Y A Y A S A N P E R G U R U A N C I K I N I
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Jl. Moh. Kahfi II, Bhumi Srengseng Indah, Jagakarsa, Jakarta Selatan 12640 Telp. (021) 727 0090, 787 4645, 787 4647 Fax. (021) 786 6955, http://WWW.istn.ac.id E-mail:rektorat@istn.ac.id

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Untuk melaksanakan tugas sebagai berikut:

Bidang	Perincian Kegiatan	Tempat	Jam/ Minggu	Kredit (SKS)	Keterangan
I PENDIDIKAN DAN PENGAJARAN	MENGAJAR DI KELAS (KULIAH/RESPONSI DAN LABORATORIUM)				
	Biokimia (A)	R-HC4		1	Senin, 13:00 s.d 15:10
	Analisis Sediaan Farmasi (A), Praktikum	R-HC7. Lab		1,3	Senin, 08:00 s.d 13:00
	Analisis Sediaan Farmasi (C), Praktikum	R-H.A, Lab		1,3	Senin, 08:00 s.d 13:00
	Analisis Sediaan Farmasi (K), Praktikum	R-HC7. Lab		1,3	Senin, 17:00 s.d 21:00
	Analisis Sediaan Farmasi (L), Praktikum	R-HC8, Lab		1,3	Sabtu, 08:00 s.d 13:00
	Bimbingan Skripsi		3 Jam/Minggu	1	
	Menguji Tugas Akhir		3 Jam/Minggu	1	
	Pengembangan Bahan Ajar		3 Jam/Minggu	1	
	B. MENDUDUKI JABATAN PERGURUAN TINGGI				
	Kepala Laboratorium Farmasi (struktural)		9 Jam/Minggu	3	
II PENELITIAN	Penulisan Karya Ilmiah		6 Jam/Minggu	2	
III PENGABDIAN DAN MASYARAKAT	Pelatihan dan Penyuluhan		3 Jam/Minggu	1	
IV UNSUR UNSUR PENUNJANG	Pertemuan Ilmiah		3 Jam/Minggu	1	
	Jumlah Total			**16.1**	

Kepada yang bersangkutan akan diberikan gaji/honorarium sesuai dengan peraturan penggajian yang berlaku di Institut Sains Dan Teknologi Nasional

Penugasan ini berlaku dari tanggal 03 Maret 2025 sampai dengan tanggal 31 Agustus 2025

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1. Wakil Rektor Bidang Akademik - ISTN
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Antibacterial Activities of n-Hexane and Ethyl Acetate Extracts from young leaves of Red Lip (Syzygium myrtifolium (Roxb.) Walp.) (AbstractView.aspx?PID=2025-18-4-55)

Author(s): Vilya Syafriana (search.aspx?key=Vilya Syafriana), Yasman (search.aspx?key=Yasman), Windri Handayani (search.aspx?key=Windri Handayani), Dwi J. Ningsih (search.aspx?key=Dwi J. Ningsih), Catur D. Anjani (search.aspx?key=Catur D. Anjani), Rosario T. Manalu (search.aspx?key=Rosario T. Manalu), Ratna Yuniati (search.aspx?key=Ratna Yuniati), Munawarohthus Sholikha (search.aspx?key=Munawarohthus Sholikha)

Email(s): mona.farmasi@istn.ac.id (mailto:mona.farmasi@istn.ac.id)

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Address: Vilya Syafriana1,2, Yasman1,3, Windri Handayani1,3, Dwi J. Ningsih2, Catur D. Anjani2, Rosario T. Manalu2, Ratna Yuniati1,3, Munawarohthus Sholikha2*
1Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Kampus UI Depok, West Java, Indonesia.
2Faculty of Pharmacy, National Institute of Sciences and Technology, Jl. Moh. Kahfi II, Srengseng Sawah, Jagakarsa, South Jakarta 16420. Phone number: (021) 7270090.
3Research Group of Metabolomics and Chemical Ecology, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Kampus UI Depok, West Java, Indonesia.
*Corresponding Author

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Antibacterial Activities of n-Hexane and Ethyl Acetate Extracts from young leaves of Red Lip (*Syzygium myrtifolium* (Roxb.) Walp.)

Vilya Syafriana[1,2], Yasman[1,3], Windri Handayani[1,3], Dwi J. Ningsih[2], Catur D. Anjani[2], Rosario T. Manalu[2], Ratna Yuniati[1,3], Munawarohthus Sholikha[2]*

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Kampus UI Depok, West Java, Indonesia.

[2]Faculty of Pharmacy, National Institute of Sciences and Technology, Jl. Moh. Kahfi II, Srengseng Sawah, Jagakarsa, South Jakarta 16420. Phone number: (021) 7270090.

[3]Research Group of Metabolomics and Chemical Ecology, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Kampus UI Depok, West Java, Indonesia.

*Corresponding Author E-mail: **mona.farmasi@istn.ac.id**

ABSTRACT:

Syzygium myrtifolium, commonly known as *pucuk merah* in Indonesia, is not only an ornamental plant but also has the potential as a source of antibacterial raw material. Despite several studies reporting its antimicrobial activity against various bacteria and fungi, the antibacterial potential of its young leaf extract remains largely unexplored. The present study aimed to investigate the antibacterial activity of the leaf extract using two solvents with different polarities, namely n-hexane and ethyl acetate, and to determine their respective yield values. The sequential maceration method was employed to extract the bioactive compounds from the young leaves. The results showed that the ethyl acetate extract had a higher yield value (17.71%) compared to the n-hexane extract (5.46%) and exhibited stronger antibacterial activity against all five bacteria at concentrations of 10% and 20%. The observed variability in antibacterial activity could be attributed to differences in the metabolite compositions of the two extracts, as the n-hexane extract lacked tannins and saponins, which are known to possess antibacterial properties. The findings of this study suggest that the ethyl acetate extract of young *S. myrtifolium* leaves may be a promising source of antibacterial agents, particularly against pathogenic bacteria such as *Salmonella* sp., *S. epidermidis*, *S. mutan*, *S. pyogenes*, and *P. acnes*. However, further research is necessary to determine the exact mechanism of action and potential toxicity of these extracts before considering their use in medicine, cosmetics, or food industries.

KEYWORDS: Antibacterial, Ethyl acetate, Maceration, n-Hexane, *Pucuk merah*, Young leaves.

INTRODUCTION:

The *Syzygium* genus is the largest in the Myrtaceae family, encompassing between 1000 to 1800 species that can be found worldwide, primarily in Southeast Asia, China, and Australia[1,2]. These plants have a long history of traditional use in treating various ailments[3].

Its therapeutic potential holds a prominent place in the realm of natural remedies. These plants have garnered attention for their well-documented antimicrobial and antioxidant properties. Their antioxidant capabilities have raised interest in the field of health and nutrition, as these properties can contribute to the prevention of oxidative stress-related diseases. Furthermore, several studies have consistently reported the effectiveness of *Syzygium* spp. in combating various pathogens, making them a valuable resource in the quest for novel antimicrobial agents[4,5,6,7,8].

Universitas Indonesia, located at Depok, West Jawa, is home to at least three species of *Syzygium*, including *S. aromaticum*, *S. polyantum*, and *S. myrtifolium*. While the former two have been extensively studied for their bioactive potential, the latter remains relatively unknown. Although *S. myrtifolium* belongs to the same taxonomic genus as previously mentioned species, it remains relatively unknown, despite its potential for use in food and medicine. Locally known as red lip or *pucuk merah* due to the striking red colour of its young leaves, *S. myrtifolium* (Roxb.) Walp. (Figure 1) is presently the most commonly found ornamental plant in parks, residential and commercial buildings, and almost every highway. It is also easy to cultivate and maintain, making it increasingly popular[9]. Nonetheless, the plant is underutilized in the field of pharmaceutical activities, which is unfortunate, as it represents a missed opportunity for exploiting its health benefits.

This red lip plant has the potential to be a source of antibacterial raw materials due to the presence of flavonoids, tannins, and saponins, all of which have antimicrobial properties[10,11]. Additionally, previous studies have shown that this plant has antimicrobial activity against various bacteria and fungi, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Candida* spp., *Propionibacterium acnes*, and *Cryptococcus neoformans*[10,11,12,13]. Our previous study also found that the methanol extract of *S. myrtifolium* leaf has antibacterial activity against *P. aeruginosa*

previous study also found that the methanol extract of *S. myrtifolium* leaf has antibacterial activity against *P. aeruginosa*, *Staphylococcus epidermidis*, and *Streptococcus mutans* growth[10,14]. This study aims to explore further the antimicrobial activity of *S. myrtifolium* young leaves extract using n-hexane and ethyl acetate solvents towards various pathogenic bacteria, including *Propionibacterium acnes*, *Salmonella* sp., *Staphylococcus epidermidis*, *Streptococcus mutans*, and *Streptococcus pyogenes*, and to determine their respective yield values. When selecting the pathogens for this study on the antimicrobial activity of *S. myrtifolium* young leaves extract, several key considerations were taken into account. *Propionibacterium acnes* was chosen due to its role in acne formation, making it a relevant target for dermatological applications[15]. *Salmonella* sp. was included for its significance as a common cause of foodborne illnesses[16], while *Staphylococcus epidermidis*, a skin commensal, was selected for its potential to cause opportunistic infections, particularly in immunocompromised individuals[17,18]. *Streptococcus mutans* and *Streptococcus pyogenes* were also chosen due to their roles in dental caries and streptococcal infections, respectively[19]. These diverse bacterial strains provide a comprehensive evaluation of the extracts' antimicrobial efficacy across different types of pathogens, reflecting both clinical and environmental relevance.

To obtain the extracts, the maceration method was employed, utilizing a series of two solvents (n-hexane and ethyl acetate). This method was chosen due to its simplicity as a sample extraction method[20,21]. The antibacterial activity was determined using the disk diffusion method (Kirby-Bauer disk diffusion), where the clear zone formed around the disc was measured as the Inhibition Zone (IZ)[22,23]. Hopefully, the findings of this study may serve as a foundation for utilizing *S. myrtifolium* not only as an ornamental plant but also as a medicinal plant, providing valuable preliminary information about the potential use of *Syzygium* plant diversity at the UI Depok Campus.

Figure 1. Red lip (*Syzygium myrtifolium*) plant

MATERIALS AND METHODS:

Materials:

In this study, various chemicals and reagents were employed, including Nutrient Agar (NA) [Oxoid], Mueller Hinton Agar (MHA) [Oxoid], Aquadest [MPLab], 70% Ethanol [Dwinika], n-Hexane [PMP], Ethyl Acetate [PMP], FeCl3 [Merck], Wagner reagent [CDH], Mayer reagent [CDH], Dragendorff reagent, Ammoniak [Merck], Acetic acid anhydride [Merck], NaNO2 [Merck], AlCl3 [Merck], HCl [Merck], Chloroform [Merck], H2SO4 [Merck], DMSO [Merck], immersion oil, Crystal violet [Merck], Safranin, Lugol's iodine, 0.9% NaCl [Braun], Blank disc [Oxoid], the antibiotic disk of Amoxicillin 25µg [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.

Preparation and extraction of sample *Syzygium myrtifolium* leaf:

To obtain the *S. myrtifolium* young leaves, three kilograms of fresh leaves were sourced from the Research Agency of Spices and Medicinal Plants (Balittro) in Bogor. These leaves were then thoroughly cleaned with water and air-dried for three days. Next, the dried leaves were crushed with a blender[24] and passed through mesh 44 to ensure a homogeneous size of simplicia. This step was important to facilitate equal interaction between the leaf powders and the solvent, as suggested in previous studies[25,26,27].

A total of 250g of *S. myrtifolium* young leaves were extracted using the maceration method with a nonpolar solvent (n-hexane) followed by a semi-polar solvent (ethyl acetate) in a 1:10 ratio. The maceration process was conducted for 24 hours and repeated once using the same procedure[21]. The resulting extract was filtered using filter paper and evaporated using a vacuum rotary evaporator until a crude extract was obtained.

Specific and non-specific parameters of extracts:

The parameters for the analysis include organoleptic test, water and ethanol soluble compound content, phytochemical screening, total ash content, and acid insoluble ash content[20,21]. The tests were conducted at Testing Laboratory of the Research Institute for Medicinal and Aromatic Plants, Balittro, in Bogor, Indonesia.

Antibacterial Activity Test:

To perform the antibacterial test, the disk diffusion method was employed to measure the diameter of the inhibition zone (IZ) of the extract against various pathogenic bacteria, including *Propionibacterium acnes*, *Salmonella* sp., *Staphylococcus epidermidis*, *Streptococcus mutans*, and *Streptococcus pyogenes*. This method has been previously described in various literatures[23,28,29,30].

a) Bacterial Suspension Preparation:

The bacteria, aged for 24hours, were taken in 3-4 doses and transferred into a test tube containing 9mL of 0.9% NaCl. The test tube was then vortexed to ensure homogeneity. The bacterial suspension was adjusted to McFarland 3(9.0×10^8 CFU/mL) as the turbidity reference[29,30,31,32].

b) The Extract Concentrations Preparation:

The extracts were prepared at four different concentrations (w/v), which are 2.5%, 5%, 10%, and 20%, based on our previous study with modification[10,14]. A negative control using 50% DMSO was included, while a positive control was

prepared using the antibiotic amoxicillin[30,31,33].

c) Diameter of Inhibition Zone (IZ) Test:

Each bacterial suspension was pipetted onto a Petri dish containing Mueller Hinton agar (MHA) at a volume of 0.1mL, which was then evenly spread using a sterile L-shaped rod. After the media and bacterial suspension had dried, a sterile paper disk was placed onto the agar. Each concentration of the extract (2.5%, 5%, 10%, and 20%) was applied to the disk using a dropper, with approximately 20µL of extract per application. The Petri dishes were then incubated at 37°C for 24hours. The clear zone formed around the disk was observed and measured as the Inhibition Zone (IZ)[23]. A negative control using 50% DMSO (v/v) and a positive control using the antibiotic amoxicillin 25µg were included in the experiment.

d). Data Analysis:

Data analysis was conducted using a two-way ANOVA test to determine the effects of bacterial type and extract concentration. A P-value of less than 0.05 was considered to indicate a significant difference in each treatment, with the analysis performed using MINITAB 18. Following the ANOVA, Tukey's post-hoc test was applied to compare all pairs of treatment means.

RESULT:

Sample extraction:

The resulting extract yields value of the *S. myrtifolium* young leaves extract was found to increase with the polarity of the solvent. The ethyl acetate extract exhibited a higher yield value of 17.71% as compared to the n-hexane extract, which had a yield value of 5.46% (Table 1).

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

Sample	Solvent	Sample weight (g)	Extract weight (g)	Yield (%)
Young leaves (red lip)	n-Hexane	250	13.65	5.46
	Ethyl acetate	250	44.28	17.71

Specific and non-specific parameters of extracts:

a) Organoleptic observations:

The organoleptic observations conducted in this study included tests for odor, color, and texture (Table 2). The results showed that both extracts had a distinct odor that resembled the scent of the original leaf samples. The color of the extracts was reddish-brown, with the n-hexane extract having a deeper hue than the ethyl acetate extract. The texture of the n-hexane extract was thick, while the ethyl acetate extract was liquid in form.

Table 2. Organoleptic observations of *Syzygium myrtifolium* young leaf using n-hexane and ethyl acetate as solvents

Organoleptic observation	n-Hexane extract	Ethyl acetate extract
Odor	Typical	Typical
Color	Reddish-brown	Reddish-brown
Texture	Thick	Liquid

b) Phytochemical screening:

Phytochemical screening was carried out to identify the presence of various secondary metabolites in the n-hexane and ethyl acetate extracts of *S. myrtifolium* young leaves. Table 3 demonstrated that the ethyl acetate extract of *S. myrtifolium* young leaves contained alkaloids, saponins, tannins, phenolics, flavonoids, steroids, triterpenoids, and glycosides. On the other hand, the n-hexane extract exhibited negative results in the saponins and tannins tests.

Table 3. Phytochemicals screening of n-hexane and ethyl acetate extracts of *Syzygium myrtifolium* young leaves

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

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

c) Water and ethanol soluble compound content:

To estimate the quantity of polar compounds (water soluble) and semi-polar or nonpolar compounds (ethanol soluble) in the extracts, compound content analysis was performed and presented in Table 4. Both n-hexane and ethyl acetate extracts exhibited greater solubility in alcohol solutions than in water solutions.

Table 4. 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

d) Total ash content and acid insoluble ash content:

The total ash content is a useful indicator for determining the mineral content in extracts, including minerals that are not soluble in acids. The maximum range of total ash content can also indicate the level of contaminants and purity in the extract. The total ash content of the n-hexane extract was found to be approximately 8.34%, while that of the ethyl acetate extract was only 0.06%, as shown in Table 5. Acid insoluble ash content was detected only in the n-hexane extract, and not in the ethyl acetate extract. The presence of minerals in both extracts was confirmed by the total ash content, while the acid insoluble ash content indicated the presence of sand or other impurities.

Table 5. Total ash and acid insoluble ash content of n-hexane and ethyl acetate extracts of *Syzygium myrtifolium* young leaves

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

ND: Not Detected

Antibacterial activity:

In this study, five pathogenic bacteria were examined, consisting of one Gram-negative bacteria (*Salmonella* sp.) and four Gram-positive bacteria (*Propionibacterium acnes*, *Staphylococcus epidermidis*, *Streptococcus mutans*, and *Streptococcus pyogenes*). The results can be seen in Figures 2 and 3, as well as in Table 6.

Figure 2. Antibacterial activity of n-hexane extract (NHE) of *Syzygium myrtifolium* young leaves (AML: Amoxicillin; DMSO: dimethyl sulfoxide)

Figure 3. Antibacterial activity of ethyl acetate extract (EAE) of *Syzygium myrtifolium* young leaves (AML: Amoxicillin; DMSO: dimethyl sulfoxide)

Based on the data presented in Figures 2 and 3, it can be concluded that the ethyl acetate extract exhibited stronger antibacterial activity compared to the n-hexane extract. The results of the Tukey test ($P < 0.0500$) shown in Table 6 indicate that the ethyl acetate extract provides significantly greater bacterial growth inhibitory activity than the n-hexane extract across all tested bacteria. Notably, the 10% and 20% ethyl acetate extracts demonstrated the highest inhibition zone (IZ) values against *P. acnes*, with significant differences when compared to the n-hexane extract. Similar strong inhibitory activity was observed with the ethyl acetate extract against *Salmonella* sp., *S. epidermidis*, and *S. pyogenes*. The only exception was with *S. mutans* at a 10% extract concentration, where the IZ values between the two extracts were not significantly different. Overall, the ethyl acetate extract consistently showed the strongest antibacterial inhibitory activity among all types of bacteria tested, outperforming the n-hexane extract.

The potential of the n-hexane extract to inhibit *P. acnes*, *S. mutans*, and *S. pyogenes* was observed at concentrations of 10% and 20%. However, it failed to suppress the growth of *Salmonella* sp. and *S. epidermidis* (Figure 2). On the other hand, the ethyl acetate extract displayed a higher inhibition zone compared to the n-hexane extract, and it effectively inhibited the growth of all five bacteria at 10% and 20% concentrations (Figure 2 and Figure 3).

The n-hexane extract's activity revealed that it was incapable of inhibiting either *S. epidermidis* or *Salmonella* sp. at any of the tested concentrations. This indicates that these two bacteria can maintain the resilience of their cells, preventing damage from the n-hexane extract. Gram-negative bacteria, such as *Salmonella* sp., are generally more resistant to antimicrobial agents than Gram-positive bacteria. In contrast, *S. epidermidis*, although it is a Gram-positive bacterium, this bacterium has the ability to produce biofilms which can reduce the permeability and penetration of antibiotics into cells. This was confirmed by the results of the *S. epidermidis* test on positive control of the antibiotic amoxicillin which showed resistance because the diameter of growth inhibition value was below 28mm (Table 6).

The ethyl acetate extract exhibited different results against *Salmonella* sp. as compared to the n-hexane extract. The ethyl acetate extract was able to inhibit the growth of *Salmonella* sp. with a diameter range of inhibition from 8-14mm (Table 6). In addition to *Salmonella* sp., differences were seen in the activity of the extracts against *S. pyogenes* and *P. acnes*. The n-hexane extract inhibited bacterial growth at concentrations of 10% and 20%, while the ethyl acetate extract displayed inhibition activities from the lowest concentration (2.5%) to the highest concentration (20%). This suggests that the metabolite compounds present in the ethyl acetate extract but absent in the n-hexane extract played a role in inhibiting the growth of *Salmonella* sp., *S. pyogenes*, and *P. acnes*.

DISCUSSION:

A sequential extraction using different polarity organic solvents, n-hexane and ethyl acetate, was conducted using the maceration method, chosen for its simplicity and ability to preserve heat-resistant bioactive compounds. Stirring facilitated contact between the solvent and plant cell cavity, while improved solvent circulation enabled efficient extraction. The methods used were in accordance with the guidelines provided by the Indonesian National Agency of Drug and Food Control[34] and similar to those used in prior research[35]. The higher yield value of the ethyl acetate extract compared to the n-hexane extract suggests a greater amount of active compounds may have been extracted by the solvent. A higher yield value implies a higher amount of active compounds extracted[36,37]. These findings are consistent with those of previous studies[38] that reported a higher yield value for the ethyl acetate extract compared to the n-hexane extract in their investigation of *S. polyanthum* leaf extract, which were 1.40g and 0.33g, respectively.

Table 6. Antibacterial activities of n-hexane and ethyl acetate extracts of *S. myrtifolium* young leaves

Bacteria	Inhibition Zone (IZ) (mm)									
	NHE Concentrations				EAE Concentrations				Control	
	2.5%	5%	10%	20%	2.5%	5%	10%	20%	AML	DMSO
P. acnes	0^{G}	0^{G}	7.05 ± 0.19^{F}	11.15 ± 0.49^{D}	9.55 ± 0.50^{E}	11.50 ± 0.30^{D}	13.16 ± 0.62^{C}	16.25 ± 0.65^{B}	24.73 ± 0.17^{A}	0^{G}
***Salmonella* sp.**	0^{E}	0^{E}	0^{E}	0^{E}	8.51 ± 0.80^{D}	11.44 ± 1.20^{C}	12.88 ± 0.52^{C}	14.72 ± 1.07^{B}	18.57 ± 0.73^{A}	0^{E}
S. epidermidis	0^{C}	0^{C}	0^{C}	0^{C}	0^{C}	0^{C}	6.82 ± 0.30^{B}	6.85 ± 0.30^{B}	14.80 ± 0.42^{A}	0^{C}
S. mutans	0^{D}	0^{D}	7.32 ± 0.15^{C}	7.9 ± 0.11^{C}	0^{D}	0^{D}	7.42 ± 0.08^{C}	9.85 ± 1.25^{B}	43.03 ± 0.87^{A}	0^{D}
S. pyogenes	0^{E}	0^{E}	7.36 ± 0.76^{D}	8.7 ± 0.86^{D}	8.59 ± 0.96^{D}	10.92 ± 0.90^{C}	12.08 ± 0.88^{B}	12.38 ± 1.22^{B}	21.37 ± 0.13^{A}	0^{E}

NHE: n-hexane extract; EAE: ethyl acetate extract; AML: amoxicillin 25 µg (positive control); DMSO: dimethyl sulfoxide 50% (negative control); Superscript letters: results of comparative statistical tests between extracts of the same species. Means that do not share a letter are significantly different

Both n-hexane and ethyl acetate extracts had a distinct odor resembling the original leaf samples, but the n-hexane extract was darker than the ethyl acetate extract. The color and odor of the extracts were showed similarity to the 96% ethanol extract performed by Moerfiah et al.[39]. Moreover, the n-hexane extract's texture was similar to that reported by Hasti et al.[40], while for the ethyl acetate extract, this study is the first to report its organoleptic properties. The physical properties of plant extracts and the presence of certain compounds can be determined through organoleptic observations as conducted in this study. Such observations can aid in evaluating the purity of the extracts, detecting any contamination or alteration, and determining the sensory attributes, which are crucial for their application in fields such as cosmetics and pharmaceuticals[41]. The n-hexane extract had a remarkably higher total ash content and was the sole extract to exhibit the presence of acid-insoluble ash, signifying impurities such as sand or soil in herbal extracts. Total ash content is also an important indicator of mineral content that helps determine the level of contaminants and purity in extracts[42,43]. The Indonesian Herbal Pharmacopoeia (FHI) standards specify a maximum of 13.3% total ash content[21], which the n-hexane extract complied with despite having a higher total ash content of approximately 8.34%.

The analysis of compound content showed that the extracts had a greater solubility in alcohol solutions than in water solutions, suggesting that alcohol-based solvents would be more effective in extracting *S. myrtifolium* young leaves[43]. The estimation of polar and semi-polar/nonpolar compounds in extracts can aid in selecting the most appropriate solvent for extracting desired compounds from plant material and provide information on the bioactivity of the extracts, indicating their potential applications in pharmaceuticals and cosmetics.

Phytochemical screening was carried out to identify the presence of secondary metabolites such as alkaloids, flavonoids, glycosides, phenolics, saponins, steroids, tannins, and triterpenoids in the n-hexane and ethyl acetate extracts of *S. myrtifolium* young leaves, as described in previous studies[44,45]. In contrast to the ethyl acetate extract, the n-hexane extract did not show the presence of saponins and tannins during the phytochemical screening. These results were consistent with previous studies[10,11], which identified flavonoid and phenolic compounds in their *S. myrtifolium* extracts. It is worth noting that the flavonoid content in these extracts has been reported to possess antibacterial properties[46].

The weaker antibacterial activity of the n-hexane extract as compared to the ethyl acetate extract may be attributed to its higher impurity level and lack content of saponins and tannins. Furthermore, the observed differences in the activity of the extracts against the tested bacteria suggest that the presence of metabolite compounds such as saponins and tannins in the ethyl acetate extract, which were absent in the n-hexane extract, played a role in inhibiting the growth of *Salmonella* sp., *S. pyogenes*, and *P. acnes*. As Gram-negative bacteria, such as *Salmonella* sp., are generally more resistant to antimicrobial agents than Gram-positive bacteria due to the presence of lipopolysaccharides in their outer membrane, which can block the penetration of antibiotics or other undesirable compounds into the bacterial cell[47,48], the ability of the ethyl acetate extract to inhibit the growth of *Salmonella* sp. with a diameter range of 8-14 mm is noteworthy, as it indicates the potential of the extract as a natural antimicrobial agent against this bacterium.

In addition, the present study also highlights the challenge of treating *S. epidermidis*, which can reduce the permeability and penetration of antibiotics into cells as indicated by the positive control of the antibiotic amoxicillin that showed resistance against *S. epidermidis* because the diameter of growth inhibition value was below 28mm[49]. *Staphylococcus epidermidis*, although it is a Gram-positive bacterium, this bacterium has the ability to produce biofilms which can reduce the permeability and penetration of antibiotics into cells[17]. Amoxicillin is a class of beta-lactam antibiotics. *Staphylococcus* bacteria are renowned for their ability to neutralize beta-lactam groups through the release of beta-lactamase enzymes. These enzymes bind to the beta-lactam rings found in antibiotic compounds and effectively break the amide bond within these rings. The ability of *Staphylococcus* bacteria to produce beta-lactamase enzymes represents a significant challenge in the treatment of bacterial infections. The development of beta-lactamase inhibitors, such as clavulanic acid, has been a key mechanism in enhancing the efficacy of beta-lactam antibiotics[50]. This, in turn, has opened up opportunities to explore new

antibiotics from natural products. For instance, the ethyl acetate extract of *S. myrtifolium* young leaves exhibited growth inhibition at higher concentrations (as shown in Table 6). Although its inhibitory activity is only half that of amoxicillin, which was used as a positive control, the crude ethyl acetate extract in this study provides hope for further exploration of the compounds responsible for the antimicrobial activity. Thus, continuing to search and develop new antibiotics and alternative treatment strategies to counteract the threat of antibiotic resistance is crucial and ongoing[51,52].

These results are consistent with previous studies by Veranita et al.[53] and Armansyah et al.[54], which reported that the n-hexane extracts of breadfruit and red betel leaves were unable to inhibit the growth of *E. coli*, while the ethyl acetate extracts of these leaves exhibited inhibitory effects. Similarly, a study by Nuraskin et al.[55] showed that the ethyl acetate extract of *Vitex pinnata* leaves was more effective in inhibiting the growth of *S. mutans* than the n-hexane extract. Interestingly, the present study also found that the ethyl acetate extract exhibited a similar inhibitory effect against *S. mutans* as against *S. epidermidis*, which required a higher concentration for inhibition, as shown in Table 6.

Staphylococcus bacteria have a specific resistance mechanism against antibiotics: they produce penicillin-binding proteins (PBPs) that alter the metabolic pathway for peptidoglycan formation in the bacterial cell wall. The penicillin-binding domains of PBPs are transpeptidases or carboxypeptidases involved in peptidoglycan metabolism, which modifies the cell wall structure and makes it difficult for antibiotics to target the bacterial cell, thus conferring resistance[56,57,58]. *Streptococcus mutans*, a Gram-positive bacterium, also forms biofilms, which can reduce sensitivity to antimicrobial agents[59,60]. These findings underscore the importance of developing alternative treatment strategies for infections caused by biofilm-forming bacteria, including those with PBP-mediated resistance mechanisms.

Unlike *Staphylococcus*, streptococci bacteria such as *Streptococcus pneumoniae* does not have a penicillin-resistant PBP resembling *Staphylococcus aureus* PBP2a. Instead, resistance to β-lactams in *Streptococcus pneumoniae* arises from alterations in the sequence and structure of its PBPs[57]. In resistant streptococci, the evolution from penicillin-susceptible to reduced susceptibility and then to nonsusceptible occurs through the progressive accumulation of amino acid substitutions in HMM PBPs rather than single-event horizontal gene transfer of β-lactamase or low β-lactam affinity HMM PBPs[56,61,62,63,64].

This phenotype-to-genotype workflow has dominated the molecular basis of antibiotic resistance research for decades, facilitating the identification of genetic polymorphisms in pathogenic streptococci leading to reduced antibiotic susceptibility and the discovery of novel mechanisms. Group A *Streptococcus* (GAS) with reduced susceptibility to β-lactams have acquired mutations in genes encoding PBPs, including PBP1a, PBP2a, PBP2b, and PBP2x[65]. The most common mutation in GAS results in the substitution of an amino acid in PBP2x transpeptidase for T553K[66].

CONCLUSION:

In conclusion, the present study demonstrated that ethyl acetate is a more effective organic solvent for the extraction of *S. myrtifolium* young leaves when compared to n-hexane. Furthermore, the ethyl acetate extract exhibited greater antibacterial activity than the n-hexane extract, effectively inhibiting the growth of all five bacteria at concentrations of 10% and 20%. Conversely, the n-hexane extract showed no ability to inhibit either *S. epidermidis* or *Salmonella* sp. at any concentration tested. These discrepancies in antibacterial activity between the two extracts may be attributed to differences in metabolite composition, with the n-hexane extract lacking the tannins and saponins that are known to disrupt bacterial cell membranes and inhibit protein synthesis. The study's implications suggest that the ethyl acetate extract of *S. myrtifolium* young leaves may serve as a promising source of antibacterial agents, particularly against pathogenic bacteria such as *Salmonella* sp., *Staphylococcus epidermidis*, *Streptococcus mutans*, *Streptococcus pyogenes*, and *Propionibacterium acnes*. However, further research is needed to further exploration of the compounds responsible for the antimicrobial activity and to determine the exact mechanism of action and potential toxicity of these extracts before they can be considered for use in medicine, cosmetics, or food industries.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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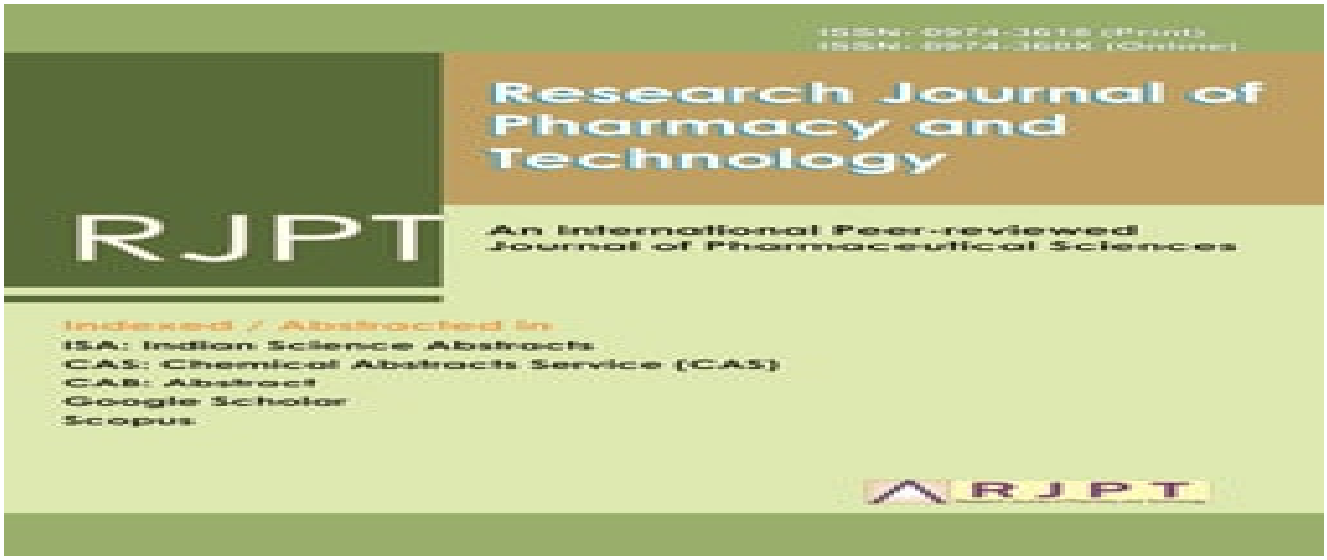

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