INDONESIAN JOURNAL OF LIFE SCIENCES

Volume 5, No. 2, September 2023

RESEARCH ARTICLE

Identification, Extraction, Phytochemical Screening and Study of Antimicrobial Activities of Native Papuan Tree Bark Extract: *Dysoxylum alliaceum* and *Aglaia sp*.

Edwin Hadrian[1], Kelly Anvolia[1], Gabriela Lysette Rahardja[1], Rayyane Mazaya Syifa Insani[2*]

[1]Department of Food Science and Nutritio, Institut Bio Scientia Internasional Indonesia, Jakarta, Indonesia

[2]Department of Food Technology, Institut Bio Scientia Internasional Indonesia, Jakarta, Indonesia

**Corresponding author: rayyanesyifa@gmail.com*

A B S T R A C T

Indonesia has one of the most diverse groups of ethnicities and high levels of biodiversity. Accordingly, this leads to a great wealth of ethnobotanical knowledge, where different ethnic groups develop their own methods for utilizing local plants. In Sorong, West Papua, locals often use tree barks for medication, two of which are known locally as Kayu Bawang (KB) and Kayu Teh (KT). KB and KT were morphologically identified in this study and their tree barks were extracted. Crude extracts of the tree barks were obtained via maceration using different solvents (hexane, chloroform, ethyl acetate, acetone, and methanol) with a ratio of 1:15 (w/v). Later, the extract was screened for phytochemicals (alkaloids, tannins, phlobatannins, saponins, terpenoids, flavonoids, and cardiac glycosides) followed by an assessment of antimicrobial properties. KB and KT were identified as *Dysoxylum alliaceum* and *Aglaia sp.* using the leaves, tree barks, and roots. The extraction yields of KT were highest in methanol (11.4%), while KB showed the highest yield in chloroform (1.83%). KT extracts were shown to contain all seven phytochemicals, while KB extracts lacked in tannins and flavonoids. Antimicrobial activity against *E. coli* and *S. aureus* was exhibited by 5% of KB ethyl acetate extract. These findings confirm the potential for KB and KT tree bark applications as functional food in the food industry.

K E Y W O R D S

Extraction, Antimicrobial properties, Phytochemical, Tree bark, Papuan tree bark

H I G H L I G H T S

- Kayu Bawang (KB) and Kayu Teh (KT) are used for medication in West Papua.
- KB and KT were identified as *Dysoxylum alliaceum* and *Aglaia sp*., using the leaves, tree barks, and roots.
- KB extracts contained seven phytochemicals (alkaloids, tannins, phlobatannins, saponins, terpenoids, flavonoids, cardiac glycosides), while KB extracts lacked in tannins and flavonoids.
- KB and KT extracted with ethyl acetate exhibited superior antimicrobial properties against *E. coli* and *S. aureus*.

INTRODUCTION

Phytochemical compounds are bioactive ingredients that provide health benefits beyond essential nutrition to decrease the risk of major chronic disease (Jimenez-Garcia et al., 2018). One example of phytochemicals is phenolic compounds with prominent antioxidant activity that can be utilized for health purposes, such as flavonoids, tannins, and phenolic acid (Szwajkowska et al., 2020). Flavonoids are a group of natural substances with various phenolic structures. It is widely available in plant compounds such as fruits, grains, bark roots, stems, flower tea, and wine. Different types of flavonoids compounds found in plants, such as alkaloids, flavonols, tannins, and phenolic compounds, are believed to be responsible for the health benefits associated with food and medicine.

Tree bark is one of phytochemical sources available in plants. Not only does it serve as a defense mechanism for trees, most tree barks contain biopolymers, tannins, lignin, suberin, suburban, and polysaccharide, although different species might have different compounds. Some tree bark also contain phenolic compounds, which are the most abundant secondary metabolites of plants that are said to have antioxidant, anticancer, antibacterial, and anti-inflammation properties (Vane et al., 2006). Other than phenolic compounds, trees may also have terpenoids, alkaloids, lectins, and polypeptides. However, not all tree bark is thoroughly investigated, as there are still a lot of unknown/unidentified plants that local people only utilize, rendering bioprospecting of said ingredients especially important.

Currently, West Papua contains one of the highest levels of vascular plant diversity globally (Mutke & Barthlott, 2005). With over 70 distinct tribes inhabiting the region (Ronsumbre, 2020), the wealth of ethnobotanics in this region is vast. Several native plants that are locally used as food sources have been shown to be highly nutritious and can be used medically, such as *Pandanus considers* (Rahanra & Samber, 2022), *Haplolobus monticola* (Toja et al., 2020), *Laportea document* (Thalib et al., 2021), Pometia pinata (Thalib et al., 2021), and *Phaleria macrocarpa* (Alara et al., 2016).

In Sorong, West Papua, people of the Omi tribe commonly use the bark of *Dysoxylum alliaceum* and *Aglaia* sp, locally known as Kayu Teh Kamlowelen (KT) and Kayu Bawang Gesikisik (KB) respectively, for food supplements to increase vigour or treat mild flu symptoms. The bark of KT is steeped in hot water and drank as tea, while KB is used as food seasoning due to its aromatic similarity with garlic. Both can be either used fresh or sun-dried. In addition, both trees often grow in hard-to-reach areas within the dense tropical rainforests of the region.

Studies in the properties of *Dysoxylum alliaceum* and *Aglaia sp* have been limited, especially in its bark. Previously, it has been known that *Dysoxylum alliaceum* exude an onion-like smell when the barks are slashed. Nevertheless, the onion smell is stronger in *D. alliaceum* when the leaves and seeds are crushed (Mabberley et al., 1995). Meanwhile, *D. alliaceum* has been mainly studied for its pharmacologic potential. It has been shown to possess antibacterial activity through (+)-8-hydroxycalamenene from its seed and anticancer activity through alliance with 6,7-dimethoxy-2H-chromane-2-one from the bark (Nishizawa et al., 1983; Nurcahyati et al., 2015). Additionally, sesquiterpenoids are found to be present in *Aglaia sp*. They are known to have biological activity, such as antifeedant, antifouling, antimicrobial, antiviral, insect repellent, and cytotoxic activity (Milawati et al., 2020).

Therefore, the characterisation of *Dysoxylum alliaceum* and *Aglaia sp* compounds along with their antimicrobial activities are further assessed in this study. The study encompasses a few aims: identification of KT and KB through morphological characters, screening of phytochemical compounds, as well as determination of antimicrobial properties.

MATERIAL AND METHODS

Plant samples and identification

The outer bark of KT and KB was obtained from a number of trees located within a dense tropical jungle in Sorong, West Papua. The samples were harvested from the wild with the aid of several locals from the Omi tribe. Leaf, bark, and root samples were then sent to Herbarium Bogoriense of BRIN (National Research and Innovation Agency) in Cibinong for morphological identification with the number B-1665/II.6.2/DI.05/07/6/2022 and B-2354/II.6.2/DI.05/07/7/2022. For other tests such as phytochemical screening and assessment of antimicrobial properties, the tree barks were allowed to dry at room temperature before extraction.

Maceration and extraction

Extracts of the bark were done largely in accordance to Gujjeti and Mamidala (2013). Dried tree barks were grounded using a Klaz CG9100 coffee grinder to obtain the coarse powder, 15 g of which was macerated using several solvents with a 1:15 (w/v) ratio. The solvents included hexane, chloroform, ethyl acetate, acetone, and methanol (Merck). The maceration was done at room temperature inside a dark cabinet for seven days. The resulting liquid was then filtered using cheesecloth. Subsequently, the filtrates were dried under pressure at 40°C using Rotavapor® R-100. After the removal of the solvent, the extract was weighed and stored in an airtight container at room temperature for KB extracts and a chiller for KT extract. The yield was calculated by dividing the weight of the extract with the amount of starting material (Co et al., 2012).

Phytochemical screening

The extracts were screened for the presence of alkaloids, tannins, phlobatannins, saponins, flavonoids, terpenoids, and cardiac glycosides through qualitative and colorimetric means. The presence of alkaloids was determined using Mayer's reagent and Dragendorff's reagent, following the methods of Mondal (2017) and Houghton and Raman (2012). The presence of alkaloid was determined by mixing 2 mL of the extract solution with 5 mL of 1% hydrochloric acid, then added with 1 mL of Mayer's reagent and Dragendorff's reagent in a separated test tube. The presence of precipitate with white or yellow or creamy white color (Mayer's reagent) and orange-brown (Dragendorff's reagent) indicated the presence of alkaloids (Mondal, 2017).

Analysis of the tannin content was performed by ferric chloride test, in which the tree bark's extract (0.3 g) was boiled for 10 minutes in 30 mL of water in a water bath. Filtration was then carried out by filtering the solution using a Whatman filter paper. Then, to 5 mL of the filtrate, 0.1% Ferric chloride (3 drops) was added, wherein the appearance of a brownish-green or blue-black color indicated the presence of tannins (Wijaya & Ekowati, 2021). Frothing test was performed to analyse saponins. About 30 mL of distilled water was added to the 0.3 g extracted tree bark samples and boiled for 10 minutes in a water bath. The solution was filtered using a Whatman filter paper, where the resulting solution (10 ml) was mixed with 5 mL distilled water and agitated vigorously to form stable foam. After 5 minutes, if the foam pertained, it indicated the presence of saponin (Owoseni et al., 2010). Salkowski test was used to determine the presence of terpenoids. 5 mL of the tree bark samples' extract was added with chloroform (2 mL) along with 3 mL of concentrated sulphuric acid to form a layer. Formation of precipitate at an interface with reddish-brown color indicated a positive result (Shantabi & Jagetia, 2015). For ascertaining the presence of flavonoids, a different test was conducted. First, 5 mL of dilute ammonia solution (1M) was added to 10 mL of the aqueous tree bark extract. Then, 5 mL of sulphuric acid was added (Ejikeme et al., 2014; Radhia et al., 2018). In these tests, the presence of yellow color indicated the presence of flavonoids. Presence of cardiac glycoside was determined by the

Keller-Killani test. Aqueous extract of the tree bark samples (5 mL) was added with 2 mL glacial acetic acid that contained 1 drop of ferric chloride solution layered with concentrated sulphuric acid (1 mL). The establishment of a brown ring among the layers showed the existence of deoxysugar, which is a trait for cardenolides (Belay & Makonnen, 2020).

Bacterial culture and media preparation

Gram-positive and negative bacteria were used to check the antimicrobial activity of the extracts, which were *Staphylococcus aureus* cultured in Tryptic soy broth (TSB) and *Escherichia coli* (E. coli) cultured in nutrient broth (NB). Both bacterial cultures were obtained from the Indonesia International Institute for Life Sciences (i3L) Laboratory. After 18-24 hours of incubation, the bacteria were standardized with a 0.5 McFarland standard, prepared by adding 0.05 ml of 0.048 M $BaCl_2$ to 9.95 ml of 0.36 NH_2SO_4. The bacteria used must meet or exceed the turbidity of 0.5 MacFarland standard, demonstrating that the bacteria had a concentration of 1.5×10^8 CFU/ml (Goodwin et al., 2007; Ruangpan, 2004). Mueller Hinton Agar was used as the media plate for antimicrobial testing (MHA). 1.7% (w/v) bacteriological agar (oxoid LP0011) and 2.1% (w/v) dehydrated Mueller-Hinton Broth (Merck 1.10293.0500) were used to make the agar plate. For 100-mm plates, the agar must be poured to a depth of 4 mm within the plate, which is roughly 25-30 ml of liquid agar (Hudzicki, 2009). The plates were stored sealed in plastic bags and stored in a refrigerator at a temperature 2-8°C when not in use, with a shelf life of four weeks (Ruangpan, 2004).

Determination of antimicrobial activities

Antimicrobial properties were determined using disk diffusion or the Kirby-Bauer method. The discs (Whatman No. 1: 5 mm diameter) were sterilized using an autoclave. After sterilization, the discs were impregnated with sterile diluted extracts (10% and 5% of extraction yield) or positive control (1% chloramphenicol). The extracts were sterilized by passing through a syringe filter of 0.22 um with a PES membrane. A spreader was used to inoculate 100 µL of bacterial culture into the MHA plate. The extract or standard drug-containing discs were gently pressed into place on the designated section to achieve complete contact with the medium. In a 9-10 cm petri dish, the maximum number of disks that can be inserted is six. When measured from centre to centre, the disks should be 30 mm apart and no closer than 24 mm apart to reduce overlap between inhibition zones (Goodwin et al., 2007). After that, the plates were inverted and incubated for 18-24 hours at 37°C. The outcome was evaluated by measuring the inhibition zone around the disc.

Statistical analysis

The statistical analysis in this journal article utilized the Welch ANOVA method with 95% confidence intervals using IBM SPSS Statistics 24.0.

RESULTS AND DISCUSSION

Morphological identification of KT and KB

KB was identified as *Dysoxylum alliaceum* (Blume) Blume ex A. Juss through morphological characters from roots, barks, and leaves. *D. alliaceum* is a large tree growing up to 38 m tall, with buttresses up to 60 cm tall and 1 m around the tree. The thin bark sheds irregular strips, with red-brown inner bark which becomes yellower within and usually accompanied by a strong smell of onions. The leaves are arranged in three to six dark green elliptic leaflets, each measuring up to 7.5-25 in length and 2.5-7.5 cm in width with a similar onion scent. In addition to the Malesian region, which includes Indonesia, its distribution ranges from

the Andamans and peninsular Thailand to the Solomon Islands and Queensland (Mabberley et al., 1995). Like other members of *Meliaceae Juss, Dysoxylum Blume* possesses many pharmacological activities, including anti-inflammatory, analgesic, anti-microbial, antitumor, CNS, molluscicidal, and cardiac activities (Pham et al., 2004). As such, *D. alliaceum* has been shown to possess antibacterial activity through (+)-8-hydroxy calamenene from its seed and anticancer activity through alliacene and 6,7-dimethoxy-2H-chromane-2-one from the bark (Nishizawa et al., 1983; Nurcahyati et al., 2015). Nevertheless, compared with other *Dysoxylum* species, the pharmacological activities of *D. alliaceum* are still poorly characterized.

Figure 1. Herbarium sample KB (a) and KT (b) obtained in Papua

Unlike KB, identification of KT can only be ascertained up until the genus level: *Aglaia sp*. This could be because morphological characters from flowers and fruits are essential to determine the species, which was lacking in our study. Aglaia is also a member of Meliaceae, and thus the genus contains members showing various pharmacological activities, such as anticancer, antituberculosis, antiviral, anti-inflammatory, and antifungal activities; up to 34% of the members show cytotoxic and insecticidal properties (Harneti & Supratman, 2021). Aglaia is a widely distributed, large, and diverse genus with up to 100 described species, ranging from shrubs to tall trees (Mabberley et al., 1995). Out of these, 22 species are known to occur in New Guinea (Heads, 2001). To date, only one study reported the usage of Aglaia tree barks as a medicinal tea in West Papua (Korain et al., 2014).

Extraction

Table 1. Extraction yield of KT and KB using different solvents (n=3).
Solvents are listed with a descending polarity index.

Solvent	Relative Polarity	KT yield (%)	KB yield (%)
Methanol	0.762	11.44 ± 5.08^{b}	0.996 ± 0.05^{a}
Chloroform	0.259	4.97 ± 1.94^{ab}	1.828 ± 0.73^{a}
Ethyl acetate	0.228	3.98 ± 0.92^{ab}	0.695 ± 0.45^{a}
Acetone	0.355	8.85 ± 4.64^{ab}	0.636 ± 0.31^{a}
Hexane	0.09	0.86 ± 0.16^{a}	0.894 ± 0.47^{a}

Means in each row followed by the same letter are not statistically different at a $p < 0.05$ level

KT and KB extractions were done using different solvents and in triplicate. The extraction yields of KT were highest in methanol (11.4%), followed by acetone (8.85%), chloroform (4.97%), ethyl acetate

(3.98%), and hexane (0.86%). The polarity of the solvents affected the extraction yield, as higher polarity solvents yielded higher extraction yield (Wakeel et al., 2019). Statistical analysis also showed there was a significant difference between methanol and hexane in terms of extraction yield, indicating the influence of polarity in extracting phytochemical compounds. As a result, polar solvents are often used to extract polar phytochemicals, including alkaloids, phenolic compounds, flavonoids, and glycosides (Yusnawan, 2013)

In contrast, the extraction of KB showed the highest yield in chloroform (1.83%), followed by methanol (1%), hexane, ethyl acetate, and acetone. This was unexpected, as more polar solvents were expected to yield higher extraction (Nawaz et al., 2020). Statistical analysis showed a p-value of under 0.05, indicating the absence of any significant differences between the solvents. This result might be due to the lower concentration of biologically active compounds in plants. Hence, an extraction technique which is able to obtain extracts with high yield and minimal changes to the functional properties of the extract is required (Quispe-Condori et al., 2008). Additionally, some phytochemical compounds may have limited solubility in the chosen solvent, leading to lower extraction yields. If the solubility of the target compounds is low, they may not fully dissolve in the solvent, resulting in a lower extraction efficiency (Chebil et al., 2007). According to Brennan et al. (2020), different parts of bark affect total yield extract, as yield increases from the base of the tree to the top. The inner and outer barks also contain various amounts of stilbene chemicals. Jyske et al. (2022) discovered that the proportions of these components, as well as the proportion of residual stem wood (no stilbenes) after debarking, determine extraction yield. This means that phytochemical compounds can have an impact on extraction yield.

Phytochemical identification

Table 2. Result of the phytochemical screening from various extract of KT and KB.

	Extract	Alkaloid	Tannin	Phlobatannin	Saponin	Terpenoid	Flavonoid	Cardiac glycoside
KT	Acetone	+	+	+	+	+	+	+
	Chloroform	-	+	+	+	+	+	+
	Methanol	+	+	+	+	+	+	+
	Ethyl acetate	-	+	-	+	+	+	+
	Hexane	-	+	+	+	+	+	+
KB	Acetone	+	-	+	+	+	-	+
	Chloroform	-	-	+	+	+	-	+
	Methanol	+	-	+	+	+	-	+
	Ethyl acetate	-	-	-	+	+	-	+
	Hexane	-	-	+	+	+	-	+

Phytochemical screening was performed on extracts of the tree barks KT and KB to identify the classes of phenolic compounds present. Table 1 shows that methanol and acetone were the best solvents

for extracting from KT, as they were able to extract all seven tested phytochemical compounds. Hexane and chloroform were able to extract all phenolic compounds except alkaloids, and ethyl acetate only extracted tannins, saponins, terpenoids, flavonoids, and cardiac glycosides. In regards to KB, methanol and acetone were again the best choices, as they contained most of the compounds tested, including alkaloids, phlobatannins, saponins, terpenoids, and cardiac glycosides. Chloroform and hexane extracted phlobatannins, saponins, terpenoids, and cardiac glycosides, while ethyl acetate only extracted saponins, terpenoids, and cardiac glycosides. The different solvents used have different polarities, and this is likely the reason for the diversity of extracted phytochemical compounds. Methanol and acetone are polar solvents and are usually used to extract polar compounds such as alkaloids and phenolic compounds, while chloroform and hexane are nonpolar and are used to extract lipophilic compounds such as terpenoids and alkaloids (Redha et al., 2021)

KT extracted with methanol and acetone contains all of the phytochemicals tested (alkaloids, tannins, phlobatannins, saponins, flavonoids, terpenoids, and cardiac glycoside). Higher polarity showed the capability to extract those compounds. Reducing polarity in chloroform impacts its ability to extract the alkaloids compound. KT extracted with ethyl acetate did not contain alkaloids and phlobatannins. This might be due to alkaloid solubility in polar organic solvents (Dewick, 2009). Ethyl acetate cannot extract phlobatannin from the sample due to its properties, which when combined with hot dilute acids, yields phlobaphene. and ethyl acetate is a weak acid, the other solvents were either acids or weak acids.

Both methanol and acetone serve as effective solvents for extracting a wide range of phytochemical compounds from plants. Methanol, a polar solvent, exhibits high efficacy in extracting low molecular weight phenolic compounds with medium polarity, as well as flavonoids, terpenoids, and saponins (Yusnawan, 2013). Acetone, an intermediate polar compound, is commonly employed for extracting phenolic compounds, flavonoids, proanthocyanidins, and saponins (Ngo et al., 2017). Consequently, these solvents offer broad suitability for extracting diverse phytochemical compounds. It is worth noting that plants contain a diverse array of medicinal compounds, albeit with varying qualities among different species. It is also important to recognize that the absence of bioactivity in extracts does not necessarily indicate a complete absence of bioactive compounds; it may be attributed to lower concentrations of these compounds or the presence of other constituents that counteract their functions. Thus, it is recommended to perform quantitative analysis of phytochemical compounds to enable comparisons among different extracts. Natural bioactive substances derived from plants are increasingly utilized as functional foods to enhance human health and address various ailments. Furthermore, the results emphasize that methanol is particularly efficient in extracting bioactive compounds from KB and KT, likely due to the higher solubility of these compounds in methanol solvent.

Antimicrobial Properties of Tree Bark Extract

According to the results presented in Table 3, the ethyl acetate extract of (KB) had the potential for antimicrobial activity, as there was an increasing inhibition zone in conjunction with the increase in KB extract concentration from 0% to 5%. However, there was a statistically significant difference between all treatments and positive control (1% chloramphenicol) ($p>0.05$). KB extracted using acetone also showed an increase in the inhibition zone compared to the negative control. However, the results of these tests resulted in an inhibition zone that is not clear, implying bacteriostatic activity (presence of bacteria in the inhibition zone). Bacteriostatic antimicrobials inhibit bacterial protein synthesis or growth but do not directly cause bacterial death. Other extracts, such as those extracted using methanol and acetone, showed potential antimicrobial activity against *S. aureus.* The mechanisms underlying the potential antimicrobial activity of these extracts is

likely due to the presence of phytochemical alkaloids, a diverse group of compounds that have been used as scaffolds for important antibacterial drugs, which were only found in these two and not in the other KB extracts.

Table 3. Inhibition zone radius of KB extract against *E. coli* and *S. aureus*

Solvent	*E. coli*				*S. aureus*			
	Concentration of KB extract				Concentration of KB extract			
	Solvent only (mm)	5% Extract (mm)	10% Extract (mm)	Positive control	Solvent only (mm)	5% Extract (mm)	10% Extract (mm)	Positive Control
Methanol	0.50 ± 0.00[a]	0.50 ± 0.00[a]	0.50 ± 0.00[a]	8.94 ± 0.68[b]	0.50 ± 0.00[a]	0.50 ± 0.00[a]	1.25 ± 1.06[a]	9.44 ± 0.16[b]
Acetone	0.85 ± 0.49[a]	2.75 ± 3.18[a*]	4.25 ± 5.30[a*]		0.75 ± 0.35[a]	0.50 ± 0.00[a]	0.50 ± 0.00[a]	
Ethyl acetate	1.25 ± 1.06[a*]	5.00 ± 1.41[a*]	4.25 ± 2.47[a*]		0.75± 0.35[a]	5.50 ± 0.71[a*]	5.00 ± 1.41[a*]	
Chloroform	1.50 ± 0.70[a]	1.5 ± 0.70[a]	0.00 ± 0.00[a]		0.75 ± 1.06[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	
Hexane	0.50 ± 0.70[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]		0.75 ± 0.35[a]	1.05 ± 0.70[a]	0.50 ± 0.70[a]	

*Result indicates bacteriostatic

Means in each row followed by the same letter are not statistically different at a $p < 0.05$ level

Table 4. Inhibition zone radius of KT extract against *E. coli and S. Aureus*

Solvent	*E. coli*				*S. aureus*			
	Concentration of KB extract				Concentration of KB extract			
	Solvent only (mm)	5% Extract (mm)	10% Extract (mm)	Positive control	Solvent only (mm)	5% Extract (mm)	10% Extract (mm)	Positive Control
Methanol	0.167 ± 0.29[a]	1.33 ± 1.04[a]	1.08 ± 0.38[a]	8.94 ± 0.68[b]	0.67 ± 0.76[a]	0.65 ± 0.77[a]	0.43 ± 0.77[a]	9.44 ± 0.16[b]
Acetone	0.62 ± 0.20[a]	0.33 ± 0.58[a]	0.25 ± 0.43[a]		1.58 ± 0.72[b]	0.5 ± 0.00[a]	0.67 ± 0.29[a]	
Ethyl acetate	1.45 ± 0.43[ab]	2.67 ± 0.58[b]	1 ± 0.43[a]		0.750 ± 0.00[a]	0.5 ± 0.00[a]	0.67 ± 0.29[a]	
Chloroform	0.45 ± 0.17[a]	0.33 ± 2.25[a]	0.25 ± 0.87[a]		1.58 ± 0.00[b]	0.5 ± 0.00[a]	0.67 ± 0.29[a]	
Hexane	0.25 ± 0.25[a]	0.33 ± 0.58[a]	0.25 ± 0.43[a]		0.33 ± 0.38[a]	0 ± 0.00[a]	0 ± 0.00[a]	

*Result indicates bacteriostatic

Means in each row followed by the same letter are not statistically different at a $p < 0.05$ level

According to Table 4, the methanol extract of KT may have a slight potential for antimicrobial activity against *E. coli*. Statistically, there was a significant difference between treatments, as ethyl acetate showed greater inhibition zone against *E. coli*. However, the hexane extract of KT also showed a lesser antimicrobial property, with the inhibition zone of the 5% extract showing activity. The extract of KT shows no antimicrobial activity against *S. aureus* culture. Gram-negative bacteria are typically more resistant to antibiotics than gram-positive bacteria due to their outer membrane (Breijyeh et al., 2020).

CONCLUSION

The results of the study demonstrate that ethyl acetate extracts of KB and KT exhibit superior antimicrobial properties against *E. coli*, a gram-negative bacterium, and *S. aureus*, a gram-positive bacterium. Literature supports the presence of saponins, terpenoids, and cardiac glycosides in both KB and KT, which are known to possess natural antibacterial activity (Table 2). These findings suggest that the majority of the

antimicrobial compounds present in KB and KT are lipophilic in nature, as evidenced by their higher activity in lower polarity solvents. However, further study to confirm the quantity of the compound such as using high performance liquid chromatography as well as study to exhibit the efficacy of the extracts are necessary to verify current findings.

REFERENCES

Alara, O. R., & Olalere, O. A. (2016). A critical overview on the extraction of bioactive compounds from Phaleria macrocarpa (Thymelaceae). Nat Prod Chem Res, 4(5), 1000232. DOI:10.4172/2329-6836.1000232

Belay, R., & Makonnen, E. (2020). Anti-inflammatory activities of ethanol leaves extract and solvent fractions of Zehneria scabra (Cucurbitaceae) in rodents. Asian Journal of Natural Product Biochemistry, 18(1). https://doi.org/10.13057/biofar/f180105

Breijyeh, Z., Jubeh, B., & Karaman, R. (2020). Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules, 25(6), 1340. doi: 10.3390/molecules25061340

Brennan, M., Fritsch, C., Cosgun, S. et al. (2020). Quantitative and qualitative composition of bark polyphenols changes longitudinally with bark maturity in Abies alba Mill.. *Annals of Forest Science 77*, 9. https://doi.org/10.1007/s13595-019-0916-x

Chebil, L., Humeau, C., Anthoni, J., Dehez, F., Engasser, J. M., & Ghoul, M. (2007). Solubility of flavonoids in organic solvents. *Journal of C*hemical *& Engineering D*ata, 52(5), 1552-1556. https://doi.org/10.1021/je7001094

Co, M., Fagerlund, A., Engman, L., Sunnerheim, K., Sjöberg, P. J., & Turner, C. (2012). Extraction of antioxidants from spruce (Picea abies) bark using eco-friendly solvents. *Phytochemical A*nalysis, 23(1), 1-11. DOI: 10.1002/pca.1316

Dewick, P. M. (2009). Medicinal natural products: a biosynthetic approach (3rd ed.). John Wiley & Sons.

Ejikeme, C., Ezeonu, C. S., & Eboatu, A. N. (2014). Determination of Physical and Phytochemical Constituents of some Tropical Timbers Indigenous to nigerdelta area of nigeria. *European Scientific Journal, 10*(18), 247-270. http://eprints.gouni.edu.ng/id/eprint/1312

Goodwin, A. C., Steele-Moore, L., & Schwalbe, R. (2007). Antimicrobial Susceptibility Testing Protocols. United States: CRC Press.

Gujjeti, R. P., & Mamidala, E. (2013). Phytochemical screening and thin layer chromatographic studies of Aerva lanata root extract. *International Journal of I*nnovative Research in Science, Engineering and Technology, 2(10), 5725-30. PHYTOCHEMICAL-SCREENING-AND-THIN-LAYER-CHROMATOGRAPHIC-STUDIES-OF-AERVA-LANATA-ROOT-EXTRACT.pdf (researchgate.net)

Harneti, D., & Supratman, U. (2021). Phytochemistry and biological activities of Aglaia species. *Phytochemistry, 181*, 112540. DOI: 10.1016/j.phytochem.2020.112540

Heads, M. (2001). Regional patterns of biodiversity in New Guinea plants. *Botanical Journal of the* Linnean Society, 136(1), 67-73. https://doi.org/10.1006/bojl.2000.0403

Houghton, P., & Raman, A. (2012). Laboratory handbook for the fractionation of natural extracts. Springer Science & Business Media.

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American Society for Microbiology, 15,* 55-63.https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-DiffusionSusceptibility-Test-Protocol-pdf.pdf

Jimenez-Garcia, S. N., Vazquez-Cruz, M. A., Garcia-Mier, L., Contreras-Medina, L. M., Guevara-González, R. G., Garcia-Trejo, J. F., & Feregrino-Perez, A. A. (2018). Phytochemical and pharmacological properties of secondary metabolites in berries. *Therapeutic Foods*, 397-427. https://doi.org/10.1016/B978-0-12-811517-6.00013-1

Jyske, T., Brännström, H., Halmemies, E., Laakso, T., Kilpeläinen, P., Hyvönen, J., ... & Saranpää, P. (2022). Stilbenoids of Norway spruce bark: does the variability caused by raw-material processing offset the biological variability?. *Biomass Conversion and Biorefinery*, 1-15. https://doi.org/10.1007/s13399-022-02624-9

Korain, A., Sinaga, N., & Ungirwalu, A. (2014). Etnobotani Pemanfaatan Kulit Kayu oleh Suku Maybrat-Papua.http://repository.unipa.ac.id:8080/xmlui/bitstream/handle/123456789/376/03.%20Antoni%20Jurnal%20Beccariana%20Vol%2016_1_Kulit%20Kayu.pdf?sequence=1&isAllowed=y

Mabberley, D. J., Pannell, C. M., & Sing, A. M. (1995). Flora Malesiana: Series I. Spermatophyta Volume 12, part 1, 1995. Meliaceae. Flora Malesiana: Series I. Spermatophyta Volume 12, part 1, 1995. https://www.cabdirect.org/cabdirect/abstract/19960605484

Milawati, H., Sukmawati, W., Harneti, D., Maharani, R., Nurlelasari, N., Hidayat, A. T., ... & Shiono, Y. (2020). Cytotoxic Sesquiterpenoids from the Stem Bark of Aglaia harmsiana (Meliaceae). *Indonesian Journal of Chemistry*, *20*(6), 1448-1454. https://doi.org/10.22146/ijc.47808

Mondal, S. (2017). Screening of bioactive compounds from Cucurbitaceae family edible plants of Bangladesh–Cucurbita pepo Linn.: A case study. Anchor Academic Publishing.

Mutke, J., & Barthlott, W. (2005). Patterns of vascular plant diversity at continental to global scales. Biologiske Skrifter*, 55*(4), 521-531. https://www.researchgate.net/profile/Jens-Mutke/publication/215672861_Patterns_of_vascular_plant_diversity_at_continental_to_global_scale/links/587f244408aed3826af479dd/Patterns-of-vascular-plant-diversity-at-continental-to-global-scale.pdf

Nawaz, H., Shad, M. A., Rehman, N., Andaleeb, H., & Ullah, N. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Phaseolus vulgaris) seeds. *Brazilian Journal of Pharmaceutical Sciences*, *56*. https://doi.org/10.1590/s2175-97902019000417129

Ngo, T. V., Scarlett, C. J., Bowyer, M. C., Ngo, P. D., & Vuong, Q. V. (2017). Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of Salacia chinensis L. *Journal of Food Quality*, *2017*. https://doi.org/10.1155/2017/9305047

Nishizawa, M., Inoue, A., Sastrapradja, S., & Hayashi, Y. (1983). (+)-8-Hydroxycalamenene: A fish-poison principle of Dysoxylum acutangulum and D. Alliaceum. Phytochemistry*, 22*(9), 2083-2085. https://doi.org/10.1016/0031-9422(83)80052-1

Nurcahyanti, O., Julaeha, E., & Mayanti, T. (2015). Senyawa Steroid Dari Kulit Batang Dysoxylum Alliaceum Dan Aktivitasnya Terhadap Sel Kanker Payudara Mcf-7. Chimica et Natura Acta*, 3*(2). https://doi.org/10.24198/cna.v3.n2.9184

Owoseni, A. A., Ayanbamiji, T. A., Ajayi, Y. O., & Ewegbenro, I. B. (2010). Antimicrobial and phytochemical analysis of leaves and bark extracts from Bridelia ferruginea. *African Journal of Biotechnology, 9*(7), 1031-1036. DOI:10.5897/AJB09.1072

Pham, N. K., Bui, H. T., Tran, T. H., Hoang, T. N. A., Vu, T. H., Do, D. T., ... & Nguyen, M. C. (2021). Dammarane triterpenes and phytosterols from Dysoxylum tpongense Pierre and their anti-inflammatory activity against liver X receptors and NF-κB activation. Steroids, 175, 108902. DOI: 10.1016/j.steroids.2021.108902

Quispe-Condori, S., Foglio, M. A., Rosa, P. T., & Meireles, M. A. A. (2008). Obtaining β-caryophyllene from Cordia verbenacea de Candolle by supercritical fluid extraction. *The Journal of Supercritical Fluids*, *46*(1), 27-32. https://doi.org/10.1016/j.supflu.2008.02.015

Radhia, A., Hanen, N., Abdelkarim, B. A., & Mohamed, N. (2018). Phytochemical screening, antioxidant and antimicrobial activities of Erodium glaucophyllum (L.) L'Hérit. Journal of Biomedical Sciencies*, 7*(4). DOI: 10.4172/2254-609X.100092

Rahanra, R. M., & Samber, L. (2022). Upaya Peningkatan Tanaman Buah Merah Papua (Pandanus conoideus) Dengan Berbagai Perlakuan Pupuk Organik Cair dan Pupuk Kompos di Kampung Mariadei. *BIO-EDU: Jurnal Pendidikan Biologi, 7*(1), 9-24. https://doi.org/10.32938/jbe.v7i1.2529

Redha, A., Siddiqui, S. A., & Ibrahim, S. A. (2021). Advanced extraction techniques for Berberis species phytochemicals: A review. *International Journal of Food Science & Technology*, *56*(11), 5485-5496. https://doi.org/10.1111/ijfs.15315

Ronsumbre, A. (2020). Ensiklopedia Suku Bangsa Di Provinsi Papua Barat.

Ruangpan, L. (2004). Minimal inhibitory concentration (MIC) test and determination of antimicrobial resistant bacteria. In *Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria*

isolated from aquatic animals and environ ment (pp. 31-55). Aquaculture Department, Southeast Asian Fisheries Development Center. http://hdl.handle.net/10862/1637

Shantabi, L., & Jagetia, G. C. (2015). Phytochemical profiling of Kam-sabut, Croton caudatus Geiseler. *Res Rev J Bot* Sci*, 4, 5-14.* https://api.semanticscholar.org/CorpusID:73644498

Szwajkowska-Michałek, L., Przybylska-Balcerek, A., Rogoziński, T., & Stuper-Szablewska, K. (2020). Phenolic compounds in trees and shrubs of central Europe. *Applied Sciences*, *10*(19), 6907. DOI:10.3390/app10196907

Thalib, Z., Wulandari, S., Nugraha, A., & Himmah, A. (2021). Ethnobotanial study of Laportea decumena in West Papua. *Journal of Ethnobiology and Ethnomedicine, 17*(1), 1-7. https://doi.org/10.3889/oamjms.2021.7759

Toja, Y. T., Suprayitno, E., & Yanuhar, U. (2020). In silico potential black fruit seeds (haplolobus monticola) wondama local plant west papua barat as antibacterial Aeromonas hydrophila. EurAsian Journal of BioSciences*, 14*(2).

Vane, C. H., Drage, T. C., & Snape, C. E. (2006). Bark decay by the white-rot fungus Lentinula edodes: Polysaccharide loss, lignin resistance and the unmasking of suberin. *International Biodeterioration & Biodegradation*, *57*(1), 14-23. https://doi.org/10.1016/j.ibiod.2005.10.004

Wakeel, A., Jan, S. A., Ullah, I., Shinwari, Z. K., & Xu, M. (2019). Solvent polarity mediates phytochemical yield and antioxidant capacity of Isatis tinctoria. *PeerJ*, *7*, e7857. DOI:10.7717/peerj.7857

Wijaya, T. H., & Ekowati, N. (2021). The Antibacterial Activity of Acanthus ilicifolius L. n-Hexane Fraction. *Journal of Science and Technology Research for Pharmacy, 1*(2), 48-56. DOI 10.15294/JSTRP.V1I2.49615

Yusnawan, E. (2013). The effectiveness of polar and non polar fractions of ageratum conyzoides L. to control peanut rust disease and phytochemical screenings of secondary metabolites. *Jurnal Hama dan Penyakit Tumbuhan Tropika*, *13*(2), 159-166. DOI: 10.23960/j.hptt.213159-166