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INTRODUCTION Indonesia is an archipelago country consisting of more than 17.000 islands. Indonesia's geographic and historical conditions make this country one of the countries with high biodiversity, otherwise known as mega biodiversity1,2. This immense biodiversity has the potential for nutritious and medicinal plants. The World Conservation Monitoring Centre from the UN has reported that Indonesia is an area where various types of medicinal plants are found, with 2.518 species of plants that have been used3. One of the medicinal plants known in Indonesia is the Dilleniaceae family. Dilleniaceae is native to tropical and warm-temperate regions such as Asia, Australia, and the Indian Ocean Islands4,5.

Dilleniaceae are known for their edible fruit and medicinal applications, such as for arthritis, dysentery, diabetes, gastrointestinal disorder, and wound healing6. The most investigated species for its potential as medicinal plants from this family is Dillenia indica. At the same time, there are many other species that also potential. One of it is Dillenia suffruticosa4,7. Dillenia suffruticosa has few local names such as sempur, simpor, simpoh, simpur air, and simpur bini4,8-11. The name sempur is derived from the hissing sound when the trunk tree is cut4. However, most residents in Indonesia call it sempur.

Dillenia suffruticosa is a native Asian plant that grows in tropical forests from Malaysia, Indonesia, the Philippines, and Brunei Darussalam. Dillenia suffruticosa in Indonesia can be found in Sumatra and Kalimantan (Borneo) Islands. Local societies in Brunei and Malaysia are used D. suffruticosa leaves to promote wound healing, treat fever, and relieve rheumatism8,9. The people in Bangka-Belitung, Sumatra, usually used the boiled water of D. suffruticosa leaves to treat diabetes mellitus10. Besides that, the local community also used boiled water of D. suffruticosa leaves as an anti-diarrhea.

However, the study of this potential plant against pathogenic microorganisms is still underreported, and none of the studies reported using ethanol solvents. Research on the activity of D. suffruticosa leaves against pathogenic microorganisms was reported by Wiart et al12. They reported that methanol extract of D. suffruticosa leaves was inhibited the growth of Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, and Candida albicans. However, it did not affect the growth of Escherichia coli and Staphylococcus aureus. Otherwise, Yakop et al11. reported that the methanol extract of D. suffruticosa leaves could inhibit S. aureus but did not affect B. subtilis, E. coli, and P. aeruginosa.

Another research showed an antifungal activity from D. suffruticosa leaves extract with methanol, acetone, and chloroform against Colletotrichum gloeosporioides13. According to Goh et al.9, the cytotoxic activities of this plant could be attributed to the presence of phytochemicals such as saponins, triterpenes, sterols, and polyphenols compounds. However, more studies should be performed to validate their traditional

uses for such diseases fully. This research was conducted to explore the antimicrobial activity of D. suffruticosa leaves extract against several pathogenic microorganisms.

This research used 70% ethanol as a solvent since its lower toxicity than methanol14. The tested microorganisms were S. aureus (Gram-positive bacteria), E. coli (Gram-negative bacteria), and C. albicans (Fungi). MATERIALS AND METHODS Materials The materials used include D. suffruticosa leaves, Nutrient agar (NA), Sabouraud Dextrose agar (SDA), distilled water (Brataco), 70% ethanol (Brataco), FeCl3 (Merck), Wagner's reagent, Mayer's reagent, Dragendorff's reagent, ammonia (Merck), acetic acid anhydride (Merck), NaNO2 (Merck), AlCl3 (Merck), HCl (Merck), chloroform (Merck), H2SO4 (Merck), DMSO, immersion oil, crystal violet (Merck), safranin, Lugol's iodine, 0.9% NaCl, blank antimicrobial susceptibility discs (Oxoid), and the antibiotic susceptibility discs of nystatin (Oxoid) and amoxicillin (Oxoid).

The main instruments used in this study were analytical balance (Excellent), oven (Memmert), blender (Phillips), autoclave, incubator, vacuum rotary evaporator, hot plate, and laminar airflow. Methods Preparation and extraction of D. suffruticosa leaves Dillenia suffruticosa leaves were obtained from Pedindang Village, Pangkalan Baru, Central Bangka District, Bangka-Belitung Island. The sample was identified in the Research Center for Plant Conservation and Botanic Gardens, Indonesian Institute of Sciences, Bogor, with report number B-848/IPH.3/KS/VII/2020. The fresh leaves of the D.

suffruticosa plant were weighed as much as 6 kg were cleaned with clean water from the tap. The leaves were dried for several hours under the sun to avoid moisture after shipping, so it was not easily contaminated by the fungus. After that, the leaves were sorted and chopped (about 2-3 cm) to speed up the drying process. The leaves were dried with a wind-dried method for 15 days10,15. The dried leaves then being crushed using a blender and followed by sieving using mesh 60. The sieving produces simplicia of D. suffruticosa leaves powder. This procedure was to gain a homogeneous size of simplicia, so the interaction between the D. suffruticosa leaves powders and the solvent would be optimal.

Besides, homogeneous size particle could optimize the extraction process16. The D. suffruticosa leaves powder was weighed as much as 100 g then extracted with the maceration method using 70% ethanol as a solvent with a ratio of 1 : 10. The maceration was done for 24 hours and re-macerated twice with the same procedure. The maceration results were filtered with filter paper. The filtrate was evaporated using the vacuum rotary evaporator until it produces a thick extract17. Organoleptic observation The organoleptic observation of ethanolic extract of D. suffruticosa leaves aimed to determine the physical form of color, smell, shape, and taste using the senses.

This observation includes color checking by looking at the evaporated extract visually, checking the odor by smelling the evaporating extract on filter paper, and checking the taste by dropping extract on the tip of the tongue and then discarding it18. Phytochemical screening and antimicrobial activity tests The extract was tested for phytochemical screening in Lux Chemicals Laboratory (Chemicals Product and Chemical Analysis Service), Depok. The screening test included alkaloids (with Mayer's, Wagner's, and Dragendorff's reagents), flavonoids, saponins, tannins, steroids, and triterpenoids19.

The extract also tested for antimicrobial activity using the Kirby-Bauer Disk Diffusion Susceptibility Test method in Testing Laboratory of Biotechnology Center, Agency for the Assessment and Application of Technology, Serpong20. The microorganisms tested were S. aureus ATCC 25923 (representative of Gram-positive bacteria), E. coli ATCC 25922 (representative of Gram-negative bacteria), and C. albicans ATCC 10231 (representative of fungi). The S. aureus and E. coli were incubated for 24 hours, while C. albicans for 48 hours. The differences in incubation time were based on the optimum growth of the microorganisms. Our previous study also used incubation time of 18-24 hours for S. aureus and E.

coli as well as 48 hours for C. albicans21-23. RESULTS AND DISCUSSION Preparation and yield extract of D. suffruticosa leaves Dillenia suffruticosa leaves were categorized as broad leaves (15-35 cm) in a plant4. Due to its enormous size, the leaves were chopped into smaller pieces and consumed about 15 days to gain dried leaves (Figure 1). The leaves were dried without direct contact with the sun to avoid damaging compounds, such as thermosensitive polyphenols24. According to some references, D. suffruticosa leaves contain polyphenols9,10,15,25,26. This dried method was suitable with Priamsari et al.27, which stated that the total flavonoid content was higher in wind-dried leaves than the oven method.

It also corresponded with Rivai et al.28, which proved that the wind-dried method was the optimum method to gain phenolics. The wind-dried method also had another advantage: retaining chlorophyll, so the sample still looks greenish, not brown29. The D. suffruticosa leaves powder in this research was showed a greenish color (Figure 2). However, this method had limitations, such as time-consuming27-29. This could be seen from the drying time, which took more than two weeks. The wind-dried method could take time about 3-7 days to months and up to a year, depending on the types of samples dried24. The extraction method in this research was done by maceration with 70% ethanol as a solvent.

Solvents with high polarities, such as ethanol, were pretty efficient to attract active

compounds from plants30. Maceration was chosen because it was a straightforward method and could be used to extract thermolabile compounds31,32. Hasnaeni et al.33 also reported that the maceration method produced a higher yield than reflux and soxhlet extraction. The yield of ethanol extract of D. suffruticosa leaves was about 65.5% (Table I). Yield extract showed some active compounds that are trapped during the extraction process27,33. The high percentage yield indicates the high content of the active compounds in a sample. The ethanol extract of D.

suffruticosa leaves showed a high yield (more than 50%). This was probably due to the influence of the solvent used. The higher the solvent polarity, the yield obtained will also increase34. / a / b Figure 1. Wide fresh leaves (a) and chopped dry leaves (b) of D. suffruticosa / Figure 2. Dillenia suffruticosa leaves powder showed greenish color with a wind-dried method Table I. Yield of ethanol extract of D. suffruticosa leaves Dillenia suffruticosa leaves powder (g) _Thick extract (g) _Yield (%) _ _100 _65.5 _65.5 _ _ Organoleptic observation The organoleptic observation involved eight respondents.

Each respondent was asked to observe the shape and color of the extract. Other than that, respondents were also asked to smell and taste the extract (Figure 3). The respondents agree that the extract was in thick, blackish-green color, had a distinctive smell of D. suffruticosa leaves, and had an astringent taste (Table II). The findings of organoleptic observations have never been published, so this article was a preliminary report for future studies as a guide. / Figure 3. Thick extract of D. suffruticosa leaves Table II. Organoleptic observation of D.

suffruticosa leaves extract Organoleptic indicator _Observation _ _Shape Color Odor Taste _Thick Blackish-green Distinctive odor Astringent _ _ Phytochemical screening Phytochemical screening was an essential step in uncovering the potential of medicinal plant resources as antibiotics, antioxidants, and anticancer. The compounds contained in the extract were analyzed qualitatively based on the color change reaction with several reagents35. The screening results from Table III showed that the ethanol extract of D. suffruticosa leaves contained alkaloids, flavonoids, tannins, and saponins. Meanwhile, the test for steroids and triterpenoids showed a negative result.

The positive tests of flavonoids, tannins, and saponins were similar to those obtained by Yuningtyas et al10. The presence of flavonoids and tannins indicates that the ethanol extract of D. suffruticosa leaves contains polyphenols36. Ethanol was known as a solvent that was best for extracting polyphenols from plants37. Besides the flavonoids and tannins, the extract also contains saponins. Saponins were triterpene glycosides that had polar tendencies in their glycosidic bonds38. Based on the law of similarity and intermiscibility (like dissolves like), a solvent with a polarity near the polarity of the

solute was likely to perform and vice versa32. This explains why ethanol as a polar solvent could attract saponin from D.

suffruticosa leaves. Another compound found in D. suffruticosa leaves extract was alkaloids. This research using three different reagents to test the alkaloid compounds. Two of the tests were showed positive results (Wagner's and Dragendorff's), while Mayer's showed a negative result. Based on Surbakti et al.39, a sample could contain alkaloids if there were at least two positive qualitative test results. Meanwhile, Yuningtyas et al.10 reported the opposite; their extract showed negative at alkaloid test. This difference in result probably due to regional differences in sample acquisition. The D.

suffruticosa leaves were obtained from Pedindang Village, Pangkalan Baru, Central Bangka District, while Yuningtyas et al.10 obtained their sample from Jebus Village, West Bangka District. According to Verma et al.40, plants from the same species might have differences in the concentration of a particular secondary metabolite. The main factor affecting this phenomenon was the abiotic stress in the plant environment. Different season or different environmental condition could encourage plants to produce specific compounds to survive in the unfavorable condition and to protect against extinction. Table III. Phytochemical screening of D.

suffruticosa leaves extract Phytochemical _Results _Conclusion _ _Alkaloids _Wagner’s _A brown precipitate was formed _Positive _ _ _Mayer’s _No sediment formed _Negative _ _ _Dragendorff’s _A thick red precipitate was formed _Positive _ _Flavonoids _A red solution formed _Positive _ _Tannins _A greenish black solution formed _Positive _ _Saponins _Formed a stable foam after shaking _Positive _ _Steroids _No blue or green color formed _Negative _ _Triterpenoids _No red color formed _Negative _ _ Antimicrobial activity The antimicrobial activity was done using a Kirby-Bauer disk diffusion method. This method was used to determine the sensitivity or resistance of pathogenic microorganisms to various antimicrobial compounds.

The clear zone that appears around the disk was measured as the inhibition zone20,41. The results of the antimicrobial activity showed in Table IV. The results in Table IV showed that the D. suffruticosa leaves extract did not affect the growth of E. coli and C. albicans. It only affected S. aureus growth at concentrations 10%, 20%, and 40%. The result of this study against S. aureus was the same as Yakop et al11. Thus, the result against E. coli was the same with Yakop et al11. and Wiart et al12. However, the result against C. albicans was inconsistent with Wiart et al12., in which their research showed a growth inhibition zone, while this study did not. The antibacterial activity against S.

aureus showed a higher inhibition zone along with higher concentrations. The higher the extract concentration, the active substance in the extract increases so that the antibacterial activity would be greater42. The antibacterial activity against S. aureus was presumed due to the synergistic mechanisms among chemicals compounds found in extract ethanol of D. suffruticosa leaves. Based on the literature, alkaloids were known could intercalate with DNA. In general, alkaloids work with interfering the DNA synthesis43. Flavonoids and saponins were work by disrupting the bacterial cell membrane of microorganisms44.

Meanwhile, tannins act by disturbing the cell protein, either bind and precipitate or shrink proteins45. These conjectures were in line with the literature, which stated that the antibacterial activity could be grouped into four main mechanisms: disturbing bacterial cell wall, disrupting cell membrane, interfering protein biosynthesis, and inhibiting nucleic acid biosynthesis43,45. Bacteria based on their cell wall structure were differentiated into Gram-positive and Gram-negative bacteria.

Gram-positive bacteria have a simple cell wall structure composed of peptidoglycan, while in Gram-negative bacteria, they have an additional structure called an outer membrane. The outer membrane contains lipopolysaccharide and could secrete endotoxin. The outer membrane acts as a protection, including keeping the bacterial cells from penetrating antibiotics or other unwanted compounds. This layer causes Gram-negative bacteria to generally more resistant than Gram-positive bacteria46,47. The description could explain why in this study, the extract was only affecting S. aureus, which was Gram-positive bacteria, while it did not affect E. coli.

Escherichia coli was classified as Gram-negative bacteria and known to have developed multi-drug resistance47,48. This study showed that E. coli were resistant to D. suffruticosa leaves extract. Another microorganism tested against the D. suffruticosa leaves extract in this study was C. albicans. The result showed that the ethanol extract of D. suffruticosa leaves neither could inhibit the C. albicans growth. As a fungus, the cell wall of C. albicans was composed of chitin, glucan, and mannoprotein. The cell wall forms a two-layer structure with mannoproteins in the outer, while chitin in the inner layer. Glucans lie in the inner layer and connecting the inner and outer layers.

The mannoproteins in the outer layer have low permeability and porosity, so they could not easily pass by some compounds, including antifungal agents. This structure made the C. albicans resistant to antifungal drugs or host defense mechanisms49,50. This finding was corresponding with Lima et al.51, which report that a more mannan structure in fungi could develop the resistance in Candida against antimicrobial agents. This was probably could explain why the D. suffruticosa leaves extract could not inhibit

the C. albicans growth. Table IV. Antimicrobial activity of D. suffruticosa leaves extract Extract concentration _Inhibition zone (mm) _ _ _S. aureus _E. coli _C. albicans _ _5% _- _- _- _ _10% _8.35±0.05 _- _- _ _20% _9.34±0.32 _- _- _ _40% _10.52±0.22 _- _- _ _Positive control _42.72±0.14 _28.04±0.82 _9.44±0.11 _ _Negative control _- _- _- _ _(-): no activity; positive control: amoxicillin (S. aureus and E. coli), nystatin (C.

albicans); negative control: 10% DMSO CONCLUSION The ethanolic extract of D. suffruticosa leaves could inhibit the growth of S. aureus, while E. coli and C. albicans showed no activity. Further research about the ethanolic extract of D. suffruticosa leaves against other pathogens still being suggested, especially the gastrointestinal pathogen.

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