangkan pada daun dewasa nilai rendemen ekstrak dengan pelarut nonpolar n-heksan lebih besar (25,33%) dibandingkan dengan pelarut semi polar etil asetat (4%). Hasil ini menunjukkan bahwa daun muda memiliki lebih banyak metabolit semi polar dibandingkan senyawa nonpolar, dan hal sebaliknya pada daun dewasa.

Nilai rendemen suatu ekstrak secara umum menggambarkan jumlah senyawa aktif yang berhasil disari oleh pelarut pada sampel. Semakin besar nilai rendemn, maka senyawa aktif yang terkandung semakin tinggi [6,7]. Nilai rendemen paling tinggi baik pada ekstrak daun muda maupun dewasa ditunjukkan oleh pelarut etanol 96%. Hasil ini sesuai dengan penelitian Syafriana *et al.* (2020) yang melakukan ekstrak biji anggur dengan pelarut n-heksan, etil asetat, dan etanol 70% juga menunjukkan bahwa pelarut etanol menghasilkan nilai rendemen paling tinggi dibandingkan dua pelarut lainnya. Tingginya nilai rendemen pada pelarut etanol dikarenakan etanol diketahui sebagai pelarut universal, sehingga mampu menarik senyawa-senyawa polar dan nonpolar [8].

3. Hasil Fraksinasi

Fraksinasi merupakan suatu metode pemisahan senyawa organic berdasarkan kelarutan senyawa-senyawa tersebut dalam dua pelarut yang tidak saling bercampur, biasanya antara pelarut air dengan pelarut organik [9-10]. Ekstrak etanol dari daun merah dan daun hijau selanjutnya dilakukan fraksinasi cair-cair dengan pelarut n-heksan dan etil asetat. Hasil fraksinasi kedua ekstrak dapat dilihat pada Tabel 4.

Tabel 4. Hasil fraksinasi ekstrak etanol daun muda dan daun dewasa tanaman pucuk merah dengan pelarut n-heksan dan etil asetat

Ekstrak	Pelarut	Berat (g)	Warna	Tekstur
Ekstrak etanol daun muda (dm42)	n-heksan	3,53	Merah kecokelatan	kental
	Etil asetat	1,58	Hijau kecokelatan	padat
Ekstrak etanol daun dewasa (dh42)	n-heksan	1,22	Hijau kehitaman	Kental
	Etil asetat	1,94	Hijau kehitaman	kental

Berdasarkan data pada Tabel 4, tampak bahwa fraksi n-heksan baik pada daun muda maupun daun dewasa memiliki berat ekstrak lebih besar dibandingkan fraksi etil. Hasil ini berbeda dengan hasil ekstrak kasar yang menunjukkan nilai rendemen ekstrak etil lebih tinggi dibandingkan ekstrak n-heksan (Tabel 3). Hasil ini menunjukkan bahwa daun pucuk merah lebih banyak mengandung senyawa nonpolar dibandingkan semi-polar [10].

4. Hasil Penapisan Fitokimia

Penapisan fitokimia dilakukan untuk mengetahui kandungan metabolit sekunder yang meliputi uji alkaloid, flavonoid, saponin, tanin, steroid/triterpenoid [11-14]. Hasil penapisan fitokimia ekstrak daun muda dan daun dewasa tanaman pucuk merah dapat dilihat pada Tabel 4.

Tabel 4. Hasil penapisan fitokimia ekstrak daun muda dan daun dewasa tanaman pucuk merah

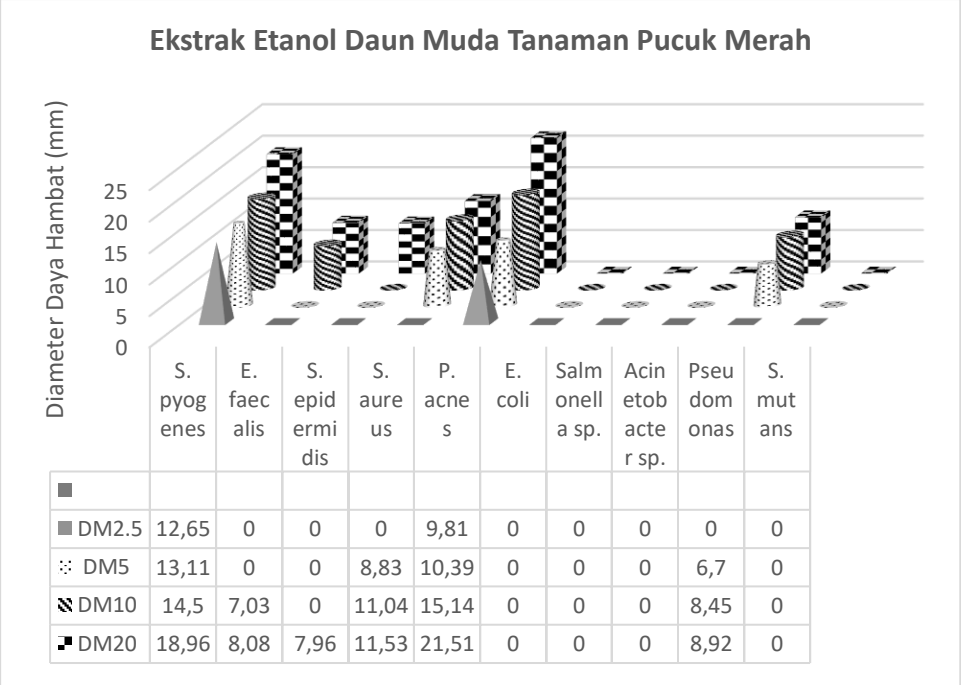
Senyawa	Hasil Pemeriksaan Fitokimia					
	Ekstrak daun muda			Ekstrak daun dewasa		
	n-heksan	Etil asetat	Etanol 96%	n-heksan	Etil asetat	Etanol 96%
Alkaloid	(+)	(+)	(+)	(-)	(+)	(+)
Flavonoid	(+)	(+)	(+)	(+)	(+)	(+)
Tanin	(-)	(+)	(+)	(+)	(+)	(+)
Saponin	(-)	(+)	(+)	(+)	(-)	(+)
Steroid	(+)	(+)	(-)	(-)	(-)	(-)
Triterpenoid	(+)	(+)	(+)	(-)	(-)	(-)

(+): mengandung senyawa yang dimaksud; (-): tidak mengandung senyawa yang dimaksud

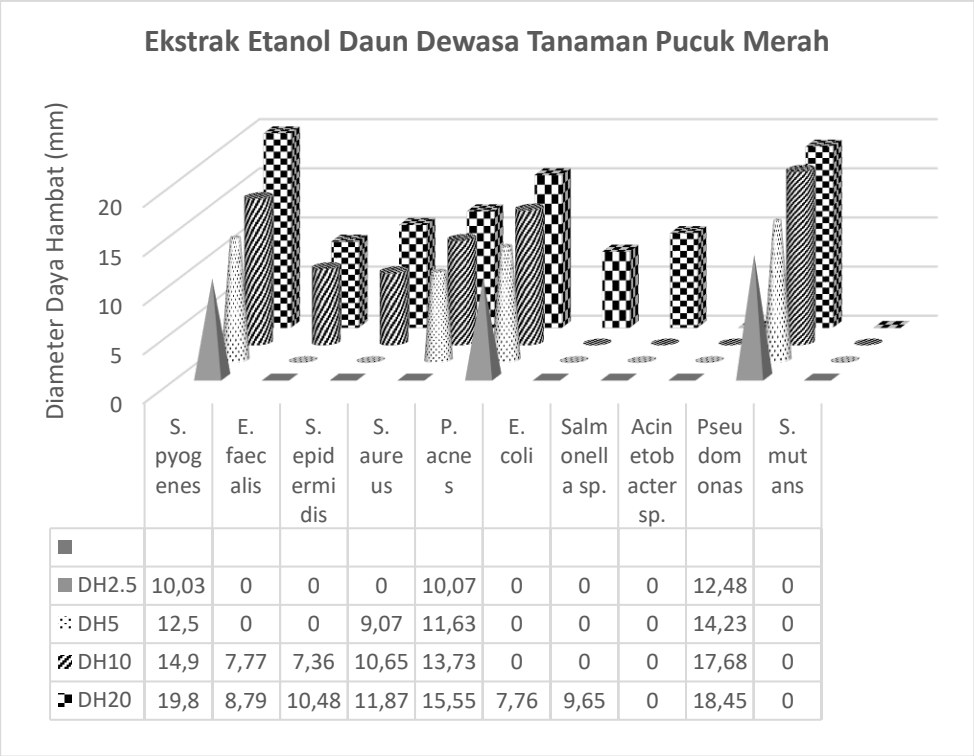
Hasil penelitian yang diperoleh pada Tabel 4 menunjukkan adanya kesamaan hasil uji pada keenam ekstrak daun pada uji flavonoid (menunjukkan hasil porsitif), sedangkan hasil uji alkaloid, tanin, saponin, steroid, dan triterpenoid menunjukkan perbedaan hasil. Kandungan flavonoid yang dimiliki ekstrak daun muda dan daun dewasa sesuai dengan penelitian yang dilakukan oleh Haryati *et al.* (2015), Syafriana *et al.* (2019), serta Ahmad *et al.* (2022) yang juga menunjukkan adanya senyawa polifenol termasuk flavonoid dalam ekstrak daun muda dan dewasa tanaman pucuk merah [15-17]. Kandungan flavonoid yang dimiliki ekstrak daun tanaman pucuk merah inilah yang diduga dapat berperan sebagai agen antibakteri [18].

5. Hasil Uji Aktivitas Antibakteri

Uji antibakteri dari ekstrak etanol daun muda dan daun dewasa tanaman pucuk merah dilakukan terhadap sepuluh bakteri patogen, yaitu *Streptococcus pyogenes*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Propionibacterium acnes*, *Escherichia coli*, *Salmonella* sp., *Acinetobacter* sp., *Pseudomonas* sp., dan *Streptococcus mutans.* Uji aktivitas antibakteri dilakukan dengan metode difusi kertas cakram. Aktivitas antibakteri ditunjukkan dengan terbentuknya zona bening di sekitar kertas cakaram [19,20]. Hasil uji aktivitas antibakteri dari ekstrak ini dapat dilihat pada Gambar 1dan 2.



Gambar 1. Aktivitas antibakteri ekstrak etanol daun muda tanaman pucuk merah



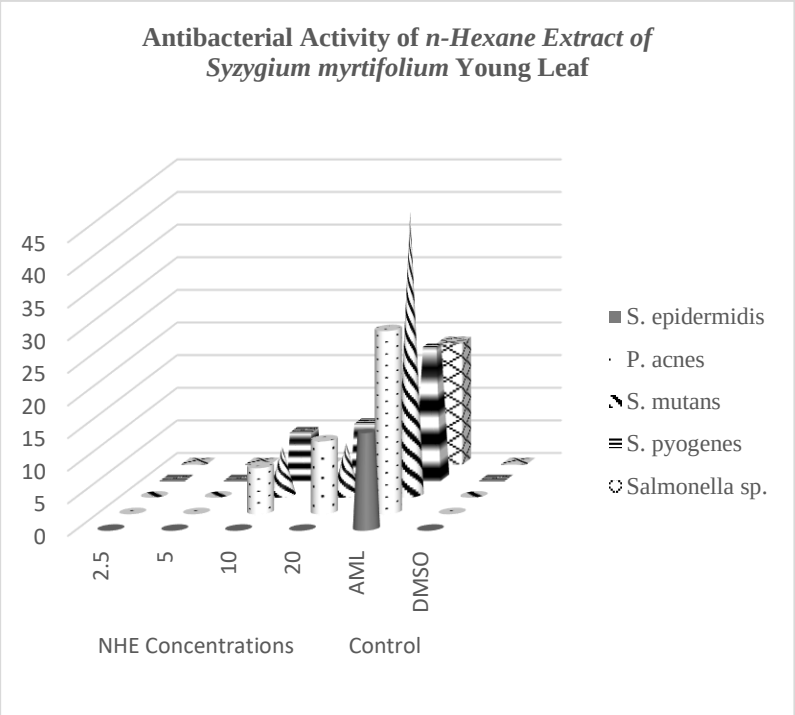
	S. pyogenes	E. faecalis	S. epidermidis	S. aureus	P. acnes	E. coli	Salmonella sp.	Acinetobacter sp.	Pseudomonas	S. mutans
DH2.5	10,03	0	0	0	10,07	0	0	0	12,48	0
DH5	12,5	0	0	9,07	11,63	0	0	0	14,23	0
DH10	14,9	7,77	7,36	10,65	13,73	0	0	0	17,68	0
DH20	19,8	8,79	10,48	11,87	15,55	7,76	9,65	0	18,45	0

Gambar 2. Aktivitas antibakteri ekstrak etanol daun dewasa tanaman pucuk merah

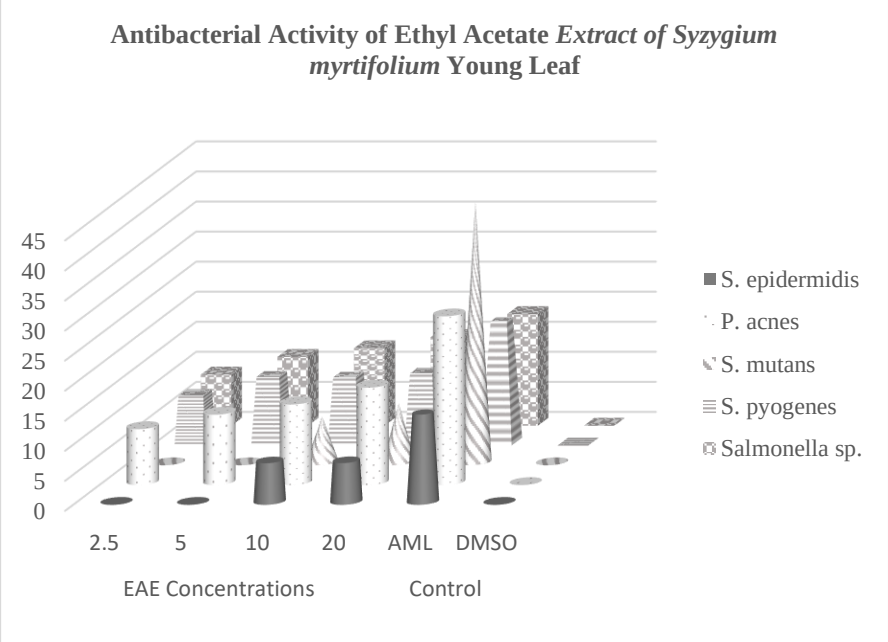
Hasil yang ditunjukkan pada Gambar 1 dan 2 tampak bahwa *Acinetobacter* dan *S. mutans* tidak terpengaruh pertumbuhannya dengan adanya ekstrak etanol daun pucuk merah. Begitu pun dengan *E. coli* dan *Salmonella* sp., meskipun pada ekstrak daun dewasa tampak ada penghambatan pada konsentrasi 20%, namun nilai daya hambatnya masih tergolong lemah. Ekstrak daun muda tanaman pucuk merah tampak memiliki daya hambat yang kuat terhadap *S. pyogenes* dan *P. acnes*, sedangkan terhadap *S. aureus*, *S. epidermidis*, dan *E. faecalis* masuk dalam kategori sedang. Penggolongan kategori daya hambat dikemukan oleh Nazri et al. (2011), yaitu nilai DDH 0-9 mm tergolong dalam aktivitas lemah; 10-14 mm tergolong kategori sedang, dan 15-20 mm tergolong kategori kuat [21]. Berdasarkan pernyataan ini, maka aktivitas ekstrak etanol daun muda tanaman pucuk merah terhadap *Pseudomonas* sp. tergolong lemah, sedangkan ekstrak etanol daun dewasa tergolong sedang hingga kuat.

Adanya aktivitas penghambatan pertumbuhan dari ekstrak etanol daun pucuk merah diduga karena adanya senyawa aktif yang terkandung dalam ekstrak tersebut. Berdasarkan data penapisan fitokimia (Tabel 4), ekstrak tersebut diketahui mengandung beberapa senyawa yang berfungsi sebagai antibakteri, diantaranya adalah alkaloid, flavonoid, tanin, dan saponin sehingga dapat memberikan diameter daya hambat sebagai aktivitas penghambatan terhadap pertumbuhan bakteri. Alkaloid diketahui dapat menghambat pertumbuhan bakteri dengan cara menghambat sintesis asam nukleat [22]. Flavonoid memiliki aktivitas untuk menghambat pertumbuhan bakteri, yaitu dengan cara mendenaturasi protein yang menyebabkan terhentinya aktivitas metabolisme sel bakteri, dengan terhentinya aktivitas metabolisme mengakibatkan kematian pada sel [23]. Tanin merupakan senyawa yang dapat merusak membran sel bakteri dengan cara mengkerutkan dinding sel sehingga menggangu permeabilitas sel, akibatnya pertumbuhan bakteri akan terhambat atau mati [24]. Saponin merupakan senyawa yang mempunyai kemampuan untuk melisiskan dinding sel bakteri karena zat aktif permukaannya mirip dengan detergen. Saponin akan menurunkan tegangan permukaan dinding sel bakteri dan merusak permeabilitas membran [25].

Selain uji ekstrak etanol kami juga menguji aktivitas esktrak n-heksan dan etil asetat dari daun muda tanaman pucuk merah. Pengujian dilakukan terhadap lima bakteri, yaitu *S. epidermidis*, *P. acnes*, *S. mutans*, *S. pyogenes*, dan *Salmonella* sp. Hasil uji dapat dilihat pada Gambar 3 dan 4.



Gambar 3. Aktivitas antibakteri ekstrak n-heksan daun muda tanaman pucuk merah



Gambar 4. Aktivitas antibakteri ekstrak etil asetat daun muda tanaman pucuk merah

Data pada Gambar 3 dan 4 menunjukkan bahwa ekstrak etil asetat memiliki kemampuan daya hambat yang lebih tinggi dibandingkan ekstrak n-heksan. Pertumbuhan kelima bakteri tampak tidak terganggu dengan adanya ekstrak n-heksan pada konsentrasi 2,5% dan 5%, sedangkan ekstrak etil asetat pada konsnetrasi yang sama menunjukkan penghambatan pertumbuhan terhadap bakteri *P. acnes*, *Salmonella*, dan *S. pyogenes*. Ekstrak n-heksan mulai menunjukkan kemampuan daya hambatnya pada konsentrasi 10% dan 20% terhadap *Salmonella* dan *S. epidermidis*. Hasil ini menunjukkan pola penghambatan yang sama dengan penelitian Nuraskin *et al*. (2020), yaitu bahwa ekstrak etil asetat dari daun *Vitex pinnata* memiliki daya hambat yang lebih besar dibandingkan ekstrak n-heksan terhadap *S. mutans* [26]. Penelitian lainnya terhadap bakteri *E. coli* juga menunjukkan pola yang sama, yaitu ekstrak etil asetat dari daun sukun dan daun sirih merah memiliki daya hambat lebih besar dibandingkan ekstrak n-heksan [27-28].

D. **STATUS LUARAN**: Tuliskan jenis, identitas dan status ketercapaian setiap luaran wajib dan luaran tambahan (jika ada) yang dijanjikan. Jenis luaran dapat berupa publikasi, perolehan kekayaan intelektual, hasil pengujian atau luaran lainnya yang telah dijanjikan pada proposal. Uraian status luaran harus didukung dengan bukti kemajuan ketercapaian luaran sesuai dengan luaran yang dijanjikan. Lengkapi isian jenis luaran yang dijanjikan serta mengunggah bukti dokumen ketercapaian luaran wajib dan luaran tambahan melalui BIMA.

Luaran yang dijanjikan belum dapat disubmit dikarenakan draft manuksrip masih dalam tahap review internal tim. Hasil diskusi terakhir pada hari ini diputuskan untuk menyempurnakan tulisan dan analisis yang masih dapat dikaji demi memberi informasi yang lebih berkualitas sehingga akan memberikan dampak yang luas bagi perkembangan ilmu pengetahuan.

Rencana luaran akan disubmit ke Jurnal BIODIVERSITAS (Terindeks Scopus Q3).

Berikut kami lampirkan draft manuskrip artikel kami untuk Jurnal Biodiversitas.

COVERING LETTER

Dear **Editor-in-Chief,**

I herewith enclosed a research article,

- [x] The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in Comments to the Editor).
- [x] The submission file is in OpenOffice, Microsoft Word (DOC, not DOCX), or RTF document file format.
- [x] The text is single-spaced; uses a 10-point font; employs italics, rather than underlining (except with URL addresses); and all illustrations, figures, and tables are placed within the text at the appropriate points, rather than at the end.
- [x] The text adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines.
- [x] Most of the references come from current scientific journals (c. 80% published in the last 10 years), except for taxonomic papers.
- [ ] Where available, DOIs for the references have been provided.
- [ ] When available, a certificate for proofreading is included.

SUBMISSION CHECKLIST

Ensure that the following items are present:

The first corresponding author must be accompanied with contact details:

- [x] E-mail address
- [x] Full postal address (incl street name and number (location), city, postal code, state/province, country)
- [x] Phone and facsimile numbers (incl country phone code)

All necessary files have been uploaded, and contain:

- [x] Keywords
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Effect of Solvent Polarity on Extraction Yield and Antibacterial Activities of *Syzygium myrtifolium* Young Leaves

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Pucuk merah (Syzygium myrtifolium) has only ever been grown for ornamental purposes. Despite the fact that this plant contains antimicrobial properties. We are investigating the antibacterial activity of this plant extracts against several pathogenic bacteria since research on this plant is still inadequate. The chosen extracts are those from n-hexane (nonpolar) and ethyl acetate (semi-polar).

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Vilya Syafriana

Effect of Solvent Polarity on Extraction Yield and Antibacterial Activities of *Syzygium myrtifolium* Young Leaves

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Abstract. *Syzygium myrtifolium* or known as pucuk merah plant in Indonesian is known as an ornamental plant, despite the fact that this plant has the ability to serve as a source of antibacterial raw material. Several studies showed that this plant has antimicrobial activity against some bacteria and fungi. However, the antibacterial activity of its young leaf extract has not been explored much. Therefore, this research was aimed to conduct further exploration regarding the antibacterial activity of the leaf using two different polarity-based solvents, n-hexane and ethyl acetate. Besides that, it also aimed to determine the yield value of the extracts. The extraction was done sequentially using the maceration process with two different solvents (n-hexane and ethyl acetate). The antibacterial activity was carried out using the disk diffusion method. The extraction revealed that the ethyl acetate extract had a greater yield value (17.7%) than the n-hexane extract (5.46%). The antibacterial activity tests showed that the ethyl acetate extract has a stronger ability to suppress bacterial growth than the n-hexane extract.

Key words: antibacterial, ethyl acetate, maceration, n-hexane, pucuk merah, young leaves

Abbreviations (if any): Balittro (Balai Penelitian Tanaman Rempah dan Obat); Depkes RI (Depatermen Kesehatan Republik Indonesia); Dirjem POM (Direktorat Jenderal Pengawas Obat dan Makanan); Kemenkes RI (Kementerian Kesehatan Republik Indonesia); MHA (Mueller Hinton Agar); NA (Nutrient Agar); IZ (Inhibition Zone); NHE (n-Hexane Extract); EAE (Ethyl Acetate Extract)

Running title: antibacterial activity of *Syzygium myrtifolium* young leaf

INTRODUCTION

Syzygium myrtifolium or known as pucuk merah plant in Indonesian is a plant that is currently found in parks, yards, and almost every highway, as well as in residential and commercial buildings. Due to its appealing color, this plant is referred to as an ornamental plant (**Figure 1**). Additionally, this plant is simple to maintain and grow, making it increasingly well-liked. However, this plant belongs to the same taxonomic genus as salam (*Syzygium polyanthum*) and cloves (*Syzygium aromaticum*), which are well-known plants in Indonesia due to their use in food and medicine. Therefore, it is very likely that this plant has the same potential as salam and cloves, including the ability to be a source of antibacterial raw materials (Haryati et al. 2015; Putri 2019; Batiha et al. 2020; Iskandi et al. 2021; Mudiana et al. 2021).

The phytochemical analysis of this plant's leaves revealed the presence of flavonoids, tannins, and saponins, all of which have antimicrobial properties (Syafriana et al. 2019; Ahmad et al. 2022). Several studies showed that this plant has antimicrobial activity against the bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella pneumoniae*, and *Staphylococcus* spp., as well as the fungi *Candida* spp. and *Cryptococcus neoformans* (Haryati et al. 2015; Syafriana et al. 2019; Ahmad et al. 2022). Our previous study has shown that the methanol extract of *S. myrtifolium* leaf has antibacterial activity against the *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* growth (Syafriana et al. 2019). Therefore, we intend to conduct further exploration regarding the activity of the leaf extract using two different polarity-based solvents, namely n-hexane and ethyl acetate.

The extraction was carried out by maceration method consecutively in a series of two solvents (n-hexane and ethyl acetate). Maceration was chosen as the simplest method of simplicial extraction (Depkes RI, 1995;

Kemenkes RI, 2017). The disk diffusion method (Kirby-Bauer disk diffusion) was used to test the antibacterial activity by measuring the clear zone formed around the disc as the Inhibition Zone (IZ) (Dafale et al. 2016; Hudzicki 2016). The findings are expected to serve as a model for the development of *S. myrtifolium* as medicinal plants besides as ornamental plants.

Figure 1. Pucuk merah (*Syzygium myrtifolium*) plant

MATERIALS AND METHODS

1.1. Chemicals and Reagents

Nutrient Agar (NA) [Oxoid], Mueller Hinton Agar (MHA) [Oxoid], Aquadest [MPLab], 70% Ethanol [Dwinika], n-Hexane [PMP], Ethyl Acetate [PMP], $FeCl_3$ [Merck], Wagner reagent [CDH], Mayer reagent [CDH], Dragendorff reagent, Ammoniak [Merck], Acetic acid anhydride [Merck], $NaNO_2$ [Merck], $AlCl_3$ [Merck], HCl [Merck], Chloroform [Merck], H_2SO_4 [Merck], DMSO [Merck], immersion oil, Crystal violet [Merck], Safranin, Lugol's iodine, 0.9% NaCl [Braun], *Blank disc* [Oxoid], the antibiotic disk of Amoxicillin [Oxoid], analytical balance [Excellent], blender [Phillips], aluminium foil [Klin Pak], Parafilm [Bemis], autoclave [B-one], incubator [Memmert], vacuum rotary evaporator [Buchi, Eyela], waterbath [Memmert], Hot plate[Joanlab], and Laminar Air Flow.

1.2. Preparation and Extraction of Sample *Syzygium myrtifolium* Leaf

S. myrtifolium young leaf were obtained from Research Agency of Spices and Medicinal Plants (Balittro), Bogor. Three kg of fresh leaves were cleansed with clean water. The leaves were air-dried for three days. The dried leaves then being crushed with a blender and sieved through mesh 44. This procedure was used to achieve homogenous size of simplicia, so that there would be equal interaction between the leaf powders and the solvent (Azwanida 2015; Sa`adah and Nurhasnawati 2015; Makanjuola 2017).

The *S. myrtifolium* young leaf was weighed as much as 250 g then extracted with maceration method using a nonpolar solvent (n-hexane) then followed by a semi-polar solvent (ethyl acetate) with ratio 1:10. The maceration was done for 24 hours and re-macerated once with the same procedure (Kemenkes RI 2017). The outcomes of the maceration were filtered using filter paper. The vacuum rotary evaporator was used to evaporate the filtrate until a thick extract was produced.

1.3. Specific and Non-Specific Parameters of Extracts

Specific parameters include organoleptic test, water soluble compound content, ethanol soluble compound content, and phytochemical screening. Non-specific parameters include total ash content and acid insoluble ash content (Depkes RI 1995; Kemenkes RI 2017). The tests were carried out at Balittro, Bogor.

1.4. Antibacterial Activity Test

The antibacterial test was carried out using the disk diffusion method to determine the diameter of Inhibiton Zone (IZ) of the extract against several pathogenic bacteria, including *Propionibacterium acnes*, *Salmonella* sp., *Staphylococcus epidermidis*, *Streptococcus mutans*, and *Streptococcus pyogenes* (Pratiwi 2008; Hudzicki 2016).

1.4.1. Bacterial Suspension Preparation

The bacteria aged 24 hours was taken 3-4 oses, then placed in a test tube containing 9 mL of 0.9% NaCl. The test tube was then vortexed to create homogeneity. The bacterial suspension was adjusted using McFarland no.3 (9.0×10^8 CFU/mL) as the turbidity reference.

1.4.2. The Extract Concentrations Preparation

The extracts were prepared at four different concentrations: 2.5%, 5%, 10%, and 20%. DMSO 50% was used as a negative control, while the antibiotic amoxicillin was used as a positive control.

1.4.3. Diameter of Inhibition Zone (IZ) Test

Each of bacterial suspension was pipetted into a petri dish containing MHA in an amount of 0.1 mL. The suspension was then distributed evenly using a stirring rod L-form. After the media and the bacterial suspension have dried, a sterile paper disk was placed onto the agar. Each concentration's extract was dripped for about 20 µL followed by incubation at 37^0C for 24 hours. The clear zone that formed around the disk was observed and measured as the Inhibition Zone (IZ).

RESULTS AND DISCUSSION

2.1. Sample Extraction

The extraction process in this study used two different solvents based on their polarity: n-hexane (nonpolar) and ethyl acetate (semi-polar). The extraction was carried out sequentially from nonpolar to semi-polar using the maceration method. Maceration was chosen because it is a simples and easy method that preserves the quality of heat-resistant bioactive compounds (Zhang et al. 2018). The maceration was repeated once for a total of 24 hours, with stirring in between. The stirring process aims to facilitate the contact of the solvent in the plant cell cavity so that the compounds in it can be extracted optimally by the solvent. Stirring can cause solvent circulation so that the extraction process can take place optimally (Dirjen POM 1986). The yields extracts were listed at **Table 1.**

Table 1. Yield extracts of *Syzygium myrtifolium* young leaf using n-hexane and ethyl acetate as solvents

Sample	Solvent	Simplicial weight (g)	Extract weight (g)	Yield (%)
Young leaves (red leaves)	n-hexane	250	13.65	5.46
	Ethyl acetate	250	44.28	17.7

The data in **Table 1** showed that the yield value of the young leaf extract increased with solvent polarity. Ethyl acetate extract had a yield value of 17.7%, whereas n-hexane had a yield value of 5.46%. The yield value of an extract indicates how many active compounds the solvent was able to extract from the sample. Higher yield values correspond to higher active compounds (Harborne 1987; Hasnaeni et al. 2019). These results corroborated those

of Hidayati et al. (2017) who discovered that *Syzygium polyanthum* leaf extract with n-hexane and ethyl acetate solvents produced a higher yield value of ethyl acetate extract than n-hexane extract.

2.2. Specific and Non-Specific Parameters of Extracts

2.2.1. Organoleptic Observations

Organoleptic observations include odor, color, and texture tests. Organoleptic tests revealed that both extracts have a distinct odor similar to the leaf samples. The color of the two extracts was reddish brown, with n-hexane extract having a deeper hue than ethyl acetate extracts. The texture of the n-hexane extract was thick, while the ethyl acetate extract was liquid.

2.2.2. Phytochemical Screening

Phytochemical screening was performed to determine the content of secondary metabolites, which included tests for alkaloids, saponins, tannins, phenolics, flavonoids, steroids, triterpenoids, and glycosides (Parbuntari et al. 2018; Wardani et al. 2019). **Table 2** showed the results of phytochemicals screening of n-hexane and ethyl acetate extracts of *S. myrtifolium* young leaf.

Table 2. Phytochemicals screening of n-hexane and ethyl acetate extracts of *Syzygium myrtifolium* young leaf

Metabolites	Extracts	
	n-Hexane	Ethyl acetate
Alkaloid	(+)	(+)
Saponin	(-)	(+)
Tannin	(-)	(+)
Phenolic	(+)	(+)
Flavonoid	(+)	(+)
Steroid	(+)	(+)
Triterpenoid	(+)	(+)
Glycoside	(+)	(+)

Note: (+): contains the metabolite; (-): do not contains the metabolite

The results obtained in **Table 2** showed that ethyl acetate extract contained alkaloids, saponins, tannins, phenolics, flavonoids, steroids, triterpenoid, and glycosides, whereas the n-hexane extract showed negative results in saponins and tannins test. The results were in accordance with the findings of Haryati et al. (2015), Syafriana et al. (2019), and Ahmad et al. (2020), who discovered flavonoid and phenolic compounds in their *S. myrtifolium* extracts. The flavonoid content in the extracts was deemed to have antibacterial properties (Permatasari et al. 2022).

2.2.3. Water and Ethanol Soluble Compound Content

The determination of extract compound content was aimed to estimate the approximate number of polar compounds (water soluble) and semi-polar or nonpolar compounds (soluble ethanol). **Table 3** showed that the n-hexane extract and ethyl acetate extract were more soluble in alcohol solutions than water solutions. The results indicated that the extraction of young *S. myrtifolium* leaf will be more advantageous with alcohol-based solvent (Putri et al. 2021).

Table 3. Water and ethanol soluble compound content of n-hexane and ethyl acetate extracts of *Syzygium myrtifolium* young leaf

Assay	Extracts	
	n-hexane	Ethyl acetate

Water soluble (%)	0.81	4.80
Ethanol soluble (%)	25.38	14.60

2.2.4. Total Ash Content and Acid Insoluble Ash Content

Total ash content is used to determine the mineral content in extracts and mineral content which is not soluble in acids where the maximum range relates to contaminants and purity (Syukri et al. 2020; Putri et al. 2021). The ash content and acid insoluble ash content was shown in **Table 4** below.

Table 4. Total ash and acid insoluble ash content of n-hexane and ethyl acetate extracts of *Syzygium myrtifolium* young leaf

Assay	**Extracts**	
	n-hexane	**Ethyl acetate**
Total ash content (%)	8.34	0.06
Acid insoluble ash content (%)	0.03	ND

ND: Not Detected

The n-hexane extract had a total ash content about 8.34%, while the ethyl acetate extract about 0.06% (**Table 4**). Results for acid insoluble ash content were only seen in the n-hexane extract, while they were undetectable in ethyl acetate extract. The total amount of ash in each extract demonstrated the presence of minerals, whereas the acid insoluble ash content pointed to the presence of sand or other residual impurities. According to these findings, ethyl acetate extract was purer than n-hexane extract (Syukri et al. 2020; Putri et al. 2021). The Indonesian Herbal Pharmacopoeia (FHI) states that the maximum allowed percentage of total ash content in an extract is 13.3%. These results showed that the total ash content of the n-hexane and ethyl acetate extracts complied with the FHI standards (Kemenkes RI 2017).

2.3. Antibacterial Activity

The antibacterial activity was performed against five pathogenic bacteria. The results can be seen in **Figures 2 and 3.**

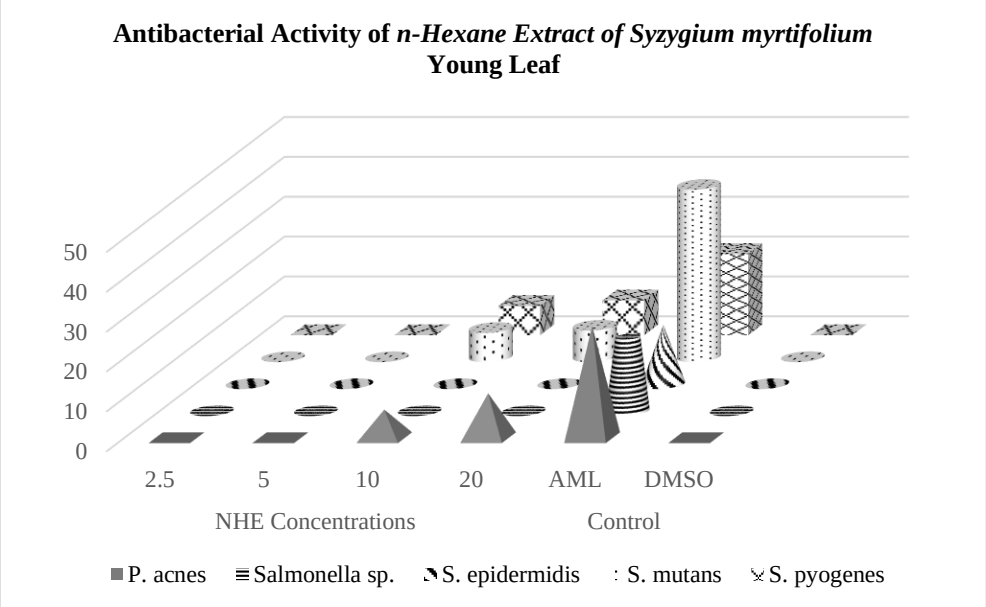

Figure 2. Antibacterial activity of n-hexane extract (NHE) of *Syzygium myrtifolium* young leaf

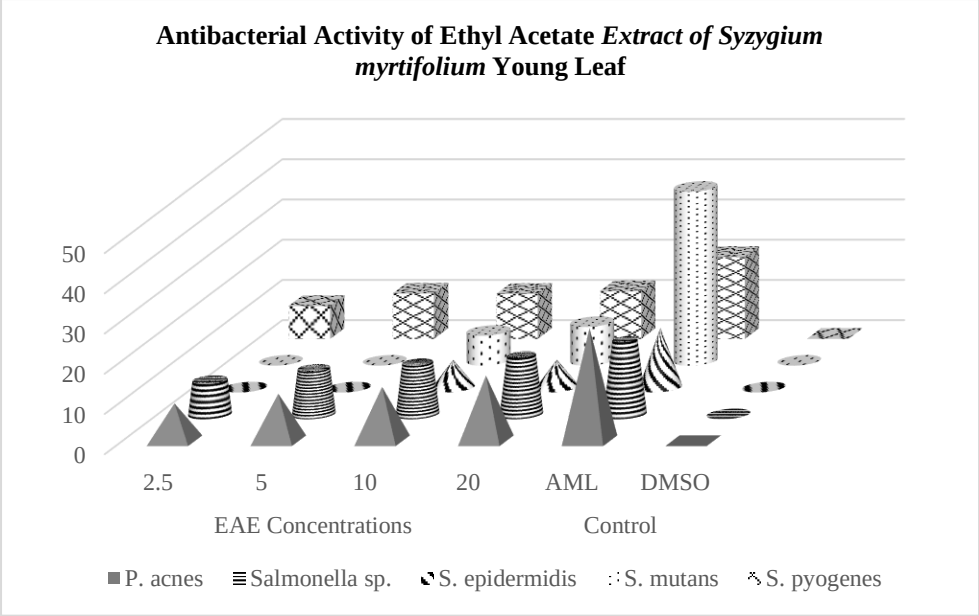

Figure 3. Antibacterial activity of ethyl acetate extract (EAE) of *Syzygium myrtifolium* young leaf

According to the data in **Figures 2 and 3**, the ethyl acetate extract has a stronger ability to suppress bacterial growth than the n-hexane extract. The five examined bacteria were still able to grow in the presence of n-hexane extract at concentrations of 2.5% and 5%. Meanwhile, the ethyl acetate extract at the same concentrations showed inhibition to the growth of *P. acnes, Salmonella* sp., and *S. pyogenes*, even though *S. epidermidis* and *S. mutans* were not affected.

N-hexane extract started to exhibit its potential to inhibit *P. acnes, S. mutans,* and *S. pyogenes* at concentrations of 10% and 20%, but it was still unable to inhibit *Salmonella* sp. and *S. epidermidis* growth (**Figure 2**). Ethyl acetate extract, which similarly showed a higher inhibition zone than n-hexane extract, could prevent the growth of the five studied bacteria at 10% and 20% concentrations (**Figure 2 and Figure 3**). These findings follow the same pattern as the research conducted by Nuraskin et al. (2020) which revealed that the ethyl acetate extract of Vitex pinnata leaf has a stronger inhibitory effect than the n-hexane extract against *S. mutans*. Another study by Veranita et al. (2020) and Armansyah et al. (2022) revealed that the n-hexane extracts of breadfruit and red betel leaves were ineffective at inhibiting the growth of *E. coli*, but the ethyl acetate extract of the two leaves could.

Different metabolite compositions in young leaves of *S. myrtifolium* extracted with n-hexane and ethyl acetate may be the cause of variability in antibacterial activity. The n-hexane extract did not contain tannins and saponins, as shown in **Table 2**. Despite the fact that saponins and tannins are known to have an impact on bacterial growth. Saponins are known to breakdown lipids in the bacterial cell membrane, reducing the membrane's surface tension and potentially leading to bacterial cell lysis. Tannins, as they are known, may prevent bacterial cells from synthesizing proteins, which could restrict the growth of the bacteria (Syafriana et al. 2019; Syafriana et al. 2021).

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Selain itu, dalam penulisan publikasi TIM Bio-UI juga sangat membantu kami dalam proses analisis dan penulisan. Salah satu masukan untuk menunda sementara submission manuskrip kami karena hasil review dari TIM Bio-UI, tulisan kami masih sangat kualitatif padahal bisa diperdalam dengan analisis kuantitatif. Saat ini, manuskrip dalam tahap review secara materi, tampilan data, dan kelugasan bahasa untuk menghasilkan publikasi yang berkualitas.

F. **KENDALA PELAKSANAAN PENELITIAN**: Tuliskan kesulitan atau hambatan yang dihadapi selama melakukan penelitian dan mencapai luaran yang dijanjikan, termasuk penjelasan jika pelaksanaan penelitian dan luaran penelitian tidak sesuai dengan yang direncanakan atau dijanjikan.

Kendala penelitian yang kami hadapi adalah kondisi kampus kami yang masih melakukan kebijakan *work from home* selama semester genap kemarin, sehingga perijinan untuk melakukan penelitian di Lab Farmasi ISTN melalui proses yang cukup lama. Hal tersebut mengakibatkan kami baru bisa melakukan penelitian sekitar bulan Juni dan Juli. Selain itu, kondisi lab yang total tidak ada aktivitas selama 2 tahun mengakibatkan banyak hal teknis yang memakan waktu terkait kondisi laboratorium yang meliputi kelayakan peralatan (ketersediaan dan kesterilan), koleksi biakan mikroorganisme (banyak yang terkontaminasi), serta ketersediaan bahan kimia dan media uji (kadaluarsa, berubah tekstur ataupun bau). Akan tetapi, hingga hari ini pelaksanaan penelitian tetap dapat dijalankan sesuai dengan ajuan tahun pertama proposal.

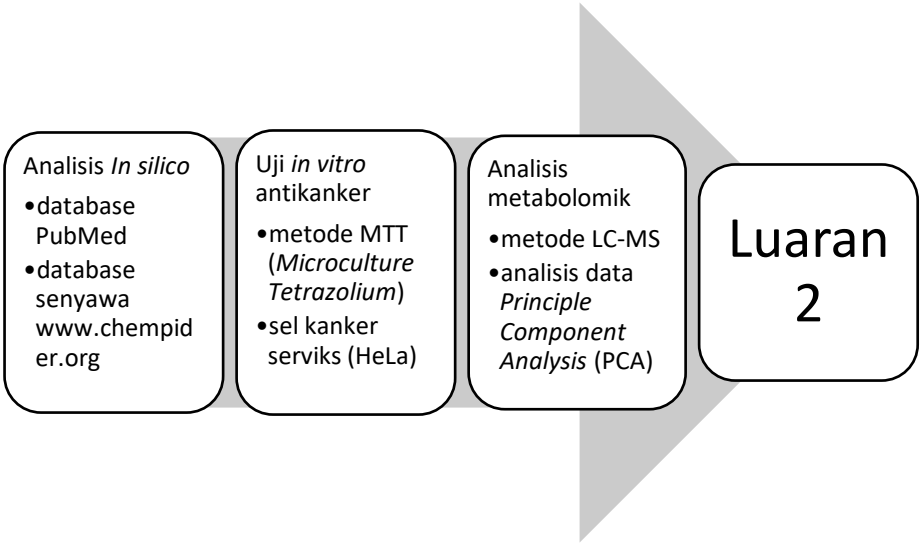
Rencana luaran berdasarkan proposal akan di-*submit* ke Jurnal HAYATI (Terindeks Scopus Q3), akan tetapi kami berencana untuk submit ke Jurnal dengan kategori indeks yang setara, yaitu BIODIVERSITAS (Terindeks Scopus Q3). Hal ini kami pilih dikarenakan jurnal Biodiversitas merupakan jurnal yang terbit secara rutin setiap bulan, sehingga kemungkinan proses perputaran artikel di Jurnal ini berlangsung cepat. Berdasarkan ini, kami berharap proses *submit* dan *review* artikel kami pun akan berlangsung cepat sehingga dapat mencapai target publikasi Jurnal Internasional sesuai ajuan proposal pada tahun pertama ini.

G. **RENCANA TAHAPAN SELANJUTNYA:** Tuliskan dan uraikan rencana penelitian di tahun berikutnya berdasarkan indikator luaran yang telah dicapai, rencana realisasi luaran wajib yang dijanjikan dan tambahan (jika ada) di tahun berikutnya serta *roadmap* penelitian keseluruhan. Pada bagian ini diperbolehkan untuk melengkapi penjelasan dari setiap tahapan dalam metoda yang akan direncanakan termasuk jadwal berkaitan dengan strategi untuk mencapai luaran seperti yang telah dijanjikan dalam proposal. Jika diperlukan, penjelasan dapat juga dilengkapi dengan gambar, tabel, diagram, serta pustaka yang relevan. Pada bagian ini dapat dituliskan rencana penyelesaian target yang belum tercapai.

Rencana penelitian di tahun berikutnya melakukan uji bioaktivitas antikanker secara *In Silico* dan *in vitro*. Analisis *In Silico* dilakukan berdasarkan Kusmardi et al. (2018) dengan target kanker adalah kanker serviks [29]. Pemodelan 2D senyawa dalam ekstrak pucuk merah dilakukan dengan database di www.chempider.org. Pencarian senyawa menggunakan database PubMed. Interaksi antara senyawa dan protein kanker serviks akan

menghasilkan nilai energi bebas (ΔG) menggunakan analisis molekuler *docking*. Uji *in vitro* bioaktivitas antikanker dilakukan dengan metode MTT (*Microculture Tetrazolium*) untuk menentukan nilai IC_{50} dari ekstrak pucuk merah menggunakan sel kanker serviks (HeLa) [30].

Analisis terakhir dari penelitian ini adalah melakukan analisis metabolomik dari ekstrak daun muda dan daun dewasa tanaman pucuk merah. Analisis metabolomik dilakukan dengan metode LC-MS [31-32]. Data metabolit yang diperoleh selanjutnya dianalisis menggunakan *Principle Component Analysis* (PCA) untuk mengelompokan data dan melihat korelasinya [30]. *Road map* penelitian tahun ke-2 dapat dilihat pada **Gambar 5** berikut.



Gambar 5. *Road map* rencana penelitian bioaktivitas antikanker daun tanaman pucuk merah di tahun ke-2

Rencana luaran di tahun ke-2 kami akan *submit* hasil penelitian di Jurnal Terindeks *Scopus Indonesian Journal of Pharmacy*. Kami menargetkan untuk menyelesaikan penelitian di bulan ke-9 dan men-*submit* artikel paling lambat di bulan ke-10.

H. DAFTAR PUSTAKA: Penyusunan Daftar Pustaka berdasarkan sistem nomor sesuai dengan urutan pengutipan. Hanya pustaka yang disitasi pada laporan akhir yang dicantumkan dalam Daftar Pustaka.

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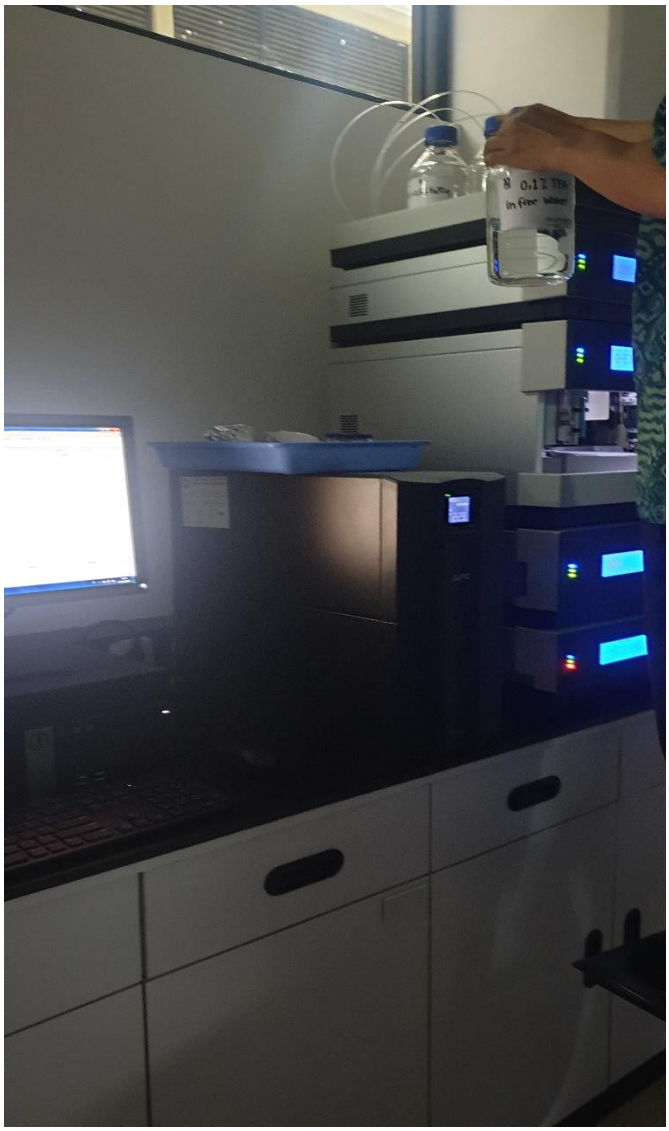
Bukti Pengerjaan Penelitian Evaporasi dan Fraksinasi di Lab Bahan Alam Bio-UI

Proses evaporasi ekstrak daun hijau tanaman pucuk merah menggunakan *vacuum rotary evaporator* merek Buchi.

Proses pengentalan ekstrak menggunakan *vacuum rotary evaporator* merek Boeco

Fraksinasi cair-cair menggunakan corong pisah



Pengenalan prinsip kerja dan operasional HPLC

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- [x] The submission file is in OpenOffice, Microsoft Word (DOC, not DOCX), or RTF document file format.
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Effect of Solvent Polarity on Extraction Yield and Antibacterial Activities of *Syzygium myrtifolium* Young Leaves

Author(s) name:

Vilya Syafriana, Yasman, Catur Dewi Anjani, Dwi Jayanti Ningsih, Nissa Maftucha, Rika Amelia, Windri Handayani, Rosario Trijuliamos Manalu, Ratna Yuniati, Munawarohthus Sholikha

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Pucuk merah (Syzygium myrtifolium) has only ever been grown for ornamental purposes. Despite the fact that this plant contains antimicrobial properties. We are investigating the antibacterial activity of this plant extracts against several pathogenic bacteria since research on this plant is still inadequate. The chosen extracts are those from n-hexane (nonpolar) and ethyl acetate (semi-polar).

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Place and date:

Jakarta, November 30th 2022

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Vilya Syafriana

Effect of Solvent Polarity on Extraction Yield and Antibacterial Activities of *Syzygium myrtifolium* Young Leaves

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Manuscript received: DD MM 2016 (Date of abstract/manuscript submission). Revision accepted: ..... 2016.

Abstract. *Syzygium myrtifolium* or known as pucuk merah plant in Indonesian is known as an ornamental plant, despite the fact that this plant has the ability to serve as a source of antibacterial raw material. Several studies showed that this plant has antimicrobial activity against some bacteria and fungi. However, the antibacterial activity of its young leaf extract has not been explored much. Therefore, this research was aimed to conduct further exploration regarding the antibacterial activity of the leaf using two different polarity-based solvents, n-hexane and ethyl acetate. Besides that, it also aimed to determine the yield value of the extracts. The extraction was done sequentially using the maceration process with two different solvents (n-hexane and ethyl acetate). The antibacterial activity was carried out using the disk diffusion method. The extraction revealed that the ethyl acetate extract had a greater yield value (17.7%) than the n-hexane extract (5.46%). The antibacterial activity tests showed that the ethyl acetate extract has a stronger ability to suppress bacterial growth than the n-hexane extract.

Key words: antibacterial, ethyl acetate, maceration, n-hexane, pucuk merah, young leaves

Abbreviations (if any): Balittro (Balai Penelitian Tanaman Rempah dan Obat); Depkes RI (Depatermen Kesehatan Republik Indonesia); Dirjem POM (Direktorat Jenderal Pengawas Obat dan Makanan); Kemenkes RI (Kementerian Kesehatan Republik Indonesia); MHA (Mueller Hinton Agar); NA (Nutrient Agar); IZ (Inhibition Zone); NHE (n-Hexane Extract); EAE (Ethyl Acetate Extract)

Running title: antibacterial activity of *Syzygium myrtifolium* young leaf

INTRODUCTION

Syzygium myrtifolium or known as pucuk merah plant in Indonesian is a plant that is currently found in parks, yards, and almost every highway, as well as in residential and commercial buildings. Due to its appealing color, this plant is referred to as an ornamental plant (**Figure 1**). Additionally, this plant is simple to maintain and grow, making it increasingly well-liked. However, this plant belongs to the same taxonomic genus as salam (*Syzygium polyanthum*) and cloves (*Syzygium aromaticum*), which are well-known plants in Indonesia due to their use in food and medicine. Therefore, it is very likely that this plant has the same potential as salam and cloves, including the ability to be a source of antibacterial raw materials (Haryati et al. 2015; Putri 2019; Batiha et al. 2020; Iskandi et al. 2021; Mudiana et al. 2021).

The phytochemical analysis of this plant's leaves revealed the presence of flavonoids, tannins, and saponins, all of which have antimicrobial properties (Syafriana et al. 2019; Ahmad et al. 2022). Several studies showed that this plant has antimicrobial activity against the bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella pneumoniae*, and *Staphylococcus* spp., as well as the fungi *Candida* spp. and *Cryptococcus neoformans* (Haryati et al. 2015; Syafriana et al. 2019; Ahmad et al. 2022). Our previous study has shown that the methanol extract of *S. myrtifolium* leaf has antibacterial activity against the *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* growth (Syafriana et al. 2019). Therefore, we intend to conduct further exploration regarding the activity of the leaf extract using two different polarity-based solvents, namely n-hexane and ethyl acetate.

The extraction was carried out by maceration method consecutively in a series of two solvents (n-hexane and ethyl acetate). Maceration was chosen as the simplest method of simplicial extraction (Depkes RI, 1995; Kemenkes RI, 2017). The disk diffusion method (Kirby-Bauer disk diffusion) was used to test the antibacterial activity by measuring the

clear zone formed around the disc as the Inhibition Zone (IZ) (Dafale et al. 2016; Hudzicki 2016). The findings are expected to serve as a model for the development of *S. myrtifolium* as medicinal plants besides as ornamental plants.

Figure 1. Pucuk merah (*Syzygium myrtifolium*) plant

MATERIALS AND METHODS

1.1. Chemicals and Reagents

Nutrient Agar (NA) [Oxoid], Mueller Hinton Agar (MHA) [Oxoid], Aquadest [MPLab], 70% Ethanol [Dwinika], n-Hexane [PMP], Ethyl Acetate [PMP], $FeCl_3$ [Merck], Wagner reagent [CDH], Mayer reagent [CDH], Dragendorff reagent, Ammoniak [Merck], Acetic acid anhydride [Merck], $NaNO_2$ [Merck], $AlCl_3$ [Merck], HCl [Merck], Chloroform [Merck], H_2SO_4 [Merck], DMSO [Merck], immersion oil, Crystal violet [Merck], Safranin, Lugol's iodine, 0.9% NaCl [Braun], *Blank disc* [Oxoid], the antibiotic disk of Amoxicillin [Oxoid], analytical balance [Excellent], blender [Phillips], aluminium foil [Klin Pak], Parafilm [Bemis], autoclave [B-one], incubator [Memmert], vacuum rotary evaporator [Buchi, Eyela], waterbath [Memmert], Hot plate[Joanlab], and Laminar Air Flow.

1.2. Preparation and Extraction of Sample *Syzygium myrtifolium* Leaf

S. myrtifolium young leaf were obtained from Research Agency of Spices and Medicinal Plants (Balittro), Bogor. Three kg of fresh leaves were cleansed with clean water. The leaves were air-dried for three days. The dried leaves then being crushed with a blender and sieved through mesh 44. This procedure was used to achieve homogenous size of simplicia, so that there would be equal interaction between the leaf powders and the solvent (Azwanida 2015; Sa`adah and Nurhasnawati 2015; Makanjuola 2017).

The *S. myrtifolium* young leaf was weighed as much as 250 g then extracted with maceration method using a nonpolar solvent (n-hexane) then followed by a semi-polar solvent (ethyl acetate) with ratio 1:10. The maceration was done for 24 hours and re-macerated once with the same procedure (Kemenkes RI 2017). The outcomes of the maceration were filtered using filter paper. The vacuum rotary evaporator was used to evaporate the filtrate until a thick extract was produced.

1.3. Specific and Non-Specific Parameters of Extracts

Specific parameters include organoleptic test, water soluble compound content, ethanol soluble compound content, and phytochemical screening. Non-specific parameters include total ash content and acid insoluble ash content (Depkes RI 1995; Kemenkes RI 2017). The tests were carried out at Balittro, Bogor.

1.4. Antibacterial Activity Test

The antibacterial test was carried out using the disk diffusion method to determine the diameter of Inhibiton Zone (IZ) of the extract against several pathogenic bacteria, including *Propionibacterium acnes*, *Salmonella* sp., *Staphylococcus epidermidis*, *Streptococcus mutans*, and *Streptococcus pyogenes* (Pratiwi 2008; Hudzicki 2016).

1.4.1. Bacterial Suspension Preparation

The bacteria aged 24 hours was taken 3-4 oses, then placed in a test tube containing 9 mL of 0.9% NaCl. The test tube was then vortexed to create homogeneity. The bacterial suspension was adjusted using McFarland no.3 (9.0×10^8 CFU/mL) as the turbidity reference.

1.4.2. The Extract Concentrations Preparation

The extracts were prepared at four different concentrations: 2.5%, 5%, 10%, and 20%. DMSO 50% was used as a negative control, while the antibiotic amoxicillin was used as a positive control.

1.4.3. Diameter of Inhibition Zone (IZ) Test

Each of bacterial suspension was pipetted into a petri dish containing MHA in an amount of 0.1 mL. The suspension was then distributed evenly using a stirring rod L-form. After the media and the bacterial suspension have dried, a sterile paper disk was placed onto the agar. Each concentration's extract was dripped for about 20 µL followed by incubation at 37^0C for 24 hours. The clear zone that formed around the disk was observed and measured as the Inhibition Zone (IZ).

RESULTS AND DISCUSSION

2.1. Sample Extraction

The extraction process in this study used two different solvents based on their polarity: n-hexane (nonpolar) and ethyl acetate (semi-polar). The extraction was carried out sequentially from nonpolar to semi-polar using the maceration method. Maceration was chosen because it is a simples and easy method that preserves the quality of heat-resistant bioactive compounds (Zhang et al. 2018). The maceration was repeated once for a total of 24 hours, with stirring in between. The stirring process aims to facilitate the contact of the solvent in the plant cell cavity so that the compounds in it can be extracted optimally by the solvent. Stirring can cause solvent circulation so that the extraction process can take place optimally (Dirjen POM 1986). The yields extracts were listed at **Table 1.**

Table 1. Yield extracts of *Syzygium myrtifolium* young leaf using n-hexane and ethyl acetate as solvents

Sample	Solvent	Simplicial weight (g)	Extract weight (g)	Yield (%)
Young leaves (red leaves)	n-hexane	250	13.65	5.46
	Ethyl acetate	250	44.28	17.7

The data in **Table 1** showed that the yield value of the young leaf extract increased with solvent polarity. Ethyl acetate extract had a yield value of 17.7%, whereas n-hexane had a yield value of 5.46%. The yield value of an extract indicates how many active compounds the solvent was able to extract from the sample. Higher yield values correspond to higher active compounds (Harborne 1987; Hasnaeni et al. 2019). These results corroborated those of Hidayati et al. (2017) who discovered that *Syzygium polyanthum* leaf extract with n-hexane and ethyl acetate solvents produced a higher yield value of ethyl acetate extract than n-hexane extract.

2.2. Specific and Non-Specific Parameters of Extracts

2.2.1. Organoleptic Observations

Organoleptic observations include odor, color, and texture tests. Organoleptic tests revealed that both extracts have a distinct odor similar to the leaf samples. The color of the two extracts was reddish brown, with n-hexane extract having a

deeper hue than ethyl acetate extracts. The texture of the n-hexane extract was thick, while the ethyl acetate extract was liquid.

2.2.2. Phytochemical Screening

Phytochemical screening was performed to determine the content of secondary metabolites, which included tests for alkaloids, saponins, tannins, phenolics, flavonoids, steroids, triterpenoids, and glycosides (Parbuntari et al. 2018; Wardani et al. 2019). **Table 2** showed the results of phytochemicals screening of n-hexane and ethyl acetate extracts of *S. myrtifolium* young leaf.

Table 2. Phytochemicals screening of n-hexane and ethyl acetate extracts of *Syzygium myrtifolium* young leaf

Metabolites	Extracts	
	n-Hexane	Ethyl acetate
Alkaloid	(+)	(+)
Saponin	(-)	(+)
Tannin	(-)	(+)
Phenolic	(+)	(+)
Flavonoid	(+)	(+)
Steroid	(+)	(+)
Triterpenoid	(+)	(+)
Glycoside	(+)	(+)

Note: (+): contains the metabolite; (-): do not contains the metabolite

The results obtained in **Table 2** showed that ethyl acetate extract contained alkaloids, saponins, tannins, phenolics, flavonoids, steroids, triterpenoid, and glycosides, whereas the n-hexane extract showed negative results in saponins and tannins test. The results were in accordance with the findings of Haryati et al. (2015), Syafriana et al. (2019), and Ahmad et al. (2020), who discovered flavonoid and phenolic compounds in their *S. myrtifolium* extracts. The flavonoid content in the extracts was deemed to have antibacterial properties (Permatasari et al. 2022).

2.2.3. Water and Ethanol Soluble Compound Content

The determination of extract compound content was aimed to estimate the approximate number of polar compounds (water soluble) and semi-polar or nonpolar compounds (soluble ethanol). **Table 3** showed that the n-hexane extract and ethyl acetate extract were more soluble in alcohol solutions than water solutions. The results indicated that the extraction of young *S. myrtifolium* leaf will be more advantageous with alcohol-based solvent (Putri et al. 2021).

Table 3. Water and ethanol soluble compound content of n-hexane and ethyl acetate extracts of *Syzygium myrtifolium* young leaf

Assay	Extracts	
	n-hexane	Ethyl acetate
Water soluble (%)	0.81	4.80
Ethanol soluble (%)	25.38	14.60

2.2.4. Total Ash Content and Acid Insoluble Ash Content

Total ash content is used to determine the mineral content in extracts and mineral content which is not soluble in acids where the maximum range relates to contaminants and purity (Syukri et al. 2020; Putri et al. 2021). The ash content and acid insoluble ash content was shown in **Table 4** below.

Table 4. Total ash and acid insoluble ash content of n-hexane and ethyl acetate extracts of *Syzygium myrtifolium* young leaf

Assay	Extracts	
	n-hexane	Ethyl acetate
Total ash content (%)	8.34	0.06
Acid insoluble ash content (%)	0.03	ND

ND: Not Detected

The n-hexane extract had a total ash content about 8.34%, while the ethyl acetate extract about 0.06% (**Table 4**). Results for acid insoluble ash content were only seen in the n-hexane extract, while they were undetectable in ethyl acetate extract. The total amount of ash in each extract demonstrated the presence of minerals, whereas the acid insoluble ash content pointed to the presence of sand or other residual impurities. According to these findings, ethyl acetate extract was purer than n-hexane extract (Syukri et al. 2020; Putri et al. 2021). The Indonesian Herbal Pharmacopoeia (FHI) states that the maximum allowed percentage of total ash content in an extract is 13.3%. These results showed that the total ash content of the n-hexane and ethyl acetate extracts complied with the FHI standards (Kemenkes RI 2017).

2.3. Antibacterial Activity

The antibacterial activity was performed against five pathogenic bacteria. The results can be seen in **Figures 2 and 3.**

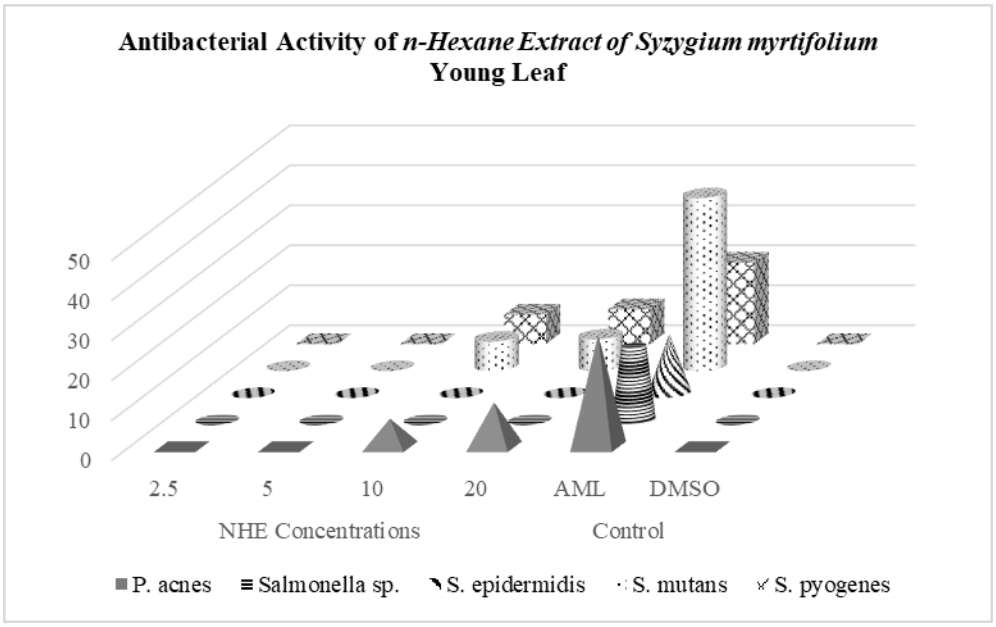

Figure 2. Antibacterial activity of n-hexane extract (NHE) of *Syzygium myrtifolium* young leaf

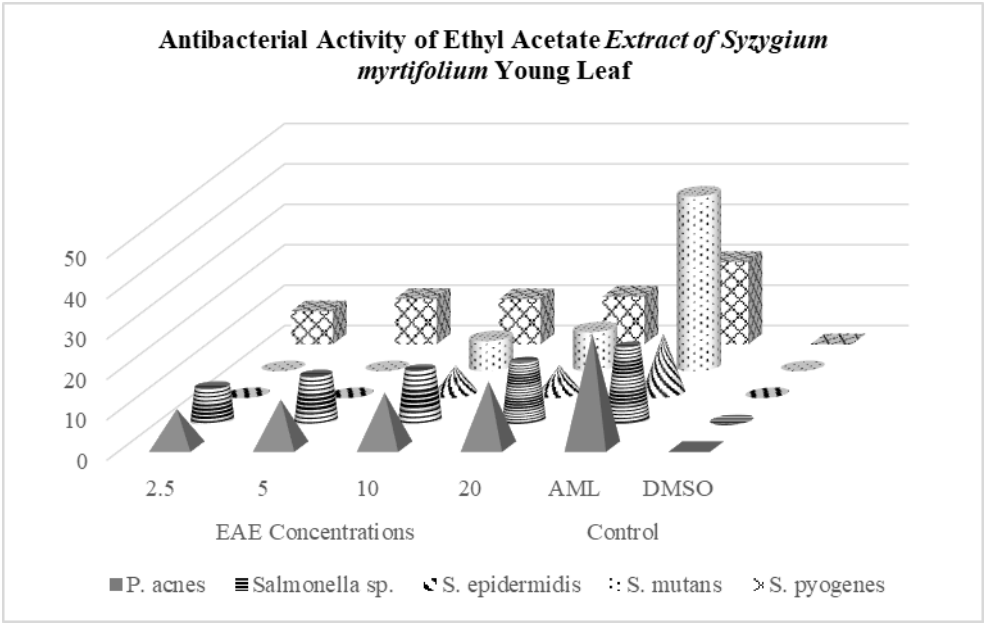

Figure 3. Antibacterial activity of ethyl acetate extract (EAE) of *Syzygium myrtifolium* young leaf

According to the data in **Figures 2 and 3**, the ethyl acetate extract has a stronger ability to suppress bacterial growth than the n-hexane extract. The five examined bacteria were still able to grow in the presence of n-hexane extract at concentrations of 2.5% and 5%. Meanwhile, the ethyl acetate extract at the same concentrations showed inhibition to the growth of *P. acnes, Salmonella* sp., and *S. pyogenes*, even though *S. epidermidis* and *S. mutans* were not affected.

N-hexane extract started to exhibit its potential to inhibit *P. acnes, S. mutans*, and *S. pyogenes* at concentrations of 10% and 20%, but it was still unable to inhibit *Salmonella* sp. and *S. epidermidis* growth (**Figure 2**). Ethyl acetate extract, which similarly showed a higher inhibition zone than n-hexane extract, could prevent the growth of the five studied bacteria at 10% and 20% concentrations (**Figure 2 and Figure 3**). These findings follow the same pattern as the research conducted by Nuraskin et al. (2020) which revealed that the ethyl acetate extract of Vitex pinnata leaf has a stronger inhibitory effect than the n-hexane extract against *S. mutans*. Another study by Veranita et al. (2020) and Armansyah et al. (2022) revealed that the n-hexane extracts of breadfruit and red betel leaves were ineffective at inhibiting the growth of *E. coli*, but the ethyl acetate extract of the two leaves could.

Different metabolite compositions in young leaves of *S. myrtifolium* extracted with n-hexane and ethyl acetate may be the cause of variability in antibacterial activity. The n-hexane extract did not contain tannins and saponins, as shown in **Table 2**. Despite the fact that saponins and tannins are known to have an impact on bacterial growth. Saponins are known to breakdown lipids in the bacterial cell membrane, reducing the membrane's surface tension and potentially leading to bacterial cell lysis. Tannins, as they are known, may prevent bacterial cells from synthesizing proteins, which could restrict the growth of the bacteria (Syafriana et al. 2019; Syafriana et al. 2021).

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Pucuk merah (Syzygium myrtifolium) has only ever been grown for ornamental purposes. Despite the fact that this plant contains antimicrobial properties. We are investigating the antibacterial activity of this plant extracts against several pathogenic bacteria since research on this plant is still inadequate. The chosen extracts are those from n-hexane (nonpolar) and ethyl acetate (semi-polar).

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Effect of Solvent Polarity on Extraction Yield and Antibacterial Activities of *Syzygium myrtifolium* Young Leaves

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Abstract. *Syzygium myrtifolium* or known as pucuk merah plant in Indonesian is known as an ornamental plant, despite the fact that this plant has the ability to serve as a source of antibacterial raw material. Several studies showed that this plant has antimicrobial activity against some bacteria and fungi. However, the antibacterial activity of its young leaf extract has not been explored much. Therefore, this research was aimed to conduct further exploration regarding the antibacterial activity of the leaf using two different polarity-based solvents, n-hexane and ethyl acetate. Besides that, it also aimed to determine the yield value of the extracts. The extraction was done sequentially using the maceration process with two different solvents (n-hexane and ethyl acetate). The antibacterial activity was carried out using the disk diffusion method. The extraction revealed that the ethyl acetate extract had a greater yield value (17.7%) than the n-hexane extract (5.46%). The antibacterial activity tests showed that the ethyl acetate extract has a stronger ability to suppress bacterial growth than the n-hexane extract.

Key words: antibacterial, ethyl acetate, maceration, n-hexane, pucuk merah, young leaves

Abbreviations (if any): Balittro (Balai Penelitian Tanaman Rempah dan Obat); Depkes RI (Depatermen Kesehatan Republik Indonesia); Dirjem POM (Direktorat Jenderal Pengawas Obat dan Makanan); Kemenkes RI (Kementerian Kesehatan Republik Indonesia); MHA (Mueller Hinton Agar); NA (Nutrient Agar); IZ (Inhibition Zone); NHE (n-Hexane Extract); EAE (Ethyl Acetate Extract)

Running title: antibacterial activity of *Syzygium myrtifolium* young leaf

INTRODUCTION

Syzygium myrtifolium or known as pucuk merah plant in Indonesian is a plant that is currently found in parks, yards, and almost every highway, as well as in residential and commercial buildings. Due to its appealing color, this plant is referred to as an ornamental plant (**Figure 1**). Additionally, this plant is simple to maintain and grow, making it increasingly well-liked. However, this plant belongs to the same taxonomic genus as salam (*Syzygium polyanthum*) and cloves (*Syzygium aromaticum*), which are well-known plants in Indonesia due to their use in food and medicine. Therefore, it is very likely that this plant has the same potential as salam and cloves, including the ability to be a source of antibacterial raw materials (Haryati et al. 2015; Putri 2019; Batiha et al. 2020; Iskandi et al. 2021; Mudiana et al. 2021).

The phytochemical analysis of this plant's leaves revealed the presence of flavonoids, tannins, and saponins, all of which have antimicrobial properties (Syafriana et al. 2019; Ahmad et al. 2022). Several studies showed that this plant has antimicrobial activity against the bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella pneumoniae*, and *Staphylococcus* spp., as well as the fungi *Candida* spp. and *Cryptococcus neoformans* (Haryati et al. 2015; Syafriana et al. 2019; Ahmad et al. 2022). Our previous study has shown that the methanol extract of *S. myrtifolium* leaf has antibacterial activity against the *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* growth (Syafriana et al. 2019). Therefore, we intend to conduct further exploration regarding the activity of the leaf extract using two different polarity-based solvents, namely n-hexane and ethyl acetate.

The extraction was carried out by maceration method consecutively in a series of two solvents (n-hexane and ethyl acetate). Maceration was chosen as the simplest method of simplicial extraction (Depkes RI, 1995; Kemenkes RI, 2017). The disk diffusion method (Kirby-Bauer disk diffusion) was used to test the antibacterial activity by measuring the

clear zone formed around the disc as the Inhibition Zone (IZ) (Dafale et al. 2016; Hudzicki 2016). The findings are expected to serve as a model for the development of *S. myrtifolium* as medicinal plants besides as ornamental plants.

Figure 1. Pucuk merah (*Syzygium myrtifolium*) plant

MATERIALS AND METHODS

1.1. Chemicals and Reagents

Nutrient Agar (NA) [Oxoid], Mueller Hinton Agar (MHA) [Oxoid], Aquadest [MPLab], 70% Ethanol [Dwinika], n-Hexane [PMP], Ethyl Acetate [PMP], $FeCl_3$ [Merck], Wagner reagent [CDH], Mayer reagent [CDH], Dragendorff reagent, Ammoniak [Merck], Acetic acid anhydride [Merck], $NaNO_2$ [Merck], $AlCl_3$ [Merck], HCl [Merck], Chloroform [Merck], H_2SO_4 [Merck], DMSO [Merck], immersion oil, Crystal violet [Merck], Safranin, Lugol's iodine, 0.9% NaCl [Braun], *Blank disc* [Oxoid], the antibiotic disk of Amoxicillin [Oxoid], analytical balance [Excellent], blender [Phillips], aluminium foil [Klin Pak], Parafilm [Bemis], autoclave [B-one], incubator [Memmert], vacuum rotary evaporator [Buchi, Eyela], waterbath [Memmert], Hot plate[Joanlab], and Laminar Air Flow.

1.2. Preparation and Extraction of Sample *Syzygium myrtifolium* Leaf

S. myrtifolium young leaf were obtained from Research Agency of Spices and Medicinal Plants (Balittro), Bogor. Three kg of fresh leaves were cleansed with clean water. The leaves were air-dried for three days. The dried leaves then being crushed with a blender and sieved through mesh 44. This procedure was used to achieve homogenous size of simplicia, so that there would be equal interaction between the leaf powders and the solvent (Azwanida 2015; Sa`adah and Nurhasnawati 2015; Makanjuola 2017).

The *S. myrtifolium* young leaf was weighed as much as 250 g then extracted with maceration method using a nonpolar solvent (n-hexane) then followed by a semi-polar solvent (ethyl acetate) with ratio 1:10. The maceration was done for 24 hours and re-macerated once with the same procedure (Kemenkes RI 2017). The outcomes of the maceration were filtered using filter paper. The vacuum rotary evaporator was used to evaporate the filtrate until a thick extract was produced.

1.3. Specific and Non-Specific Parameters of Extracts

Specific parameters include organoleptic test, water soluble compound content, ethanol soluble compound content, and phytochemical screening. Non-specific parameters include total ash content and acid insoluble ash content (Depkes RI 1995; Kemenkes RI 2017). The tests were carried out at Balittro, Bogor.

1.4. Antibacterial Activity Test

The antibacterial test was carried out using the disk diffusion method to determine the diameter of Inhibiton Zone (IZ) of the extract against several pathogenic bacteria, including *Propionibacterium acnes*, *Salmonella* sp., *Staphylococcus epidermidis*, *Streptococcus mutans*, and *Streptococcus pyogenes* (Pratiwi 2008; Hudzicki 2016).

1.4.1. Bacterial Suspension Preparation

The bacteria aged 24 hours was taken 3-4 oses, then placed in a test tube containing 9 mL of 0.9% NaCl. The test tube was then vortexed to create homogeneity. The bacterial suspension was adjusted using McFarland no.3 (9.0×10^8 CFU/mL) as the turbidity reference.

1.4.2. The Extract Concentrations Preparation

The extracts were prepared at four different concentrations: 2.5%, 5%, 10%, and 20%. DMSO 50% was used as a negative control, while the antibiotic amoxicillin was used as a positive control.

1.4.3. Diameter of Inhibition Zone (IZ) Test

Each of bacterial suspension was pipetted into a petri dish containing MHA in an amount of 0.1 mL. The suspension was then distributed evenly using a stirring rod L-form. After the media and the bacterial suspension have dried, a sterile paper disk was placed onto the agar. Each concentration's extract was dripped for about 20 µL followed by incubation at 37^0C for 24 hours. The clear zone that formed around the disk was observed and measured as the Inhibition Zone (IZ).

RESULTS AND DISCUSSION

2.1. Sample Extraction

The extraction process in this study used two different solvents based on their polarity: n-hexane (nonpolar) and ethyl acetate (semi-polar). The extraction was carried out sequentially from nonpolar to semi-polar using the maceration method. Maceration was chosen because it is a simples and easy method that preserves the quality of heat-resistant bioactive compounds (Zhang et al. 2018). The maceration was repeated once for a total of 24 hours, with stirring in between. The stirring process aims to facilitate the contact of the solvent in the plant cell cavity so that the compounds in it can be extracted optimally by the solvent. Stirring can cause solvent circulation so that the extraction process can take place optimally (Dirjen POM 1986). The yields extracts were listed at **Table 1.**

Table 1. Yield extracts of *Syzygium myrtifolium* young leaf using n-hexane and ethyl acetate as solvents

Sample	Solvent	Simplicial weight (g)	Extract weight (g)	Yield (%)
Young leaves (red leaves)	n-hexane	250	13.65	5.46
	Ethyl acetate	250	44.28	17.7

The data in **Table 1** showed that the yield value of the young leaf extract increased with solvent polarity. Ethyl acetate extract had a yield value of 17.7%, whereas n-hexane had a yield value of 5.46%. The yield value of an extract indicates how many active compounds the solvent was able to extract from the sample. Higher yield values correspond to higher active compounds (Harborne 1987; Hasnaeni et al. 2019). These results corroborated those of Hidayati et al. (2017) who discovered that *Syzygium polyanthum* leaf extract with n-hexane and ethyl acetate solvents produced a higher yield value of ethyl acetate extract than n-hexane extract.

2.2. Specific and Non-Specific Parameters of Extracts

2.2.1. Organoleptic Observations

Organoleptic observations include odor, color, and texture tests. Organoleptic tests revealed that both extracts have a distinct odor similar to the leaf samples. The color of the two extracts was reddish brown, with n-hexane extract having a

deeper hue than ethyl acetate extracts. The texture of the n-hexane extract was thick, while the ethyl acetate extract was liquid.

2.2.2. Phytochemical Screening

Phytochemical screening was performed to determine the content of secondary metabolites, which included tests for alkaloids, saponins, tannins, phenolics, flavonoids, steroids, triterpenoids, and glycosides (Parbuntari et al. 2018; Wardani et al. 2019). **Table 2** showed the results of phytochemicals screening of n-hexane and ethyl acetate extracts of *S. myrtifolium* young leaf.

Table 2. Phytochemicals screening of n-hexane and ethyl acetate extracts of *Syzygium myrtifolium* young leaf

Metabolites	Extracts	
	n-Hexane	Ethyl acetate
Alkaloid	(+)	(+)
Saponin	(-)	(+)
Tannin	(-)	(+)
Phenolic	(+)	(+)
Flavonoid	(+)	(+)
Steroid	(+)	(+)
Triterpenoid	(+)	(+)
Glycoside	(+)	(+)

Note: (+): contains the metabolite; (-): do not contains the metabolite

The results obtained in **Table 2** showed that ethyl acetate extract contained alkaloids, saponins, tannins, phenolics, flavonoids, steroids, triterpenoid, and glycosides, whereas the n-hexane extract showed negative results in saponins and tannins test. The results were in accordance with the findings of Haryati et al. (2015), Syafriana et al. (2019), and Ahmad et al. (2020), who discovered flavonoid and phenolic compounds in their *S. myrtifolium* extracts. The flavonoid content in the extracts was deemed to have antibacterial properties (Permatasari et al. 2022).

2.2.3. Water and Ethanol Soluble Compound Content

The determination of extract compound content was aimed to estimate the approximate number of polar compounds (water soluble) and semi-polar or nonpolar compounds (soluble ethanol). **Table 3** showed that the n-hexane extract and ethyl acetate extract were more soluble in alcohol solutions than water solutions. The results indicated that the extraction of young *S. myrtifolium* leaf will be more advantageous with alcohol-based solvent (Putri et al. 2021).

Table 3. Water and ethanol soluble compound content of n-hexane and ethyl acetate extracts of *Syzygium myrtifolium* young leaf

Assay	Extracts	
	n-hexane	Ethyl acetate
Water soluble (%)	0.81	4.80
Ethanol soluble (%)	25.38	14.60

2.2.4. Total Ash Content and Acid Insoluble Ash Content

Total ash content is used to determine the mineral content in extracts and mineral content which is not soluble in acids where the maximum range relates to contaminants and purity (Syukri et al. 2020; Putri et al. 2021). The ash content and acid insoluble ash content was shown in **Table 4** below.

Table 4. Total ash and acid insoluble ash content of n-hexane and ethyl acetate extracts of *Syzygium myrtifolium* young leaf

Assay	Extracts	
	n-hexane	Ethyl acetate
Total ash content (%)	8.34	0.06
Acid insoluble ash content (%)	0.03	ND

ND: Not Detected

The n-hexane extract had a total ash content about 8.34%, while the ethyl acetate extract about 0.06% (**Table 4**). Results for acid insoluble ash content were only seen in the n-hexane extract, while they were undetectable in ethyl acetate extract. The total amount of ash in each extract demonstrated the presence of minerals, whereas the acid insoluble ash content pointed to the presence of sand or other residual impurities. According to these findings, ethyl acetate extract was purer than n-hexane extract (Syukri et al. 2020; Putri et al. 2021). The Indonesian Herbal Pharmacopoeia (FHI) states that the maximum allowed percentage of total ash content in an extract is 13.3%. These results showed that the total ash content of the n-hexane and ethyl acetate extracts complied with the FHI standards (Kemenkes RI 2017).

2.3. Antibacterial Activity

The antibacterial activity was performed against five pathogenic bacteria. The results can be seen in **Figures 2 and 3.**

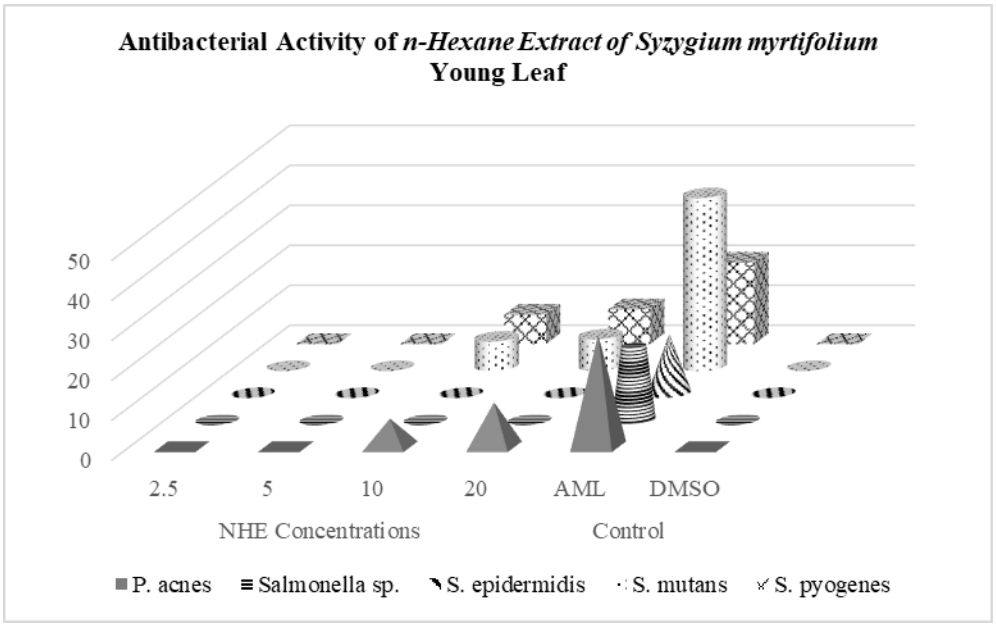

Figure 2. Antibacterial activity of n-hexane extract (NHE) of *Syzygium myrtifolium* young leaf

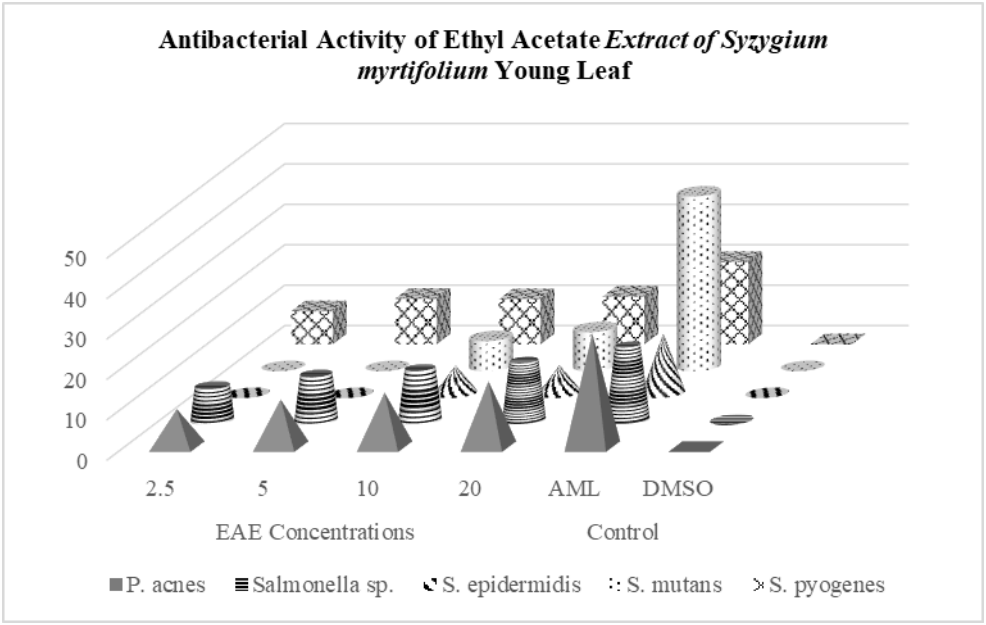

Figure 3. Antibacterial activity of ethyl acetate extract (EAE) of *Syzygium myrtifolium* young leaf

According to the data in **Figures 2 and 3**, the ethyl acetate extract has a stronger ability to suppress bacterial growth than the n-hexane extract. The five examined bacteria were still able to grow in the presence of n-hexane extract at concentrations of 2.5% and 5%. Meanwhile, the ethyl acetate extract at the same concentrations showed inhibition to the growth of *P. acnes, Salmonella* sp., and *S. pyogenes*, even though *S. epidermidis* and *S. mutans* were not affected.

N-hexane extract started to exhibit its potential to inhibit *P. acnes, S. mutans*, and *S. pyogenes* at concentrations of 10% and 20%, but it was still unable to inhibit *Salmonella* sp. and *S. epidermidis* growth (**Figure 2**). Ethyl acetate extract, which similarly showed a higher inhibition zone than n-hexane extract, could prevent the growth of the five studied bacteria at 10% and 20% concentrations (**Figure 2 and Figure 3**). These findings follow the same pattern as the research conducted by Nuraskin et al. (2020) which revealed that the ethyl acetate extract of Vitex pinnata leaf has a stronger inhibitory effect than the n-hexane extract against *S. mutans*. Another study by Veranita et al. (2020) and Armansyah et al. (2022) revealed that the n-hexane extracts of breadfruit and red betel leaves were ineffective at inhibiting the growth of *E. coli*, but the ethyl acetate extract of the two leaves could.

Different metabolite compositions in young leaves of *S. myrtifolium* extracted with n-hexane and ethyl acetate may be the cause of variability in antibacterial activity. The n-hexane extract did not contain tannins and saponins, as shown in **Table 2**. Despite the fact that saponins and tannins are known to have an impact on bacterial growth. Saponins are known to breakdown lipids in the bacterial cell membrane, reducing the membrane's surface tension and potentially leading to bacterial cell lysis. Tannins, as they are known, may prevent bacterial cells from synthesizing proteins, which could restrict the growth of the bacteria (Syafriana et al. 2019; Syafriana et al. 2021).

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SURAT PERNYATAAN TANGGUNG JAWAB BELANJA

Yang bertanda tangan di bawah ini :

Nama : MUNAWAROHTHUS SHOLIKHA S.Si, M.Si
Alamat : Jl. Darul Khoirot No. 1 E Jakarta Timur

berdasarkan Surat Keputusan Nomor SP DIPA-023.17.1.690523/2022 dan Perjanjian / Kontrak Nomor 382/LL.3/AK.04/2022 mendapatkan Anggaran Penelitian Ekplorasi Bioaktivitas dan Analisis Metabolomik Tanaman Pucuk Merah (Syzygium myrtifolium) sebagai Sumber Senyawa Antimikroba dan Antikanker Sebesar 53,500,000

Dengan ini menyatakan bahwa :

1. Biaya kegiatan Penelitian di bawah ini meliputi :

No	Uraian	Jumlah
01	**Bahan** Pembelian media uji, pembelian pelarut, pembelian akuades, pembelian kertas cakram steril dan antibiotik, pembelian pereaksi kimia, pembelian disposable tools	29,096,701
02	**Pengumpulan Data** Biaya uji sampel di Balittro, transportasi uji dan pembelian sampel, biaya konsumsi rapat, honor laboran, honor sekretaris peneliti	9,137,000
03	**Analisis Data(Termasuk Sewa Peralatan** biaya konsumsi rapat analisis hasil, FGD, monev, honor nara sumber, honor sekretaris peneliti	2,780,500
04	**Pelaporan, Luaran Wajib dan Luaran Tambahan** Pembelian ATK, materai, foto kopi dan penjilidan, biaya konsumsi, biaya publikasi jurnal, biaya proof reading	8,958,390
05	**Lain-lain** pembelian barang persediaan, APD, alat gelas, dan transportasi	3,527,409
	Jumlah	53,500,000

2. Jumlah uang tersebut pada angka 1, benar-benar dikeluarkan untuk pelaksanaan kegiatan Penelitian dimaksud.

Demikian surat pernyataan ini dibuat dengan sebenarnya.

Jakarta, 30-11-2022

Ketua,

(MUNAWAROHTHUS SHOLIKHA S.Si, M.Si)

NIP/NIK 3175084703860002