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BIOPROSPECTING OF INDIGENOUS PLANT *Syzygium Cumini* L. Skells AGAINST BACTERIA PATHOGENS

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Abstrack

Antimicrobial testing is a test to measure the ability of a substance to inhibit bacterial growth. The purpose of this study was to determine the inhibitory power of jamblang fruit extract against pathogenic bacteria. The research method used 96% ethanol extract of jamblang fruit and an inhibition zone test was carried out on 4 pathogenic bacteria, then a phytochemical test was carried out on the jamblang fruit extract. The results showed that jamblang fruit extract could inhibit the growth of pathogenic bacteria MRSA (28 mm) and S. typhi (30 mm), while L. monocytogenes and V. cholerae did not show an inhibition zone. Several bioactive compounds were successfully identified from jamblang fruit extract, namely phenolics, flavonoids, and tannins. So jamblang fruit has the potential as an antibacterial.

Keywords: *antimicrobial; bacterial; bioactive; jamblang fruit; phytochemical*

1. INTRODUCTION

Situated in the tropics, Indonesia has massive plant diversity and several of them can be used as a source of medicine (phytopharmacy). Several plants' organs could be used as medicine for instance rhizome, root, skin, flower, seeds, leaves, stems, and fruit. The Java plum *Syzygium cumini* L.Skells or locally named as "jamblang" is considered as a medicinal plant. The fruit of *S. cumini* was traditionally used as ethnomedicine to cure numerous diseases (Banerjee et al., 2005).

The indigenous plant *S. cumini* could be used as an antibacterial agent (Sikder et al., 2012). In Indonesia, this plant has been categorized as rare species that has many biological activities such as antioxidant, anti-inflammatory, hypoglycemia and antibacterial activities (De Bona et al., 2016). The previous study from (Gowri & Vasantha, 2010), methanolic extracts from the Jamblang' leaves as much as 100 µl/L could inhibit the growth of *S. typhi* with 22 mm diameter inhibition zone. Indeed, plant

extracts are considered as a source of antibiotics against various pathogenic bacteria. This is because they have antibacterial and therapeutic compounds without significant side effect compared to the synthetic antibiotics.

In the present study, both gram-positive and negative bacteria will be tested. The gram-positive bacteria *Listeria monocytogenes* known as listeriosis, septicemia and meningitis infection agent with an elevated rate of mortality (Lomonaco et al., 2009). This bacteria considered as recent bacteria thus less documented. Additionally, the *Methicillin Resistant Staphylococcus aureus* (*MRSA*) is identified as a strain from *Staphylococcus aureus* that resistant to *isoxazoyl penicillin* such as *methicillin, oxacillin,* and *flucloxacillin* as well as cross-resistance to all antibiotics in the β-lactam group (Liana, 2014). On the other hand, *Salmonella typhi* is a gram-negative microbe known as the typhoid fever agent. This disease is an infectious disease with bacteremia and inflammation that can deteriorate intestine and hepato-organs (Johnson, 1993).

Statistically, the thyphoid prevalence in Indonesia in 2008 was approximately 81.7% per 100.000 inhabitants with age from 2 to 15 years old (WHO, 2008). Additionally, *Vibrio cholera* is also a gram-negative bacteria that cause cholera disease, diarrhea, stomach cramp, nausea, vomiting and dehydration (Dziejman et al., 2001). The occurrence of this disease is considerably frequent with approximately 5.5 million cases per year in both Asia and Africa (Lesmana, 2004).

In this study, we aimed to identify a new endophytic bacterial strain from jamblang fruit capable of producing secondary metabolites as potential antibacterial agents. This research has never been conducted in Indonesia on several pathogenic bacteria. Here, we evaluate the antibacterial activity from the fruit extract, bacterial and fungal endophytes from *S.cumini* L. Skells against several pathogens such as *Listeria monocytogenes, Methicillin Resistant Staphylococcus aureus (MRSA), S. typhi* and *Vibrio cholera*.

2. METODE PENELITIAN

2.1. *Sampel preparation*

A total of 0,5 kg ripe *S. cumini* fruits were obtained from the arboretum collection Universitas Padjadjaran Jatinangor Sumedang (S 6°55’34.07556” E 107°46’28.87716).

2.2. *Extraction of Jamblang fruit*

The obtained Jamblang fruits were washed and cleaned with running water and then dried until their moisture content was less than 5%. Samples were then blended, added into the macerator containing 96% of ethanol for 3x24 h. The obtained macerate was then filtered using filter paper. Afterward, the liquid extract evaporated using a rotary evaporator to obtain concentrated extract as described in (Suryanto & Wehantouw, 2009).

2.3. Antibacterial activity of Jamblang fruit extract ethanol 90%

The ethanolic extract of Jamblang fruit was added into the well containing tested bacteria which stroke on the medium using a sterile syringe. Petridish plate was incubated at 37ºC for 24 hours for antibacterial activity and at 25ºC for 48 h for antifungal (Sayeed et al., 2012).

2.4 Phytochemical test of Jamblang fruit extract

In the present work, a phytochemical test of ethanolic extract of Jamblang fruit covers determination on phenolic, flavonoid, tannin and alkaloid compoundsas previously described in (Harborne, 1996). This test aims to identify the bioactive compounds from the secondary metabolites content in Jamblang fruit extract.

1) Phenolic

A total of 30 mg ethanolic extract of Jamblang fruit was mixed with few drops of FeCl3 1% and let stand for few minutes. A positive result was indicated by the formation of a strong black color in the sample.

2) Flavonoid

A similar amount of ethanolic extract of Jamblang fruit extract was mixed with Mg powder and few drops of H2SO4 2 N prior to shaking. A positive result was indicated by the formation of yellow color in the sample.

3) Tannin

A similar amount of ethanolic extract of Jamblang fruit extract was mixed with gelatin and the let stand for few minutes. A positive result was indicated by the formation of green-reddish color in the sample.

4) Alkaloid

A similar amount of ethanolic extract of Jamblang fruit extract was dissolved with chloroform. Afterward, H2SO4 2 N and Dragendroff reagent were added into the

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mixture. A positive result was indicated by the formation of orange-color sediments in the sample.

3. RESULT AND DISCUSSION

3.1 Antibacterial Activity

In the present work, the antibacterial experiment showed that ethanolic fruit extract of *S. cumini* could inhibit the growth of *MRSA* bacteria with 28 mm inhibition zone diameter. This result was in accordance with (Priya, 2013) who observed that the ethanolic and ethyl acetate extracts of *S. cumini* peel could inhibit the growth of *MRSA* as much as 13.5 and 10.3 mm, respectively at 10 mg/L concentration.

Table 1. Inhibition zone diameter (mm) formed during antibacterial activity test from fruit extract

No.	Type of samples		Pathogenic Bacteria (mm)			
			MRSA	***L. monocytogenes***	***S. typhi***	***V. cholera***
1.	Fruit extract		28 (SSS)	0	30 (SVT)	0
2.	Control	Amoxilin	10	*	36	*
		Chloramphenicol	*	43	*	*
		Tetracycline	*	*	*	26

Note: (* =no antibacterial activity was observed) (R = Resistant) (SSS =Semi-sensitively Strong; SS =Sensitively strong; SVT = Sensitively very strong)

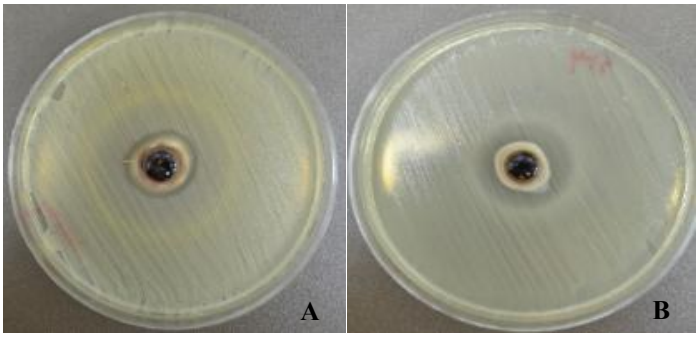

Figure 1. A. Antibacterial activity of fruit extract against *MRSA;* B. Antibacterial activityof fruit extract against *S. Typhi*

Ethanolic fruit extract of *S. cumini* hampered the growth of *S. typhi* with exhibited a very strong antibacterial activity (30 mm of inihibition zone). Indeed, (Patel & Rao, 2012) reported that *S. typhi* could is inhibited by several fruit extracts of S. cumini such

as diethyl ether (12 mm), ethyl acetate (5 mm), acetone (5 mm), methanol (13 mm) and water (13 mm). On the other hand, endophytic Bacillus sp. isolated from *S. cumini* fruit could inhibit the growth of *S. typhi* (16 mm), which categorized with strongly sensitive inhibition. This result confirmed the study from (El-Deeb et al., 2013) who reported that *Bacillus sp*. isolated from the roots of *Plectranthus tenuiflorus* significantly inhibit the growth *of S. typh*i with 15.23 mm inhibition zone.

3.2 Phytochemical analysis of fruit extract S. cumini

Our phytochemical analysis on ethanolic extract of jamblang fruit showed its phenolic, flavonoid and tannin content (Table 2). Phenolic compounds can interact with a protein that may cause a change in membran permeability and cellular damage (Yardpiroon et al., 2014). The fruit extract *Ardisia elliptica* containing tannin can inhibit the growth of *P. multocida*, *K.pneumonia* and *S. Pneumonia* (9.66, 12.66 and 7.66 mm, respectively) (Al-Abd et al., 2017). Phenolic compounds could significantly affect the antimicrobial properties on the plant extract to eliminate pathogenic bacteria in human (Cavanagh et al., 2003).

Table 2. Phytochemical analysis of fruit extract *S. cumini*

Phytochemical compounds	Presence in extract
Phenolic	+
Flavonoid	+
Tannin	+
Alkaloid	-

Tannin has the ability to inactivate the enzyme and cell transport (Godstime et al., 2014). This compound condenses and binds bacterial cell wall to hamper the growth and decrease bacterial protease activitys (Pandev & Kumar, 2013). Tannin isolated from *Solanum trilobatum* showed antibacterial activity against *S.aureus, S. pyrogens, S. typhi, E. coli, Proteus vulgaris,* and *P.aeruginosa* with minimum inhibition concentration 1.0 and 2.0 mg ml^{-1}, while the bacteriocidale minimum concentration range from 1.5 to 2.0 ml^{-1} (Doss et al., 2009).

Antibacterial mechanism of flavonoid covers: inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy

metabolism, inhibition of attachment and biofilm formation, purine inhibition in the cell membrane, change in cell membrane permeability and attenuation of pathogenicity (Xie et al., 2015). Flavonoids can inhibit DNA synthesis by intercalation or stacking of hydrogen bonds at the base of nucleic acids (Anitha and Jayraaj, 2013). A total of 4 flavonoid compounds (morin-3-O-lyxoside, morin-3-O-arabinoside, quercetine andquercetine-3-Oarabinosida) isolated from the leaves of *Psidium guajava* can inhibit the growth of pathogenic bacteria such as *Bacillus stearothermophilus, Brochothrix thermosphacta, E. coli O157: H7, L. monocytogenes, Pseudomonas fluorescens, S. enterica, S. aureus* and *V. cholerae* (Rattanachaikunsopon & Phumkhachorn, 2010)

4. CONCLUSION

Based on research results, jamblang fruit extract can inhibit the pathogenic bacteria *MRSA* and *S. typhi*. Phytochemical tests showed that jamblang fruit extract contains phenolics, flavonoids, and tannins.

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