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Antibacterial activity of faloak (*Sterculia quadrifida*) leaves extract

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Abstract. *Purwantiningsih TI, Widyobroto BP, Suranindyah YY, Artama WT. 2023. Antibacterial activity of faloak (*Sterculia quadrifida*) leaves extract. Biodiversitas 24: 6613-6620.* Restrictions on the use of antibiotics have made people start looking for alternatives to antibiotics, one of which is ingredients that come from nature. Faloak (*Sterculia quadrifida* R. Br) leaves are a source of secondary metabolites that have not been widely utilized. The aim of this study was to assess the antibacterial activity of *S. quadrifida* leaves extract in inhibiting *Staphylococcus aureus* (gram-positive bacteria) and *Escherichia coli* (gram-negative bacteria). Powdered *S. quadrifida* leaves were macerated with 96% ethanol solvent. After obtaining the crude extract, it was fractionated using the liquid-liquid method using methanol, hexane, distilled water and ethyl acetate as solvents. The crude extract of *S. quadrifida* leaves was then dissolved into several concentrations. The crude extract of *S. quadrifida* leaves and the fractions obtained were tested for antibacterial activity in vitro using the Kirby Bauer method against *Staphylococcus aureus* and *Escherichia coli*. The most active fraction in inhibiting the tested bacteria was then screened for bioactive compounds using instrument LC-HRMS. The results showed that *S. quadrifida* leaves extract and its fractions were able to inhibit *Staphylococcus aureus* and *Escherichia coli* bacteria. The ethyl acetate fraction was the most active fraction in inhibiting bacteria and the most occurring bioactive compounds appeared in LC-HRMS testing were luteolin, rutin and corchorifatty acid. The total flavonoid compound in the crude extract was 227.281 QE µg/mL and the highest i.e. 351.456 QE µg/mL total flavonoid compound was in methanol fraction.

Keywords: Antibacterial activity, *Escherichia coli*, faloak leaves, flavonoid, *Staphylococcus aureus*

INTRODUCTION

The increased use of antibiotics increases the number of bacteria that are resistant to antibiotics, reducing the effectiveness of antibiotics for livestock and humans (Poizat et al. 2017). Increasing bacterial resistance to antimicrobial agents is a threat to both humans and animals, therefore the World Organization for Animal Health (OIE) recommends monitoring and checking the resistance of pathogens and bacteria to antimicrobials. The aim of such monitoring is to provide information useful for therapeutic action and to present cases of the development of bacterial resistance as well as considerations in the use of antimicrobial drugs in an individual in practice (Holko et al. 2019). The World Health Organization reports that bacterial resistance to various types of widely used antibacterials has become a significant global health problem and is a challenge that must be addressed immediately (Shamsudin et al. 2022).

Herbal medicine and traditional medicine have great opportunities to be developed as antimicrobial agents and recently there has been very rapid increase in this field (Zhu et al. 2022). Significant improvements have occurred in the use of natural ingredients as traditional medicine, and some natural ingredients have even been produced on a large scale. The use of traditional medicines has fewer side effects compared to drugs derived from chemicals. Using natural ingredients is also more affordable (Noventi and Carolia 2016). Plants produce many secondary metabolites compound that play an important role in microbial infections. Phenolics, phenolic acids, quinones, saponins, flavonoids, tannins, coumarins, terpenoids, and alkaloids are secondary metabolite compounds that are believed to contain antibacterial compounds and play a role in antimicrobial activity (Paul et al. 2019).

The genus *Sterculia* is a member of the Malvaceae family that is widely used in traditional medicine. In Kupang, East Nusa Tenggara, Indonesia, *Sterculia quadrifida* (R. Br) is known by the regional name *faloak*. *S. quadrifida* generally grows at an altitude of 1-1000 m above sea level. *S. quadrifida* grows wild, people have not cultivated *S. quadrifida* intensively, but they can be propagated by cutting and planting seeds. In East Nusa Tenggara Province, *S. quadrifida* trees can be found on the Timor islands, Sumba, Alor, Pantar, Rote and Flores. *S. quadrifida* trees also grow in Australia and India (Saragih and Siswadi 2019). People in Kupang use the bark of this palnt as medicine to treat hepatitis/liver dysfunction, restore stamina and lower back pain (Siswadi and Saragih 2021). *S. quadrifida* bark extract contains flavonoids,

alkaloids, tannins, terpenes, and glycosides. *S. quadrifida* bark extract showed the strongest elastase inhibitory activity with an IC50 value of 73.7 g/mL compared to other parts (Radjah et al. 2021). The highest antioxidant activity was found in newly regrown *S. quadrifida* bark after harvesting. *S. quadrifida* bark can be recommended for further use as medicine because of its high phytochemical content and for cultivation with the aim of sustainable harvesting (Saragih and Siswadi 2019).

Because of its good health benefits, people in Kupang harvest *S. quadrifida* bark which exceeds the tree bark's ability to regenerate, resulting in tree death. The reduction in the number of *S. quadrifida* trees has made it difficult for people to find *S. quadrifida* bark and can eliminate the economic benefits of these trees. Therefore, it is necessary to research other parts of the *S. quadrifida* tree that can be used as antibacterials. The leaves are another alternative because their availability is quite abundant compared to the bark. *S. quadrifida* leaves can be used as medicine to treat breast cancer (Siswadi et al. 2016). Aboriginal tribes in Australia use *S. quadrifida* leaves to treat eye pain and consume the seeds (Siswadi and Saragih 2021). Of the several benefits that *S. quadrifida* leaves have, not much research has been done on *S. quadrifida* leaves. The aim of this research was to assess the ability of antibacterial compounds from *S. quadrifida* leaves extract so that they can be developed as natural antibacterials.

MATERIALS AND METHODS

Research area

Sterculia quadrifida leaves used in this research were collected from Sikumana Village, Maulafa Sub-district, Kupang, East Nusa Tenggara in September 2022 (Figure 1). Leaves were taken from one *S. quadrifida* tree with a diameter of 15 to 30 cm. Leaves taken were healthy and dark green in color.

Procedures

Extraction of Sterculia quadrifida *leaves*

Sterculia quadrifida leaves were dried in the air for a week without direct sunlight. The dried leaves were ground using a grinder (size 60 mesh) to produce *S. quadrifida* leaves powder. The extraction method used was maceration using 96% ethanol solvent. The obtained 1 kg sample powder was soaked in 12 liters of 96% ethanol for 24 hours and stirred 6 times (the duration of one stirring was 5 minutes) a day. The resulting macerate was evaporated using a rotary evaporator (Buchi Labortechnik AG, Switzerland, Model: R300 System Dynamic) and water bath until a thick extract was obtained. The crude extract of *S. quadrifida* leaves was then diluted with distilled water to concentrations of 5, 10, 20, 30, 40, and 50%. These concentrations were tested further for antibacterial activity in vitro.

Liquid-liquid partition extract of Sterculia quadrifida *leaves*

A total of 5 g of thick crude extract of *S. quadrifida* leaves was dissolved in 100 mL of methanol-water (9:1 v/v) then partitioned liquid-liquid using 100 mL of hexane until the hexane fraction was clear in a separating funnel (Pyrex, Japan). The hexane fraction was then collected, and the solvent was removed by evaporation using a rotary evaporator at 36°C. The resulting methanol fraction was also collected, and the solvent was evaporated in a water bath (because only a small amount of solvent remained). A total of 1 g of the methanol fraction was suspended little by little in distilled water until the solution volume was 100 mL. Each 100 mL suspension was partitioned using 100 mL of ethyl acetate until the ethyl acetate fraction was clear. The ethyl acetate fraction was evaporated using a rotary evaporator at 36°C. The water fraction was filtered to separate the insoluble fraction. The insoluble material adhering to the walls of separating funnel was dissolved in 96% ethanol, then the solvent was evaporated using a water bath (Munawaroh 2021). The resulting fractions were then tested for antibacterial activity.

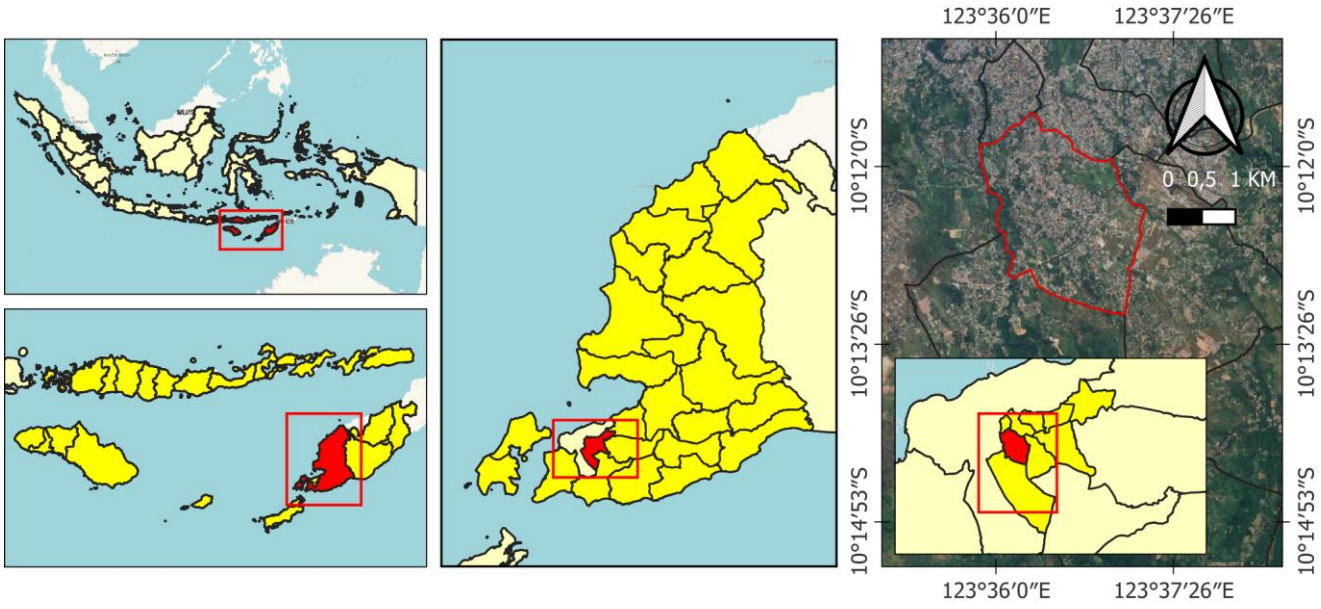

Figure 1. *Sterculia quadrifida* leaves sampling location in Sikumana Village, Maulafa Sub-district, Kupang, East Nusa Tenggara, Indonesia

In vitro antibacterial testing

The bacteria used were *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* FNCC 0091. The sterilized NA media was cooled to 40°C, then the bacterial culture was added as much as 5% of the agar volume. Media was mixed with bacterial culture and poured into a petri dish. The blank disc was placed on the surface of bacterial medium using tweezers than pressed slightly (control). 10 µl leaves extract of various concentration was dropped using a micropipette (Eppendorf, USA, Cat# 4920 0000.024) into a blank disc (diameter 5 mm). Petri dishes were incubated at 37°C for ± 24 hours. Then, the obstacle zone was measured using an automatic colony counter (Interscience, France, Model: Scan 500). The fraction of *S. quadrifida* leaves extract that showed the highest inhibitory diameter was further screened for its bioactive compounds.

Bioactive compound screening

Screening of bioactive compounds was carried out on the fraction of *S. quadrifida* leave extract which had the best antibacterial activity. A total of 0.1 mg of sample was dissolved in 1 mL of ethanol solvent, then vortexed until homogeneous then filtered with 0.2 µM millex, after which 2.5 µL sample was injected into the HRMS instrument (Thermo Scientific™ Dionex™ Ultimate 3000 RSLCnano UHPLC coupled with Thermo Scientific™ Q Exactive™ High Resolution Mass Spectrometer) with mobile phase A= water + 0.1% formic acid and mobile phase B= acetonitrile + 0.1% formic acid.

Total flavonoid compound (TFC)

Total flavonoid compounds was estimated according to the method of Mehmood et al. (2019) with minor modifications and quercetin was used as a standard. *S. quadrifida* leaves extract and its fractions were diluted to a concentration of 1.5625%. 100 µL of distilled water was added to 96 microplates, then 10 µL of NaNO2 (50 g/L) and 25 µL of *S. quadrifida* leaves extract and its fractions were added. The mixture was kept for 5 minutes, after that 15 µL of $AlCl_3$ (100 g/L) was added. After 6 minutes, NaOH (1 M) and distilled water were added in the same amount of 50 µL. Absorbance was measured using a UV-Vis Spectrophotometer (Thermo Scientific, Finland, SKY-S1119700DP) at a wavelength of 510 nm. The absorbance results obtained were calculated using the formula y=0.0019x-0.006.

Data analysis

Data on in vitro antibacterial results and TFC were analyzed using a completely randomized design and if the results were significant, they were tested further using the Duncan Test using the IBM SPSS Statistics 22 application (IBM Corp., NY, USA). Data from the bioactive compound screening was analyzed as descriptive.

RESULTS AND DISCUSSION

In vitro antibacterial test

The results of antibacterial test of *S. quadrifida* leaves extract in vitro are presented Table 1, Figure 2 and Figure 3. *S. quadrifida* leaves extract with a concentration of 5% and negative control were not able to inhibit *Staphylococcus aureus* and *Escherichia coli*. The concentration of the extract used was directly proportional to the diameter of the inhibition zone formed. The higher the concentration level of the *S. quadrifida* leaves extract tested, the larger the diameter of the inhibition zone formed. This is in line with the opinion of Hassan and El Bagoury (2018), who stated the higher the concentration of antibacterial used, the larger the inhibition zone. Statistical analysis results showed that there were significant differences in each of the treatments tested ($P<0.05$). Duncan's test results showed that 50% concentration of *S. quadrifida* leaves extract had the same ability as the positive control in inhibiting *Staphylococcus aureus* bacteria. *S. quadrifida* leaves extracts at 30, 40, and 50% concentrations had the ability to inhibit *Escherichia coli* bacteria, like the positive control.

The hexane fraction was not able to inhibit *Staphylococcus aureus* bacteria, and the water fraction was not able to inhibit the two bacteria tested. Each fraction tested showed a significant difference ($P<0.05$) on the diameter of the bacterial inhibition zone tested. The Duncan test results showed that ethyl acetate fraction was the most active fraction in inhibiting *Staphylococcus aureus* and *Escherichia coli* bacteria, as it has the largest inhibitory diameter (Table 2, Figures 4 and 5).

Table 1. Results of in vitro antibacterial test of *Sterculia quadrifida* leaf extract against bacteria

Bacteria	C+	C-	5%	10%	20%	30%	40%	50%
Staphylococcus aureus	10.9 ± 0.4^{e}	0 ± 0^{a}	0 ± 0^{a}	8.5 ± 0.3^{b}	9.5 ± 0.3^{c}	$9.8\pm0.6^{c,d}$	10.2 ± 0.6^{d}	11.0 ± 0.8^{e}
Escherichia coli	10.2 ± 0.5^{d}	0 ± 0^{a}	0 ± 0^{a}	8.4 ± 0.3^{b}	9.7 ± 0.6^{c}	10.3 ± 0.4^{d}	10.4 ± 0.6^{d}	10.5 ± 0.2^{d}

Note: C+ = positive control (antibacterial commercial) and C- = negative control (aquadest). Different superscripts in same line indicate significant differences ($p<0.05$)

Table 2. Results of in vitro antibacterial test of *Sterculia quadrifida* leaf extract fractions against bacteria

Bacteria	Methanol fraction	Hexane fraction	Water fraction	Ethyl acetate fraction	Non-dissolved fraction
Staphylococcus aureus	9.8 ± 0.5^{b}	0 ± 0^{a}	0 ± 0^{a}	13 ± 0.8^{c}	9.7 ± 0.8^{b}
Escherichia coli	10.7 ± 0.9^{c}	8.8 ± 0.9^{b}	0 ± 0^{a}	14.6 ± 0.8^{d}	9.5 ± 0.8^{b}

Note: Different superscripts in same line indicate significant differences ($p<0.05$)

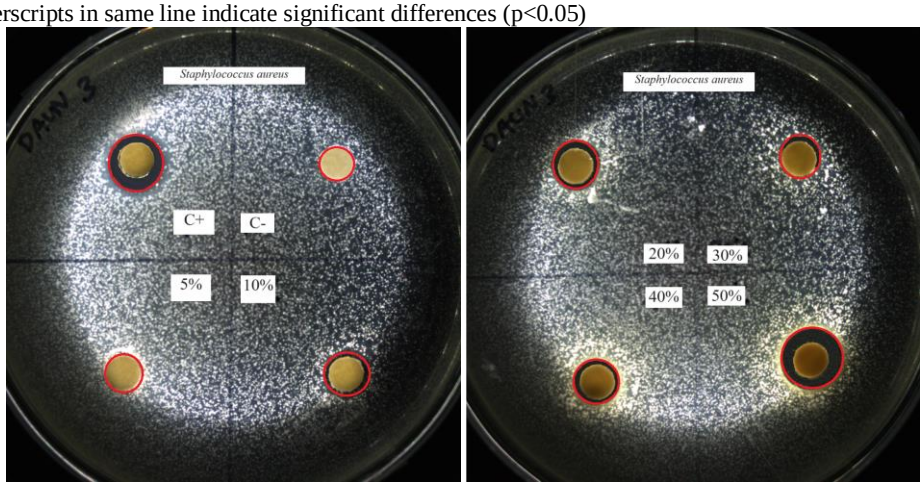

Figure 2. Diameter of the inhibition zone of *Sterculia quadrifida* leaves extract on *Staphylococcus aureus* bacteria

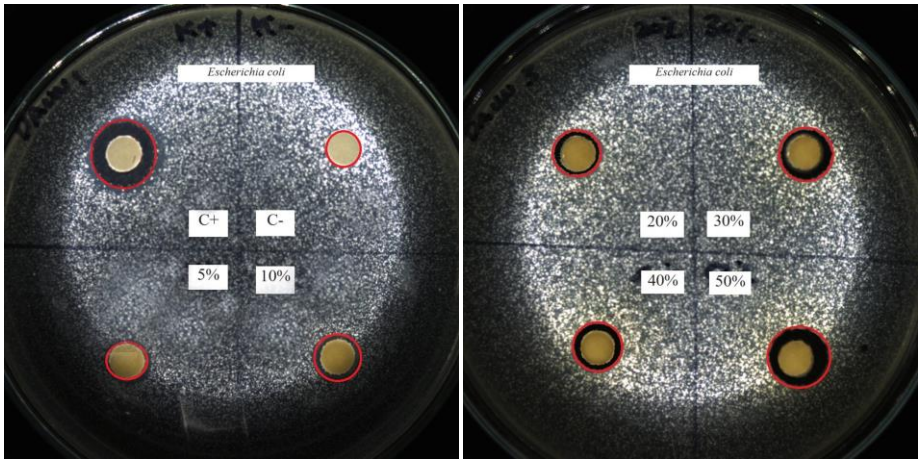

Figure 3. Diameter of the inhibition zone of *Sterculia quadrifida* leaves extract on *Escherichia coli* bacteria

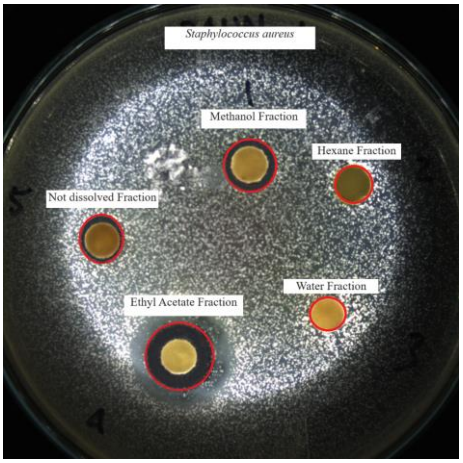

Figure 4. Diameter of the *Sterculia quadrifida* leaves extract fractions on *Staphylococcus aureus* bacteria

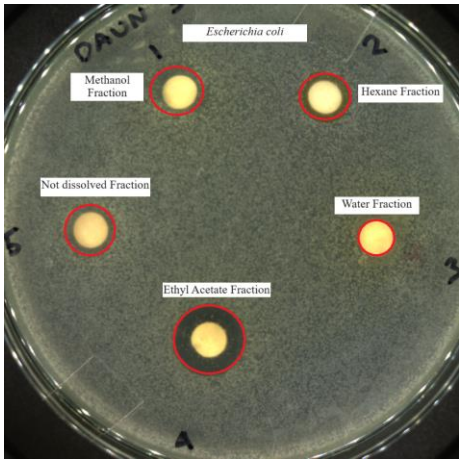

Figure 5. Diameter of the *Sterculia quadrifida* leaves extract fractions on *Escherichia coli* bacteria

Agus et al. (2017) observed that ethyl acetate was the most active fraction in inhibiting the bacteria compared to the n-hexane and methanol fractions. Masyudi et al. (2023) also reported that ethyl acetate extract of *Blumea balsamifera* leaves showed the largest inhibitory zone diameter compared to ethanol and hexane extract of *Blumea balsamifera* leaves could inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The ethyl acetate fraction attracts semi polar compounds in *S. quadrifida* leaves extract, which can inhibit bacterial growth. Semi-polar compounds can inhibit bacterial growth because bacterial cell membranes are not hydrophobic and not hydrophilic.

In the ethyl acetate fraction, there are non-polar flavonoid compounds such as flavanols, flavanones, flavone alcohols and isoflavones. Flavonoid glycosides and aglycones are flavonoids that are polar. The hexane fraction did not show antibacterial activity on *Staphylococcus aureus* bacteria. This may be because hexane fraction only contains nonpolar compounds, such as essential oils, fats, and oils. Water extract is less active compared to ethanol, ethyl acetate and acetone extracts (Stefanović et al. 2012).

Based on the results of average diameter of inhibition zone, *S. quadrifida* leaves extract and its fractions were more active in inhibiting *Escherichia coli*, compared to *Staphylococcus aureus*. This may be due to the differences in the structure of the cell membrane of gram-positive bacteria which is different from the structure of the cell membrane of gram-negative bacteria. In microscope examination, gram-positive bacteria produce a blue color after being given crystal violet dye because gram positives have a thick peptidoglycan layer where the peptidoglycan layer can retain the dye. Although there is no outer membrane on the cell wall, the plasma membrane in gram-positive is surrounded by a thick layer of peptidoglycan to protect gram-positive bacteria from various threats from outside the cell (Jubeh et al. 2020). Gram-negative bacteria have three layers of membrane, namely the outer membrane, peptidoglycan, and inner membrane. The outer membrane, the first layer, is the basis for distinguishing gram-negative bacteria from gram-positive bacteria. The second layer is peptidoglycan which is rigid and gives shape to the cell. Peptidoglycan consists of the disaccharide N-acetyl glucosamine-N-acetylmuramic acid. The inner membrane is the third layer formed from a phospholipid bilayer and is responsible for multifunctional processes such as structure, transportation, and biosynthetic functions (Breijyeh et al. 2023). Gram-positive bacteria have a thicker and tighter peptidoglycan layer than gram-negative bacteria, making it more difficult for *S. quadrifida* leave extract to penetrate the cell walls of gram-positive bacteria. Therefore, *Escherichia coli* was more sensitive so according to the diameter of the inhibition zone resulting from in vitro antibacterial tests was larger.

Bioactive screening of ethyl acetate fraction of *Sterculia quadrifida* leaves extract

Based on the results of LC-HRMS analysis, the dominant bioactive compound from the ethyl acetate fraction of *S. quadrifida* leaves extract were luteolin, rutin (flavonoid) and corchorifatty acid (corchorifatty acid). The bioactive screening result is presented in Table 3. The results showed that flavonoids were found to be the most bioactive compounds in ethyl acetate fraction of *S. quadrifida* extract. So, it can be concluded that flavonoid compounds play a role in the inhibition of bacterial.

Leaves contain polyphenolic compounds in greater quantities than other plant parts. However, research on the antimicrobial activity of leaf extracts is still limited. While most research focuses on the antimicrobial properties of herbal leaves, some researchers have looked at the antibacterial potential of the foliage of fruit trees and shrubs (Efenberger-Szmechtyk et al. 2021). Flavonol and flavone are subclasses of flavonoids that dominate the ethyl acetate fraction of *S. quadrifida* leaf extract. Flavones, flavonoids and flavonols are antimicrobial compounds that are effective against various microorganisms because of their ability to penetrate complex microbial cell wall (Papuc et al. 2017). Flavones and flavonols are synthesized by plants in response to microbial infections so there is no doubt that in vitro experiments they are antimicrobial substances that are effective against various microorganisms (Stefanović et al. 2012).

Flavonoids penetrate cell walls cause damage to the permeability of bacterial cells, leading to the destruction of microsomes and lysosomes. As antibacterial compounds, flavonoids inhibit nucleic acid synthesis, disrupt cytoplasmic membrane function, and disrupt bacterial energy metabolism (Hidanah et al. 2022). Flavonoids as antibacterial compounds work through several mechanisms, such as producing bacterial toxins, inhibiting nucleic acid synthesis and inhibiting biofilm formation (Tako et al. 2020). In starting to develop new antibacterial agents, a key factor to consider is the biofilm-forming ability of a bacterium, because within bacteria there is a biofilm that can increase resistance to antibacterial compounds by 10-1000 times (Shivaprasad et al. 2021). The availability of flavonoids is abundant in plants and does not cause antibiotic resistance when compared to conventional antibiotics (Wang et al. 2021).

Luteolin, is a flavone commonly known as 3′, 4′, 5, 7-tetrahydroxyflavone, is often found in vegetables and fruit such as parsley, celery, perilla leaves, green peppers, and chamomile tea. Luteolin possesses four major biological activities, namely antioxidant, anti-inflammatory, antibacterial and anti-cancer (Bangar et al. 2023). Luteolin induces cell dysfunction in *Escherichia coli* and *Staphylococcus aureus*, changes membrane permeability, and promotes leakage of cellular contents. Luteolin treatment affects cell structure and disrupts the integrity of cell membranes as seen from observations using confocal laser scanning microscope and scanning electron microscope (Xi et al. 2022).

Rutin shows the highest ability to scavenge free radicals, followed by kaempferol, luteolin, quercetin, apigenin, hesperidin, sinensetin, naringenin, naringin and 3,5,6,7,8,3′ ,4′ -heptamethoxyflavone. Rutin showed strong inhibitory ability against the bacteria *Klebsiella pneumoniae* ATCC700603 and *Escherichia coli* ATCC25922 in both growth curve and biofilm production (Wang et al. 2021).

Table 3. Results of bioactive screening of the ethyl acetate fraction of *Sterculia quadrifida* leave extract

Name of compounds	Formula	Molecule mass	Composition (%)	Groups
Rutin	$C_{27}H_{30}O_{16}$	610.154	14.296	Flavonol, flavonoid
Nictoflorin	$C_{27}H_{30}O_{15}$	594.158	8.056	Kaempferol, flavonoid
Kaempferol	$C_{15}H_{10}O_6$	286.048	6.816	Flavonoid
Schaftoside	$C_{26}H_{28}O_{14}$	564.148	6.022	Flavone, flavonoid
Tangeritin	$C_{20}H_{20}O_7$	372.120	4.574	Flavone, flavonoid
Tiliroside	$C_{30}H_{26}O_{13}$	594.136	3.102	Kaempferol, flavonoid
Apigenin	$C_{15}H_{10}O_5$	270.052	2.346	Flavone, flavonoid
Juglanin	$C_{20}H_{18}O_{10}$	418.089	1.858	Flavonol, flavonoid
Quercetin -3β-D-glucoside	$C_{21}H_{20}O_{12}$	464.095	1.849	Flavonol, flavonoid
Quercetin	$C_{15}H_{10}O_7$	302.042	1.814	Flavonol, flavonoid
Kaempferol-7-O-glucoside	$C_{21}H_{20}O_{11}$	448.099	1.773	Flavonol, flavonoid
Miquelianin	$C_{21}H_{18}O_{13}$	478.074	1.629	Flavonol, flavonoid
Vitexin	$C_{21}H_{20}O_{10}$	432.105	0.945	Flavone, flavonoid
Kaempferol-3-O-rhamnoside	$C_{36}H_{36}O_{17}$	740.194	0.581	Flavonoid
Apigenin-7-O-gludoronide	$C_{21}H_{18}O_{11}$	446.085	0.479	Flavonoid
Oleamide	$C_{18}H_{35}NO$	281.272	0.284	Fatty acid
Diosmetin	$C_{16}H_{12}O_6$	300.063	0.189	Flavone, flavonoid
Luteolin	$C_{15}H_{10}O_6$	286.048	22.146	Flavone, flavonoid
Corchorifatty acid	$C_{18}H_{32}O_5$	328.225	12.278	Fatty acid
Genistein	$C_{15}H_{10}O_5$	270.100	2.851	Isoflavone
Astragalin	$C_{21}H_{20}O_{11}$	448.101	2.137	Flavonol, flavonoid
Naringenin	$C_{15}H_{12}O_5$	272.069	0.262	Flavanone, flavonoid
Naringin	$C_{27}H_{32}O_{14}$	580.181	0.130	Flavone, flavonoid
Gallic acid	$C_7H_6O_5$	170.021	0.244	Phenolic acid
Palmitic acid	$C_{16}H_{32}O_2$	256.240	0.111	Fatty acid
Hispidulin	$C_{16}H_{12}O_6$	300.064	0.075	Flavone, flavonoid

Table 4. Total flavonoid concentrations in crude extract of *Sterculia quadrifida* leaves and its fractions

Samples	TFC (QE µg/mL equivalent)
Crude extract	227.281±0.734^c
Methanol fraction	351.456±3.888^d
Hexane fraction	17.789±1.811^a
Water fraction	196.298±0.427^c
Ethyl acetate fraction	107.481±8.164^b
Non-dissolved fraction	212.649±2.491^c

The antimicrobial compound of flavonoids has the potential to be developed as antibiotic drugs and for therapeutic development. Flavonoids can be used to treat various types of infections caused by microbes and have the potential to be a substitute for antibiotics, considering that the increase in microbial resistance to antibiotic treatment is very high. About 70% of infection-causing bacteria are known to be resistant to one of the drugs most used in medicine (Karak 2019). Flavonoids are natural antibacterial compounds with low toxicity, very abundant availability in plants, and affordable, therefore their use as food additives is increasing because they are able to preserve food (Dias et al. 2021).

Total flavonoid compound

The results of total flavonoid compound in *S. quadrifida* leaves crude extract and their fractions are presented in Table 4. The highest flavonoid content was found in the methanol fraction, which was equal to 351.456 QE µg/mL equivalent and the lowest 17.789 QE µg/mL equivalent flavonoid content was recorded in the hexane fraction. This is in line with the results of Nguyen et al. (2022) who extracted *Avicennia officinalis* leaves with various solvents. The highest flavonoid content was obtained in acetone and methanol solvents. El Mannoubi (2023) reported that the bioactive content in a natural material is strongly influenced by the type and concentration of the solvent. The results of statistical analysis showed that the type of solvent affected the TFC content of *S. quadrifida* leave extract fractions. Duncan's test showed that the highest TFC content was in the methanol fraction. Water fraction, crude extract (96% ethanol solvent) and insoluble fraction showed insignificant difference in total TFC. Solvent polarity plays an important role in increasing the content of phenolic compounds and flavonoids (Haminiuk et al. 2014).

Flavonoids are divided into several subclasses with different polarities, so the choice of solvent must be in accordance with the porosity of the targeted flavonoid subclass. For example, highly alkylated aglycones are better extracted with ethyl acetate. In contrast, more polar aglycones such as hydroxylated aglycones and glycosides are better extracted with acetone, water, alcohol, or a mixture of several of these solvents (Dias et al. 2021).

Result of Pearson test showed no correlation ($p>0.05$) between TFC and in vitro antibacterial test against *Staphylococcus aureus* ($r = 0.337$) and *Escherichia coli* ($r = -0.177$). The results of this study are in line with Rayan et al. (2020) who also reported that there was no correlation

between the antibacterial activity of *S. mutans* and antioxidant activity. Apart from being known for its antibacterial activity, flavonoids are also known as antioxidant compounds. Jain et al. (2021) mentioned that there is no significant correlation between total phenolic compound (TPC) and antibacterial activity of *Citrus acida* leaf extract. The antibacterial activity of a natural extract can be attributed to various mechanisms of action (Rayan et al. 2020).

In conclusion, *S. quadrifida* leaves extract and its fractions can inhibit *Staphylococcus aureus* and *Escherichia coli* bacteria. *S. quadrifida* leaves extract with a concentration of 5% was not able to inhibit the tested bacteria. *S. quadrifida* leaves extract with a concentration of 50% had the same ability as the positive control in inhibiting *Staphylococcus aureus* bacteria and *S. quadrifida* leaves extract with concentrations of 30, 40, and 50% had the same ability as the positive control in inhibiting *Escherichia coli* bacteria. The ethyl acetate fraction was the most active fraction in inhibiting the tested bacteria. Luteolin, rutin and corchorifatty acid were bioactive compounds that have the potential to act as antimicrobial agents in *S. quadrifida* leaves. The total flavonoid compound in the crude extract was 227.281 QE µg/mL equivalent and the highest i.e. 351.456 QE µg/mL equivalent total flavonoid compound was in methanol fraction.

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