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Antibacterial activity of leaves methanolic extract of Tanikkara (D*illenia excelsa* (Jack) Martelli ex Gilg) against *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*

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Abstract

Traditionally, tanikkara- *Dillenia excelsa* (Family: Dilleniaceae) is used as a cooking spice and as a medicine for diarrhea, fever and heartburn. However, the antibacterial activity of methanolic extract of the *D. excelsa* leaves has not been carried out. The purpose of this study was to determine the antibacterial activity of the methanol extract of *D. excelsa* leaves against several bacteria: *Salmonella typhi* and *Escherichia coli* as *Gram-negative*, and *Staphylococcus aureus*, *Bacillus cereus* as Gram-positive bacteria. Extraction was carried out by maceration method with 96% methanol and then screened for phytochemical constituents. The extract was dissolved using sterile distilled water to a concentration of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100%. The antimicrobial test was carried out by the well diffusion method using Nutrient Agar (NA). The diameter of the inhibition zone was measured to determine the antibacterial activity. Gentamycin Sulfate 0.1% was used as a positive control, and distilled water as a negative control. The results of phytochemical screening showed that the ethanol extract of *D. exelsa* leaves contained alkaloids, flavonoids, phenolics, tannins and triterpenoids. The antibacterial test showed that leaves extract of *D. excelsa* could inhibit the growth of *S. typhi*, *E. coli*, *S. aureus*, *B. cereus* with the inhibition zone diameters of 0.04-4.89, 0.60-7.68, 0.26-8.05, 0.04-4.89mm successively. The higher the concentration of the extract, the wider the diameter of the inhibition zone for all types of test bacteria. *Staphylococcus aureus* bacteria were more resistant to tanikkara leaf extract at the highest extract concentration.

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Introduction

Plants are used as medicine by Indonesian people, especially in East Kalimantan. One of these medicinal plants that found in East Kaliantan is tanikkara (*Dillenia excelsa* (Jack) Martelli ex Gilg). This plant has several different regional names, namely tanikkara (Kutai), ki seal (West Java), water simpor (Pontianak), and male-simpor (Belitung). Apart from having regional names, this plant also has different names in several countries; simpor (Brunei, Sabah), zinbyun or maisaman (Burma), san or masan (Thailand), san (Cambodia), katmon (Philippines), and others. So on (Mustari, 2019). The leaves, especially the tops of the leaves, are generally used by the local community as a medicine for diarrhea. Leaves soaking in hot water and then drinking the water (Mukhlisi *et al*., 2018). This plant can also use as a spice in the kitchen. Used in fresh or dry form and mixed into dishes as a flavoring agent (Setyowati *et al*., 2005).

Communities in the village of Sungai Mawang (West Kalimantan, Indonesia) use tanikkara as a food plant that is consumed as a vegetable. Tanikkara leaves used to treat fever and heartburn. The woods are used as household items to replace dishes and make poles for houses (Pradityo *et al*., 2016). In local communities, the young leaves of this plant are used as food for probosci monkeys (Mukhlisi *et al*., 2018). Agus *et al*. (2010) reported that tanikkara is a rare plant. Based on the abundance of tanikkara in PT. Sari Bumi Kusuma Camp Tontang, Sintang District, there are young plants (height > 1.5 m) to young trees (diameter <10 cm) with the lowest species diversity index, which is only around 0.9062. Hidayat and Hardiansyah (2012) reported that tanikkara are generally sub-canopy to tree canopy plants in the forest and can grow along the sides of rivers and on slightly dry soil.

Tanikkara plant (*Dillenia excelsa*) is used as a traditional medicinal plant by the people of Melintang Village (East Kalimantan). Even though it is known as a medicinal plant until now, there is no scientific information available about its bioactivity and phytochemical tests. Previous research (Prananda *et al*., 2019) used *Dillenia indica* samples with positive phytochemical test results containing alkaloids, phenols, tannins, flavonoids, steroids and triterpenoids, and saponins. Putra (2018) reported that the ethyl acetate extract of *Dillenia suffruticosa* contained secondary metabolites of the tannins, polyphenols, and triterpenoid groups. In addition Lisdiani *et al*. (2022) revealed that the methanol extract of tanikkara leaves contained secondary metabolites such as; alkaloids, phenolics, flavonoids, triterpenoids and tannins. The secondary metabolites that contained in the extract have the potential to be used as a natural antibacterials. The use of biological agents from local plants as antibacterials is increasingly attracting the attention of researchers. The presence of secondary metabolites in the tanikkara plant indicates that the plant has the potential to be used as a natural antibacterial. Therefore, testing the potential of tanikkara leaf extract as an antibacterial is still very necessary, especially for bacteria that can cause diseases/pathogens. So the study was designed to test antibacterial activity against gram-positive (*Staphylococcus aureus*, *Bacillus cereus*) and gram-negative (*Salmonella typhi*, *Escherichia coli*) pathogenic bacteria.

Material and methods

Plant Material and Extraction

The tanikkara leaves (Dillenia excelsa) were obtained from Melintang Village, Muara Wis District, Kutai Kartanegara Regency, East Kalimantan-Indonesia. It's collected into plastic, then cleaned and dried indoors for ±7 days at room temperature (±20°C), mashed using a blender, macerated with 98% methanol, shaken for three days, and stirred once a day. Maceration results were filtered using the Whatman filter paper. Filtrate evaporated using a rotary evaporator at 40°C and placed in a vacuum oven to detect the dryness of the extract. The extract stored in the refrigerator for further use for the phytochemical screening antibacterial activity test.

Phytochemial Screening.

The crude leaves methanolic extract of *Dillenia exelsa* was subjected to a qualitative phytochemical

screening using the methods describe by Harborne (1998) and Manurung *et al.* (2019). The screening test included alkaloids, phenolic, flavonoid, tannin, saponin, steroid-triterpenoid, coumarin, carotenoid, and glycoside. The results are given as the relative abundance of the respective compound.

Alkaloids

A-2mL of leaf extract was inserted into a test tube, then add 5mL of 0.005 M chloroform-ammonia, homogenized, and filtered using filter paper. The filtrate was added with a few drops of 2M sulfuric acid and then shaken to form 2 layers of acid (top) and base (bottom). The top layer is taken and added with a few drops of Dragendorff reagent. The formation of a brownish-red precipitate indicates that the positive extract contains alkaloids.

Flavonoids

TwomL of leaf extract was added into a test tube and then added with 5mL of water, boiled for 5 minutes, and then filtered using filter paper. 2mL of filtrate was added with 0.05mg of mg powder, and 1mL of concentrated Hcl, stirred until homogeneous. The formation of red, yellow, or orange colour indicates that the extract is positive for flavonoids.

Phenolic

Two mL of methanolic leaves extract of *D. exelsa* added with 3-4 drops of ferric chloride solution. The formation of a bluish-black color solution indicates the presence of phenols.

Saponin

A-2mL methanolic extract of *D. exelsa* was dissolved with 5mL of distilled hot water and shaken for 10 minutes. The formation of layer foam indicates a positive for saponins.

Steroid-Triterpenoid

A-2mL extract of *D. exelsa* was added with a few drops of acetic anhydride, boiled, cooled, and then concentrated H2SO4 added slowly through the sides of the test tube to form a layer. The formation of a red color indicates a positive extract containing steroids, a blue color indicates positive terpenoids.

Tannin

A-0.5 g of crude extract was added to 10mL of distilled water, boiled, and filtered. Then the filtrate was added with 2-3 drops of 0.1% ferric chloride. If the color of the filtrate changes to blue, it indicates positive tannins.

Glycoside

Two (2) mL of *D. excelsa* extract was put into a test tube, then added with 0.5mL of DMSO, shaken, then added with 1mL of anhydrous acetate and six drops of sulfuric acid. Positive for glycosides if a purple ring is forming.

Coumarin

One mL of methanolic leaf extract of *D. exelsa* was added with 3-4 drops of naoh dissolved with 2mL alcohol. The changes in the extract solution to yellow color indicate that the extract contains coumarins.

Carotenoid

Two (2) mL of the stock solution of *D. excelsa* leaf methanol extract put into the test tube, added 2mL of chloroform, and 3-4 drops of 85% sulfuric acid. Positive for carotenoids will form a blue color on the surface solution.

Antibacterial activity

Bacterial and Culture Condition

The leaf methanol extract of *D. exelsa* were tested for its ability to inhibit the growth of the pathogenic bacteria (procured from the Microbiology Laboratory, Deparment of Biology, Faculty of Science, Brawijaya University, Indonesia) were common gram negative *Salmonella typhi* and *Escherichia coli*, and Gram-positive *Staphylococcus aureus*, *Bacillus cereus* as bacteria. All bacteria were subcultured on nutrient agar and stored 4°C until use.

Sterilization of tools and materials

Petri dishes, test tubes, Erlenmeyer, Mueller Hinton Agar (MHA) media, and all tools and materials to be used were sterilized in an autoclave for 15 minutes at a pressure of 15 dynes per cm3 (1 atm) and a temperature of 121°C after previously washed, dried and wrapped in paper.

Preparation of Mueller Hinton Agar (MHA) Media

A-22.8 g of MHA powder was taken then dissolved with 600mL of distilled water in an Erlenmeyer, stirred using a magnetic stirrer until homogeneous, then heated to boiling. Then the solution was sterilized using an autoclave for 15 minutes at 121°C. Then the steriled solution is poured into a sterile petri dish, closed, then left to solidify.

Agar Well Diffusion Method

A sterile cotton swab is dipped in each bacterial suspension, then squeezed by rotating the cotton part to the side of the tube so that the liquid does not drip from the cotton part. Then the cotton was squashed on the surface of the MHA (Mueller Hinton Agar) media until evenly distributed. After the bacterial suspension seeped into the media, wells were made using a well-bore iron with a diameter of 6mm. Then the extract was pipetted and put into the wells according to the treatment concentration of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, positive control, and negative control. Triplicate plates for each treatment were prepared, then the Petri dishes were incubated at 37°C for 24-48 hours. The formation of an inhibition zone was observed after the incubation period was over.

Data Analysis

The data of the phytochemical content and the concentration of flavonoid and phenolic were analyzed descriptively. The inhibition zone data were expressed as means ± standard error. The data were subjected to ANOVA, followed by Duncan's post hoc test to evaluate significant differences among the groups of treatments. All significant tests were at $P<0.05$ levels and all analysis were done using SPSS 22 (SPSS, Inc., USA).

Result and discussion

Phytochemicals of Leaves Extract

A-500 grams of *D. exelsa* leaf powder was macerated using methanol and evaporated using a rotary evaporator to obtain 65.9406 grams of methanol extract with a yield of 13.2%. The results of the phytochemical test of the methanol extract of the tanikkara leaves are presented in Table 1.

Table 1. Phytochemical screening of leaves methanolic extract of tanikkara (*Dillenia excelsa*).

Compound	Results
Alkaloid	+
Flavonoid	+
Phenolic	+
Saponin	-
Triterpenoid	+
Steroid	-
Tanin	+
Glycoside	-
Coumarin	-
Carotenoid	-

Remarks: (+) = Presence (-) = Absence

Recent research showed that tanikkara leaves contain alkaloids, flavonoids, phenolics, steroids, and tannins. Several previous studies also reported that plants belonging to the same genus as tanikkara contain phytochemical compounds. Sabandar *et al.* (2020) revealed that songi stem bark extract (*Dillenia serrate* Thunb.) contains phytochemical compounds, namely flavonoids, tannins, triterpenoids, and steroids. Utami and Anjani (2020) reported that the ethanol extract of the leaves and bark of the simpur plant (*Dillenia indica* L.) contains alkaloids, flavonoids, tannins, saponins, triterpenoids, and steroids, while the ethanol extract of the roots contains alkaloids, flavonoids, and tannins. Putra *et al.* (2018) reported that the ethyl acetate extract of simpor leaves (*Dillenia suffruticosa*) positively contained tannins, polyphenols, and triterpenoids. Illing *et al.* (2017) stated that dengen fruit extract (*Dillenia serrata*) has alkaloids, flavonoids, saponins, polyphenols and triterpenoids. Prananda *et al.* (2019) reported that the ethanol extract of simpur leaves (*Dillenia indica* L.) contains alkaloids, phenolics, tannins, flavonoids, triterpenoids, steroids, and saponins.

The phytochemical compound contained in *D. exelsa* leaf extract is a source of natural ingredients for various bioactivities.

Flavonoids are polyphenolic compounds that have antibacterial activity with various actions. Flavonoids can inhibit the formation of nucleic acids; disrupt the function of cell cytoplasmic membranes and energy metabolism processes.

Flavonoid-protein interactions such as enzymes, receptors, transporters, and transcription factors can disrupt bacterial metabolism and inhibit bacterial growth (Shamsudin *et al.*, 2022; Chusnie and Lamb, 2011).

Alkaloids are one of the secondary metabolites containing nitrogen that can be isolated from various plants. This compound has been reported to have antibacterial activity. Yan *et al.* (2021); Zhou *et al.* (2017) revealed that alkaloid compounds were able to inhibit gram-positive bacteria, gram-negative bacteria, and fungi (fungi). Their antibacterial effect is similar to that of commonly used antimicrobials on the market. Alkaloids can inhibit the growth, division, and respiration of bacterial cells by attaching to various cell membrane proteins so that the metabolism of bacterial cells becomes disturbed/inhibited.

Tannins are the most effective antibacterial because of their ability to pass through and penetrate the bacterial cell wall to the internal membrane, disrupt metabolic processes, and even destroy bacterial cells (Kaczmarek, 2020). Tannins are also reported to have various unique activities such as antitumor, antimutagenic, antiviral, antioxidant, homeostatic agents and as natural antibiotics (Kim *et al.*, 2010; Gülçin *et al.*, 2010; Bouki *et al.*, 2013; Farha *et al.*, 2020).

Triterpenoids are produced by various plants as part of a self-defense mechanism, possessing chemical and pharmacological properties. Choi *et al.* (2012) reported that the terpenoid compounds found in *Clerodendron trichotomum* extract could inhibit the growth of *Staphylococcus aureus, Escherichia coli,* and *Helicobacter pylori* bacteria. Triterpenoid isolated from the root of *Gardenia ternifolia* Schumach & Thonn (Rubiaceae) was reported to have antibacterial activity against *Escherichia coli, Salmonella typhi* (Enterobacteriaceae), *Staphylococcus aureus, Pseudomonas aerogenosae* and *Vibrion cholorae* (Bernard *et al.*, 2022).

Antibacterial activity

Testing for the antibacterial activity of methanolic leaves extract of *D. exelsa* was carried out against gram-positive and gram-negative pathogenic bacteria. The results of the antibacterial activity test of the leaf methanolic extract of *D. exelsa* revealed that the extract inhibited the growth of all the tested bacteria (Tabel 2).

The inhibition zone diameters of methanolic extracts of *D. exelsa* ranged from; 0.60 ± 0.02 to 7.68 ± 0.42mm for *E. coli*, 0.00 to 7.17 ± 0.03mm for *S. typhi*; 0.04 ± 0.02 to 5.78 ± 0.01mm for *S. aureus*; and 0.26 ± 0.01 to 8.17 ± 0.03mm for *B. cereus*. The higher the concentration of extract applied to the bacterial growth medium, the higher the inhibition zone formed. The highest inhibition zone was found in *B. cereus* bacteria at 100% extract concentration and not significantly different from the positive control-gentamycin sulfate 0.1%. These results indicate that the methanol extract of tanikkara leaves has the potential to be used as a natural antibacterial that can inhibit the growth of pathogenic *B. cereus* bacteria. *B. cereus* bacteria are a pathogenic bacterium that can cause several stomach ailments, diarrhea, stomach cramps, and vomiting (Rodrigo *et al.*, 2021).

Table 2. Effect of antibacterial activity of methanol extract of *D. exelsa* on the formation of inhibition zones (mm) of pathogenic bacteria.

Extract concentration	Gram-negative bacteria		Gram-positive bacteria	
	E. coli	*S. typhi*	*S. aureus*	*B. cereus*
Negative control	0.00±0.00^{h}	0.00±0.00^{j}	0.00±0.00^{l}	0.00±0.00^{j}
10%	0.60±0.02^{g}	0.00±0.00^{j}	0.04±0.02^{k}	0.26±0.01^{i}
20%	0.75±0.01^{g}	0.68±0.10^{i}	0.67±0.04^{j}	0.83±0.01^{h}
30%	0.88±0.02^{g}	1.14±0.02^{h}	0.95±0.03^{i}	1.29±0.01^{g}
40%	1.39±0.04^{f}	1.49±0.09^{g}	1.10±0.02^{h}	1.65±0.03^{f}
50%	1.77±0.01^{e}	1.77±0.01^{f}	1.20±0.02^{g}	1.71±0.01^{f}
60%	2.11±0.11^{de}	1.97±0.04^{f}	1.61±0.02^{f}	2.08±0.13^{e}
70%	2.23±0.01^{d}	2.24±0.05^{e}	2.35±0.03^{e}	3.56±0.11^{d}
80%	2.36±0.06^{d}	2.57±0.02^{d}	2.49±0.08^{d}	4.37±0.07^{c}
90%	3.96±0.08^{c}	3.03±0.02^{c}	3.65±0.01^{c}	5.23±0.15^{b}
100%	7.68±0.42^{b}	3.92±0.032^{b}	4.90±0.09^{b}	8.05±0.20^{a}
Positive control (Gentamycin sulfate 0.1%)	8.39±0.53^{a}	7.17±0.03^{a}	5.78±0.01^{a}	8.17±0.03^{a}

Notes: Different superscript letters in the same row indicate significantly different mean values for different treatments at P<0.05. Positive control, 0.1% gentamycin sulfate.

Not only inhibit the growth of *B. cereus*, the extract also able to inhibit the growth of *E. coli*, *S. typhi*, and *S. aureus* bacteria. The recent studies are in line with Abdullah *et al.* (2017) who reported that the ethanol extract of *D. exelsa* leaves was able to inhibit the growth of *E. coli* and *Bacillus subtilis* bacteria. Besides that, Syafriana *et al.* (2021) reported that the sempur plant (*Dillenia suffruticosa* (Griff.) Martelli), which is a plant that is in the same genus as *D. exelsa*, was able to inhibit the growth of pathogenic bacteria *S. aureus* but was unable to inhibit the growth of *E. coli* bacteria and the fungus *Candida albicans*. *B. cereus* and *E. coli* bacteria were more sensitive to *D. exelsa* methanol extract at a concentration of 100% compared to *S. typhi* and *S. aureus* bacteria.

This antibacterial activity is related to the phytochemical compounds contained in the extract. Which can kill bacteria and or slow down their growth rate. The phytochemical content of *D. exelsa* leaf extract was phenolics, flavonoids, alkaloids, triterpenoids, and tannins. According to Xie *et al.* (2015), flavonoids are known antibacterial agents against a wide range of pathogenic bacteria. Fu *et al.* (2016) also revealed that phenolic extracts from some plants also have antibacterial effects against many kinds of bacteria. In addition Kaczmarek (2020) reported that tannins are the most effective antibacterials because of their ability to pass through and penetrate the bacterial cell wall to the internal membrane, disrupt metabolic processes, and even destroy bacterial cells. The results of this study indicated that the methanol extract of *D. exelsa* leaves had antibacterial activity, both against gram-negative and gram-positive bacteria.

Conclusion

The methanol extract of tanikkara leaves (*Dillenia excelsa* (Jack) Martelli ex Gilg) can inhibit the growth of gram-negative (*Salmonella typhi*, *Escherichia coli*) and gram-positive (*Staphylococcus aureus*, *Bacillus cereus*) pathogenic bacteria at extract concentrations of 10% to 100%. The higher concentration of the extract, the larger the diameter of the bacterial inhibition zone. In a recent study, leaf methanolic extract of *D. exelsa* appeared as a good source of health promotion and had beneficial effects like antibacterial activities against Gram-positive and Gram-negative.

Conflict of interest

The Authors have not declared any conflict of interest.

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