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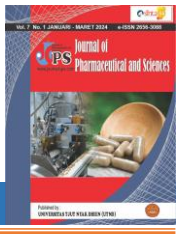

A microbiological study of *Annona muricata* Lim. Folium partitioned extract against *Propionibacterium acnes* and *Staphylococcus aureus.*

Kajian mikrobiologi ekstrak terpartisi *Annona muricata* Lim. Folium terhadap *Propionibacterium acnes* dan *Staphylococcus aureus*

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Abstract

Annona muricata is a plant broadly reported to have various medicinal benefits and has been widely used around the world. The leaves are a part reported to have antimicrobial activity. This study aimed to determine the antimicrobial properties of *A.muricata* leaves partitioned extract against *Propionibacterium acnes* and *Staphylococcus aureus.* The partitioned extract was obtained by liquid-liquid partition of ethanolic extract using N-hexane, ethyl acetate, and ethanol. An antimicrobial study was conducted using the Kirby-Bauer method. The result showed that at the highest concentration model used, antimicrobial activity was shown in N-hexane partitioned extract and ethyl acetate partitioned extract. Those properties were known to correlate with some groups of secondary metabolites, including flavonoid, steroid, phenolic, and saponin.

***Keywords*: Soursop, Leaves, Propionibacterium acnes, Staphylococcus aureus**

Abstrak

Tanaman *Annona muricata* telah dilaporkan memiliki beragam khasiat yang sejak dulu telah dimanfaatkan secara tradisional. Bagian daun dari tanaman *A.muricata* merupakan salah satu bagian tanaman yang diketahui bertanggungjawab pada aktivitas antimikroba. Penelitian ini bertujuan untuk mengetahui potensi antibakteri ekstrak terpartisi daun *A.muricata* terhadap bakteri *Propionibacterium acnes* dan *Staphyloccous aureus*. Ekstrak terpartisi dibuat dari ekstrak etanol yang dipartisi ke dalam pelarut N-heksan, etil asetat, dan etanol. Aktivitas antimikroba dari ketiga esktrak terpartisi diuji menggunakan metode Kirby-Bauer. Diperoleh hasil bahwa pada model konsentrasi yang digunakan, aktivitas antimikroba ditunjukkan pada ekstrak terpartisi N-heksan dan ekstrak terpartisi etil asetat. Hasil yang ditunjukkan tersebut berhubungan dengan golongan senyawa yang dimiliki masing-masing ekstrak terpartisi. Pada kedua ekstrak terpatisi yang memberikan respon positif pada pengujian antimikroba terhadap *P.acnes* dan *S.aureus*, terdeteksi adanya kandungan golongan senyawa flavonoid, steroid, fenolik serta saponin.

***Kata Kunci*: Soursop, Leaves, Propionibacterium acnes, Staphylococcus aureus.**

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Introduction

Annona muricata is a fruit plant widely inhabited in tropical and subtropical continents, including Indonesia. This plant belongs to the genus Annona in the Annonaceae family, order Magniliales and Magnoliophyta division. *A.muricata* is also known as soursop (English), and sirsak or nangka belanda (Indonesia). The plant is a 5-10m tall tree that blooms and fruits most of the year. The fruit is edible with white and creamy flesh and has a particular taste and aroma [1,2]. Apart from using fruit as food, *A.muricata* has a long history of medicinal traditional history. The utilisation of the whole part of the plant was reported and is still being studied. The tree, bark, root, seed, leaves and fruit were all utilised as medication traditionally for several indications. The reported usage includes arthritis, neuralgia, skin rashes, rheumatism, malaria, fever, dysentery, aches and pain, flu, asthma, diarrhoea, hypertension, diabetes, insomnia, cancer, and parasitic infection [1,3]. In Indonesia, *A.muricata is* traditionally used to treat hypertension, diabetes, cancer, and skin rashes [4].

Some indications reported were known to correlate with microbial infection. This activity is mainly shown by the leaves, seed, and fruit skin through extraction, infusion, and endophytic microbial potential [5–7]. The leaves of *A.uricata* were reported to have microbial properties by inhibiting the growth of various bacteria, either positive gram or negative gram [8–10]. To correlate the traditional use of *A.muricata* as antimicrobial medication, this study explored the microbial study of a partitioned extract of *A.muricata* leaves against *Propionibacterium acnes* and *Staphylococcus aureus* responsible for skin disease infection.

Experimental Section

Materials and Apparatus

Annona muricata Lim. was obtained from East Sakra Lombok Timur, NTB, on March 2024 and was confirmed through plant determination number 18/UN18.F7/LBL/2024 by Laboratorium Biologi Lanjut Universitas Mataram. The leaves used were the third to fifth leaves counted from shoots. The chemicals used were technical grade, including ethanol, ethyl acetate, and N-hexane. Microbiology materials were *Propionibacterium acnes* ATCC 6919, *Staphylococcus aureus* ATCC 2913, Nutrient Agar (Himedia), Nutrient Broth (Himedia), Blank disc (Oxoid), Streptomycin disc (Oxoid), and Dimethyl Sulfoxide (Merck, pro analysis). The apparatus used were the Oven (Automatic Thermo-Controller TEW IL-80EN), rotary evaporator (Heidolph Hei-Vap), and incubator (Memmert IN55).

Extraction

The leaves were washed and dried using Oven at 50°C for 48 hours. The dried leaves were then powdered before maceration in ethanol with powder: solvent ratio (1:4) for 72 hours by stirring occasionally. The filtrate obtained by filtration was further concentrated using a rotary evaporator at 50°C 48rpm. The concentrated extract was partitioned by liquid-liquid partition using N-hexane, ethyl acetate and ethanol and subjected to phytochemical screening.

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Preliminary Phytochemical screening

Flavonoid was evaluated in 5mg of each partitioned extract dissolved in ethanol and reacted with 0,1g Mg powder. The presence of flavonoid was confirmed by orange-yellow to red colouration following the addition of HCl to the mixture. Steroid content was evaluated by reacting each partitioned extract with some drops of Liebermann-Burchard reagent. The positive response of steroid content showed as a bluish-green mixture solution. The orange sediment formation confirmed Alkaloid since mixing 0,1 g of each partitioned extract with 1 mL Dragendorff reagent. Phenolic content was evaluated by forming a blackish-green colour in a mixture of 5mg of each partitioned extract with 2-3 drops of 1% $FeCl_3$. Saponin was tested using five milligrams of each partitioned extract and was shaken to induce foam formation. After reacting each sample with 1mL 2N HCl, the remaining foam indicates the presence of Saponin.

Antimicrobial evaluation

The antimicrobial properties of nutrient agar were evaluated using Kirby Bauer's methods. *P.acnes* ATCC 6919 and *S.aureus* ATCC 2913 were cultured in nutrient broth media and were diluted in sterile normal saline to obtain $1x10^8$ CFU/mL. Those cultures were then inoculated with sterile cotton swabs on sterile nutrient agar. Disc tests were prepared by soaking the 6mm blank discs into each partitioned extract at concentration levels 10%, 5%, and 2,5% (w/v) in 5% DMSO and placed immediately onto inoculated media. Streptomycin 10µg disc (Oxoid) was used as the positive control, and 5% DMSO was used as a negative control. The test was conducted in triplicate before performing incubation at 37°C overnight.

Data analysis

Antimicrobial properties were studied by observing the inhibition zone around the disc triplicate. The data is expressed as mean ± SD.

Results and Discussion

Extraction

Maceration is the simplest and oldest extraction method to obtain several contents of secondary metabolites in some dried and raw natural products, including *Annona muricata* Lim. Leaves in this study. This technique is practised at room temperature by soaking the dried plant material in a selected solvent for a longer time with frequent agitation. The absence of heating in maceration permits various metabolites to yield and secure heat-sensible metabolites from instability [11]. Maceration allows cell wall rupture and liberates the soluble secondary metabolite [12]. In this study, due to its character as the universal solvent, ethanol was used to strain metabolites contained in *Annona muricata* Lim. Leaves. Partition was conducted to the concentrated extract using the liquid-liquid partition technique using three different solvents. This technique aimed to separate the metabolites by their solubility in a particular solvent. In this study design, the three different solvents used had distinct polarity to facilitate the solubility of the metabolites. This model resulted in 3 partitions,d extracts: N-hexane, ethyl acetate, and ethanol.

Phytochemical Screening

As the preliminary examination, phytochemical screening was conducted to provide an overview of the compound content class in some natural product samples. Table 1 shows the result of some metabolites in three different partitioned extracts. Yellow, orange, and red indicate reduced benzopyrone core in flavonoids by HCl and Mg powder [13]. Positive results of steroid confirmed by the formation of a bluish colour as the esterification of Steroid by Liebermann-Burchard reagent [14]. The positive response of steroids initially appeared as red and turned blue to green end[15]. When interacting with an alkaloid, Dragendorf will form an orange precipitate due to the interaction of tetraiodobismuthate (III) ions in the reagent [16]. Identification of phenolic content was conducted using $FeCl_3$ reagent. A blackish-green stain developed during the reaction signed the presence of phenolic as Fe_3^+ ions undergo the hybridisation reaction [17]. Saponins are triterpenoids, steroid glycosides, alkaloid steroids or steroids with nitrogen [18]. In the presence of Saponin, the foam is formed by glycoside through agitation that will not subside by adding 2N HCl [18,19].

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Table 1. Phytochemical Screening of Annona muricata Lim. Folium Partitioned Extract

Class of Compound	Partitioned Extract		
	N-hexane	Ethyl Acetate	Ethanol
Flavonoid	-	+	+
Steroid	+	-	+
Alkaloid	-	-	+
Phenolic	-	+	+
Saponin	-	+	-

A current study reported the leaves of *A.muricata to* contain several classes of compounds: flavonoid, steroid, alkaloid, phenolic, and saponin [20–22]. Among those, alkaloids, flavonoids and phenolics are significant components of A. *muricata* leaves [21]. Those findings align with the result of this study in Table 1. The extract contains flavonoid, steroid, alkaloid, phenolic and saponin, partitioned into three polarity solvents. N-hexane partitioned extract showed a positive response to steroids. Subandrate et al. [23] and Pratiwi et al. [24] reported that steroids tend to draw to nonpolar solvents such as N-Hexane. In ethyl acetate partitioned extract, phenolic, flavonoid, and saponin gave a positive qualitative response. Phenolic is a class of compounds characterised by the presence of single aromatic rings coupled to a single or more hydroxyl group. Phenolics were known as the most common secondary metabolites of plants with over 8000 structures. Due to its variousity of the structures, phenolics have nonuniformity solubility [23]. As a part of phenolic classes, flavonoids were generally known to dissolve in ethyl acetate [24].

Since it is known as the sort of steroid class with a glycoside functional group, saponins are likely to draw to a more polar solvent, such as ethyl acetate, rather than N-hexane, which draws the steroids. Partitioned extract, phenolic, flavonoid, alkaloid, and steroid were detected preliminary in ethanol. Ethanol and ethyl acetate are two solvents with significant differences. Ethanol has been discovered to be more compatible with draw polar components such as phenolic and flavonoid than ethyl acetate [23]. Apart from phenolic and flavonoid, the quaternary alkaloid had good compatibility to dissolve in ethanol as a polar solvent [18]. Notwithstanding the likely steroid to non-polar solvent, a positive result was shown in ethanol partitioned extract. This might lead to the presence of some polarity steroids which dissolve in ethanol, and the residue of compounds remains. This finding supports Qorina et al. [22] that steroid was detected in ethanolic extract of *Annona muricata*.

Antimicrobial Properties

Kirby-Bauer method was conducted to study the antimicrobial properties of *A. muricata* partitioned extract. Kirby-Bauer classified it into the qualitative diffusion method, where the antibiotic sample tested in the disc spread across the plate and cultured by specific bacteria forced by diffusion force [25,26]. The antimicrobial properties were studied by observing the inhibition zone formed around the sample against *Propionibacterium acnes* and *Staphylococcus aureus*. The result showed that *Annona muricata* folium has antimicrobial properties against *Propionibacterium acnes* and *Staphylococcus aureus* (Picture 1, Table 2). Compared with *P.acnes*, it was found that *S. aureus* is more susceptible to N-hexane partitioned extract. Ethyl acetate partitioned extract showed the same completion against *P. acnes* and *S. aureus*. The clear zone around the disc formed at 10% partitioned extract. *P.acnes* and *S.aureus* showed unsusceptibility to ethanol partitioned extract even at the highest concentration exposure. These results aligned with Pinto et al. [29], who reported that *Annona muricata* has more excellent antimicrobial activity against *S. aureus* by targeting plasma and outer membrane mechanisms. The result shows that ethyl acetate partitioned extract has more excellent faculty than N-hexane and ethanolic partitioned extract. This might indicate that the active secondary metabolites responsible for this activity are likely partitioned into ethyl acetate. Since the highest concentration model level did not show superior activity, further study may explore providing more apparent properties of *Annona muricata* folium partitioned extract as antimicrobial.

According to the results in Table 2, it was known that the antimicrobial properties in this study model were shown by N-hexane and ethyl acetate partitioned extract. This material might be due to 4 compounds detected by preliminary phytochemical tests on those two partitioned extracts: steroid, flavonoid, phenolic and saponin (Table 1). That class of compounds known as responsible as active components for antimicrobial

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properties shows positive results against bacterial and fungal strains [27]. Steroids are reported to have potential biological properties, including antibacterial properties [28,29]. Steroids may induce cell lysis and blockage the nutrient's passage into the cell due to interaction with the lipid membrane layer of the bacterial cell [30,31].

The presence of flavonoid, phenolic and saponin as the preliminary screening of ethyl acetate partitioned extract revealed might allow the antimicrobial performance. As the main class of phenolic compounds, flavonoid is the most important class to be explored. It was found in various chemical diversity due to their biosynthetic pathway and ring changes. Flavonoid studies submitted reports on antibacterial mechanisms. It conducts the activity through several pathways to suppress nucleic acid synthesis, and cytoplasmic membrane function reduces biofilm formation and membrane permeability, which are important to the growth and survival of bacterial cells [32]. A diversity class in the phenolic group facilitate the diversity process for bacterial inactivation. It may be performed by damaging cell walls and disrupting cell membranes, intruding on protein synthesis through molecular disturbance, and even generating apoptosis-like death [33]. Antimicrobial activity of saponin related to their interaction with cell membranes. The interaction leads to morphological changes and cell membrane integrity destruction [34].

The primary antibacterial mechanism of alkaloids as a nitrogen-containing heterocyclic compound includes cell wall synthesis inhibition, cell membrane permeability change, metabolism inhibition, and nucleic acid and protein synthesis inhibition [35].

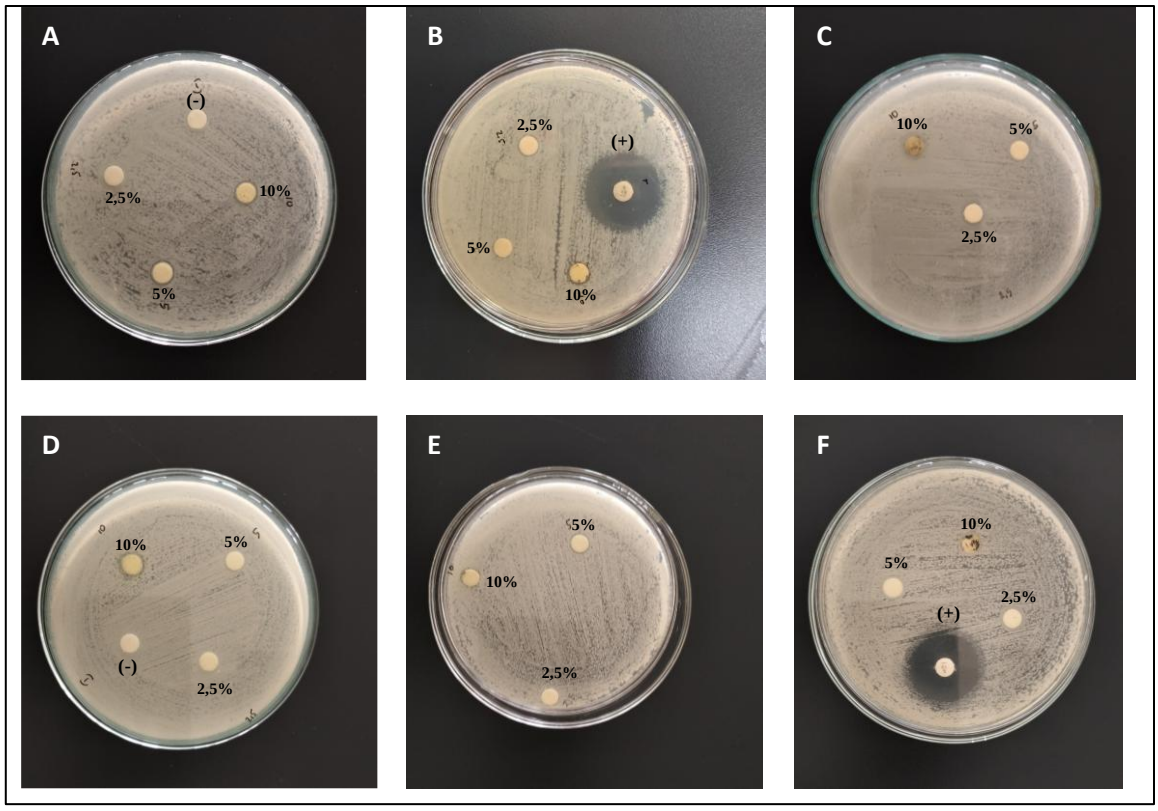

Figure 1. Kirby-Bauer Test of *Annona muricata* Lim. Folium Partitioned Extract against *P.acnes* and *S.aureus*
All treatment was conducted in triplicate. A-C (N Hexane; Ethyl acetate, Ethanol partitioned extract against P.acnes); D-F (N Hexane; Ethyl acetate, Ethanol partitioned extract against S.aureus); (-) DMSO 5%; (+) Streptomycin 10µg.

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Table 2. Antimicrobial properties of Annona muricata Lim. Folium Partitioned Extract

Sample	Concentration	Inhibition Zone (mm)	
		P.acnes	*S.aureus*
N-hexane	10 %	0 ± 0	7 ± 0
	5 %	0 ± 0	0 ± 0
	2,5%	0 ± 0	0 ± 0
Ethyl acetate	10%	7 ± 0	7 ± 0
	5%	0 ± 0	0 ± 0
	2,5%	0 ± 0	0 ± 0
Ethanol	10%	0 ± 0	0 ± 0
	5%	0 ± 0	0 ± 0
	2,5%	0 ± 0	0 ± 0
Streptomycin	10 µg	26 ± 0	23 ± 0,58
DMSO	5%	0 ± 0	0 ± 0

All values are expressed as mean ± SD (n=3). Disc diameter=6mm.

Conclusions

Two of the three partitioned extracts showed positive antimicrobial properties at the highest concentration. N-hexane partitioned extract has potency against *S. aureus*, while ethyl acetate partitioned extract positively responded to *P. acnes* and *S. aureus* through bacterial growth inhibition. Those properties were known to correlate with some groups of secondary metabolites, including flavonoid, steroid, phenolic, and saponin.

Conflict of Interest

The author has declared no conflict of interest.

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