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PHYTOCHEMICAL PROFILING OF PURNAJIWA FRUIT (*Kopsia arborea* Blume.) EXTRACTS AND ANTIBACTERIAL ACTIVITY AGAINST METHICILLIN-RESISTANT *Staphylococcus aureus* (MRSA)

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ABSTRACT

Purnajiwa (*Kopsia arborea* Blume.) contains diverse phytochemicals with notable antibacterial potential. This study aimed to characterize and quantify the phytochemicals of *Kopsia arborea* fruit extracts obtained by maceration and Soxhlet extraction with methanol and evaluate their antibacterial activity against MRSA through phytochemical screening, GC-MS analysis, spectrophotometric quantification of flavonoids, alkaloids, and phenolics, and disc diffusion assay at 100 µg/mL concentration. The findings indicated that the Soxhlet extraction produced a superior yield (19.47 ± 0.58%) compared to maceration (11.09 ± 0.65%). Phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, and triterpenoids, with no qualitative differences between the two extracts. Quantitative analysis demonstrated higher concentrations of alkaloids, flavonoids, and phenolics in the Soxhlet extract, with values of 88.58 ± 3.76, 46.50 ± 1.04, and 57.87 ± 0.44 µg/mL, respectively, compared to the maceration extract (50.46 ± 3.86, 26.22 ± 0.27, and 30.49 ± 0.31 µg/mL, respectively). GC-MS analysis identified 13 alkaloid compounds in the Soxhlet extract and 12 in the maceration extract. Antibacterial assays revealed that the mean inhibition zone diameter against MRSA was 11.69 ± 0.28 mm for the Soxhlet extract and 12.61 ± 0.27 mm for the maceration extract, respectively. In conclusion, Soxhlet extraction yielded higher concentrations of alkaloids, phenolic compounds, and flavonoids; however, GC-MS analysis revealed that the macerated extract exhibited a higher AUC of alkaloid compounds than that of Soxhlet extraction. Moreover, the macerated extract demonstrated superior antibacterial activity, indicating that maceration has greater potential for development as an antibacterial agent than Soxhlet extraction.

Keywords: *Kopsia arborea*, maceration, Soxhlet extraction, phytochemical profiling, MRSA

INTRODUCTION

Antibiotic resistance is a global problem that requires critical treatment. Cases of bacterial resistance are rapidly developing compared to the pace at which new antibiotics are developed. Antibiotic resistance in bacteria is a natural phenomenon and a form of bacterial adaptation to antimicrobial agents (Chandra *et al*., 2017). The rapid development of bacterial resistance has become a concern in the medical world, prompting the search for alternative treatments or the development of new drug compounds to fight resistant bacteria. Research on alternative antimicrobials has been conducted intensively (Havenga *et al*., 2019). Plants are a source of antimicrobial agents. Various studies have shown the effectiveness of plant metabolite compounds in fighting bacteria. Compounds commonly found in plants, such as quinine, alkaloids, lectins, polypeptides, flavonoids, manganese, terpenoids, essential oils, and tannins, have been shown to exhibit antibacterial activity (AlSheikh *et al*., 2020; Chandra *et al*., 2017).

Purnajiwa (*Kopsia arborea* Blume.) is a plant with potential antimicrobial properties. *Kopsia arborea* Blume is a species of the genus *Kopsia* (family *Apocynaceae*). *Kopsia* is known as one of the plants that produces alkaloid compounds. *Kopsia* species generally contain highly potent indole alkaloid compounds with a wide range of bioactivities (Chen *et al*., 2020; Hop & Son, 2022; Wong *et al*., 2021). Alkaloid compounds in *Kopsia* generally have a unique skeleton with significant bioactivity (Kogure *et al*., 2012). Research on the ethanol extracts of *Kopsia arborea* Blume. shows the presence of several alkaloids, such as aspidospermidin, kopsinine, quebrachamin, and tabersonin (Ariati *et al*., 2024).

Various studies have shown the effectiveness of alkaloid compounds as antibacterials. Alkaloid compounds naturally have inhibitory activity against Methicillin-resistant *Staphylococcus aureus* (MRSA). The indole alkaloid compounds dionemycin and 6-CH_3O-7,7'-dichorochromopyrrolic acid, derived from *Streptomyces* sp., demonstrated inhibition against six strains of MRSA isolated from humans and pigs, with MIC in the range of 0.5–2µg/mL (Song *et al*., 2020). Other alkaloid compounds include isoconcuressine and N-formylconessimine, isolated from the seeds of *Holarrhena antidysenteriaca* Wall. ex A. DC also shows inhibition against MRSA (Zhou *et al*., 2 017). Myoporumine A and myoporumine B, alkaloids isolated from *Myoporum bontioides* A. Gray, strongly inhibit MRSA with an MIC of 6.25µg/mL (Dong *et al*., 2018).

Various studies have proven the antibacterial activity of various plants, which is produced by a variety of phytochemical compounds. This study aimed to identify and quantify the secondary metabolite components of Kopsia arborea Blume and evaluate its antibacterial activity against methicillin-resistant Staphylococcus aureus., which were extracted using two different methods: maceration and Soxhlet extraction. Both methods are conventional extraction methods commonly used to extract various secondary metabolite compounds, especially alkaloids. The antibacterial activity of a plant is attributed to its phytochemical compounds. Extraction techniques strongly influence the diversity and concentration of phytochemicals obtained from plant materials (Candra *et al*., 2021). In addition, variations in extraction techniques can also affect the interaction between solvents and soluble compounds, particularly those with similar polarity properties (Putri *et al*., 2022). Koçancı *et al*. (2022) proposed that Soxhlet extraction is a commonly used technique for extracting total alkaloids and identifying different types of alkaloid compounds. This method is also known to produce high extract yields with more economical solvent use and a relatively short duration (Marjoni 2016). Wirawan *et al*. (2023) showed that maceration can produce extracts with a wide range of phytochemical ingredients, such as flavonoids, phenols, alkaloids, and tannins. Maceration is a simple and effective method for maintaining the stability of heat-sensitive compounds, thereby minimizing the risk of damage to phytochemical compounds (Mukhriani, 2014; Rahman *et al*., 2017; Sekali *et al*., 2020). Several studies have also shown a correlation between antioxidant and antibacterial activities (Ispiryan *et al*., 2024; Todorovic *et al*., 2017).

This study presents a comparative evaluation of phytochemical content and antibacterial activity of *Kopsia arborea* fruit extracts obtained through maceration and Soxhlet methods, specifically targeting Methicillin-Resistant *Staphylococcus aureus* (MRSA). This study is among the first to directly contrast the influence of extraction techniques on both the phytochemical profile and antibacterial efficacy of purnajiwa (*Kopsia arborea* Blume.) against MRSA.

RESEARCH METHODS

Sample Collection

The harvesting of purnajiwa (*Kopsia arborea* Blume) fruit is collected in Denpasar, Bali (8°40'16.8"S 115°14'02.2"E). The fruit used was a ripe blackish-purple fruit. The sample was determined at the Mathematics and Natural Sciences Laboratory Unit, Faculty of Tarbiyah and Teacher Training, IAIN Syekh Nurjati Cirebon (27/In.08/LB.1.1/PP.009/12/2023). The samples were sorted, washed, dried, cut, and oven-dried at 50°C for 3 days until dry. The dry sample was then blended until a fine powder was obtained.

Extraction

The sample powder was extracted by maceration and Soxhlet extraction using methanol with a sample and solvent ratio of 1:15. Maceration was carried out for 24 hours with periodic stirring. Soxhlet extraction was performed using a Soxhlet apparatus for 8 hours. Filtrates from both maceration methods were concentrated using a vacuum rotary evaporator at a controlled temperature of 40°C. Crude extracts obtained using both extraction methods were used in subsequent studies. The final result obtained was weighed and compared with the mass of the material used, calculated as a percentage of the extract yield.

$$\% \text{ Yield} = \frac{final\ weight\ of\ extract}{Initial\ weight\ of\ extract}\ x\ 100\%$$

Phytochemical Screening

Phytochemical screening was performed as a preliminary identification of phytochemicals using standard colour reactions in test tubes. Screening was performed for compounds of the alkaloid group, flavonoids, phenols, tannins, quinones, saponins, steroids, and triterpenoids using a method described by Dubale *et al*. (2023).

The phytochemical content in crude extracts was also identified using the GC-MS Agilent Technologies 8860 GC system, 5977B GC/MSD (Agilent, USA) instrument, which is equipped with a column HP-5MS UI (30 m in length × 0.250 mm in diameter × 0.25 µm in film). GC-MS spectroscopic detection was performed using an electron ionization system (70eV) with a range of 50–300 m/z. The temperature of the injector was 250 °C in the splitless mode. Helium was used as the carrier gas at a flow rate of 1 mL/min. The volume of the injected sample was 2 µL. The initial temperature was set at 60–100°C with a rise rate of 4°C/min, and then increased to 290°C with a rise rate of 10°C/min. The presence of chemical compounds in the sample was characterized by the appearance of peaks in the chromatogram. The phytochemical compounds in the sample were identified by comparing the mass spectrum of the detected components with the spectral mass data of the components in the National Institute of Standards and Technology (NIST) library with a percentage similarity index of at least 60%. In this study, the NIST17 library was used. Data were analyzed using Agilent MassHunter Qualitative Analysis Navigator B.08.00 and GCMS 5977B software.

Quantification of Total Alkaloids

The total alkaloid content was quantified using UV-VIS Spectrophotometry (Shimadzu, Japan) with caffeine as the standard. Linear regression was obtained from the calculation of the standard concentrations of caffeine (10, 15, 20, and 25 ppm) to the absorbance at a maximum wavelength of 270 nm. A total of 2 mL of extract solution with a concentration of 1000 ppm (in methanol) and a standard solution of caffeine were added with a pH phosphate buffer of 4.7 mL and a bromocresol green (BCG) solution of 2 mL, then extracted three times using 3 mL of chloroform. The chloroform phase was then evaporated using a water bath and dissolved in 10 mL of chloroform. The solution was then separated using a separate funnel, and the chloroform part was collected. The absorbance was measured at the maximum wavelength of caffeine (Karim *et al*., 2022). The determination was repeated thrice.

Total Phenol Quantification

Total phenol quantification was performed using UV-VIS Spectrophotometry (Shimadzu, Japan). A calibration curve was obtained using a gallic acid standard (0–60 µg/mL). A total of 50 µg/mL of extract and 1.6 mL of gallic acid were added to 0.2 mL of Folin-Ciocalteu reagent and mixed evenly for 3 minutes. Subsequently, 0.2 mL of 10% (b/b) sodium carbonate solution was added and left for 30 minutes at room temperature. Absorbance was measured at 760 nm for total phenolate content and 640 nm for total tannin compounds (Do *et al*., 2014; McDonald *et al*., 2001; Roghini & Vijayalakshmi, 2018). The determination was repeated three times.

Total Quantification of Flavonoids

Total flavonoid quantification was performed using UV-VIS Spectrophotometry (Shimadzu, Japan) with quercetin as the standard. Quercetin concentrations of 0–100 µg/mL in methanol were used to generate a calibration curve for the total phenolate content. A total of 2.0 mL of extract was diluted in methanol to a concentration of 100 µg/mL and quercetin *was* added to 0.1 mL solution of aluminum chloride 10% b/b and solution potassium acetate 0.1 mM. The mixture was left for 30 minutes at room temperature. Absorbance was measured at 415 nm (Do *et al*., 2014). The determination was repeated thrice.

Antibacterial Activity Testing

The antibacterial activity against MRSA was determined using the agar diffusion method with Mueller-Hinton Agar. Bacterial cultures were obtained from the Kerthi Bali Sadhajiwa Health Laboratory Center, Bali (UPTD). Balai Laboratorium Kesehatan Kerthi Bali Sadhajiwa Provinsi Bali). The bacteria were suspended at a bacterial concentration of 1.5 × 108 CFU/mL, equivalent to the standard turbidity of Mc. Farland solution of 0.5, measured with Mc. Farland densitometer (DEN-1 Grant Instruments, England), and grown on a petri dish. The negative control used was dimethyl sufoxide (DMSO 5%), and the positive control was an antibiotic clindamycin 2 µg (MRSA) disc. The test solution was prepared by macerating and extracting the fruit of *Kopsia arborea* Blume using Soxhlet extraction. suspended in 5% DMSO until a concentration of 100 mg/mL was obtained. The test was replicated three times and incubated at 35–37°C for 18 hours. The inhibition zone was observed, measured, and expressed in mm, excluding the disc size (6 mm).

Data Analysis

The data obtained in this study were statistically analyzed using IBM SPSS Statistics (version 25) with a 95% confidence level. The total values of alkaloids, flavonoids, phenolics, and inhibitory zone diameters in both extracts were compared using an independent t-test to determine the differences in values between the Soxhlet and maceration extracts.

RESULTS AND DISCUSSION

Purnajiwa (*Kopsia arborea* Blume.) is a medicinal plant used in Indonesia. In this study, phytochemical and antibacterial activities were evaluated and compared for purnajiwa (*Kopsia arborea* Blume) fruit extracted using the Soxhlet and maceration methods. Qualitative phytochemical screening showed no difference in phytochemical content between the Soxhlet and maceration extracts, suggesting that both methods extracted the same type of metabolite (Table 1). Purnajiwa (*Kopsia arborea* Blume.) The fruit extracts contained alkaloids, flavonoids, phenolics, tannins, saponins, and triterpenoids. The results of this study are supported by previous research demonstrating that both maceration and Soxhlet extraction methods can isolate phytochemicals of similar types (Candra *et al*., 2021; Putri *et al*., 2024).

The higher extraction yield achieved by Soxhlet extraction compared to maceration in this study can be attributed to continuous hot solvent cycling, which enhances solvent penetration and compound diffusion from the plant matrix. The maceration method relies on passive diffusion and often requires an extended time to reach equilibrium, whereas Soxhlet extraction continuously replenishes fresh solvent over the sample, thereby increasing the mass transfer efficiency (Sun *et al*., 2025). Several studies have reported similar findings, highlighting that the yields from Soxhlet extraction were markedly higher than those obtained via maceration (Bitwell *et al*., 2023). These results also support the phytochemical quantification results of this study (Figure 1), which showed that Soxhlet extraction produces greater concentrations of alkaloids, phenolics, and flavonoids, owing to its more efficient extraction dynamics under heat and solvent circulation.

Table I. Yield and Phytochemical Screening Results on Maceration and Soxhlet Extraction Extracts of Purnajiwa Fruit (*Kopsia arborea* Blume.)

Parameters	Result	
	Soxhlet Extraction	Maceration
Extract yields	19.47 ± 0.58 %	11.09 ± 0.65 %
Phytochemical screening		
Alkaloid (Dragendorf)	+	+
Alkaloids (Mayer)	+	+
Flavonoids	+	+
Phenolic	+	+
Tannins	+	+
Quinone	-	-
Saponins	+	+
Steroids	-	-
Triterpenoids	+	+

Caption : (+) detected ; (-) Undetectable

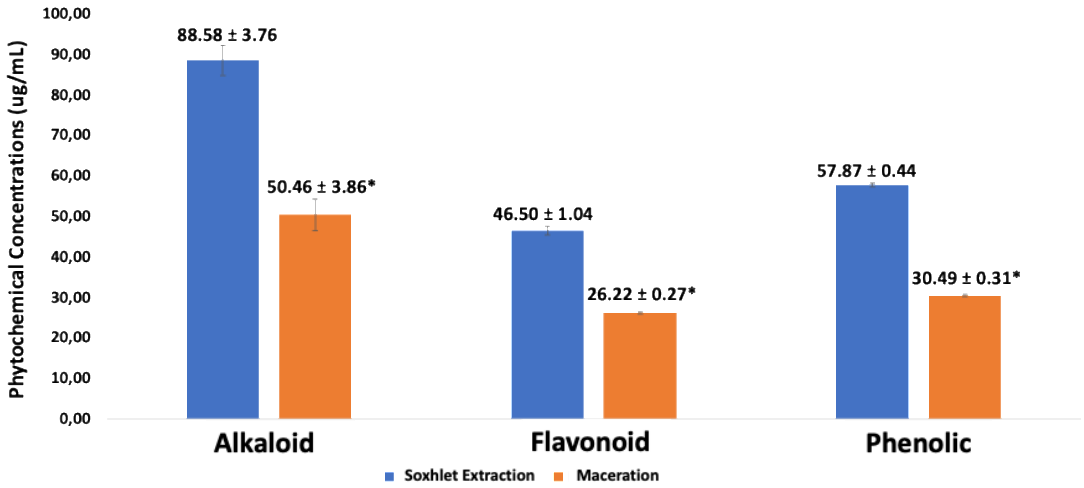

Figure 1. Concentrations of alkaloids, flavonoids, and phenolics in the maceration and Soxhlet extracts of the Purnajiwa fruit (*Kopsia arborea*). The asterisk (*) indicates a significant difference ($p < 0.05$) between the Soxhlet and maceration extract.

The quantification of alkaloids, flavonoids, and phenolic compounds in both extracts showed differences in compound levels (Figure 1). Extraction by the Soxhlet method yielded higher alkaloids, flavonoids, and phenolic compounds than maceration. Other studies, such as those on *Urtica dioica*, have shown that Soxhlet extraction is capable of producing a higher total amount of phenols and flavonoids than maceration (Horozić *et al*., 2023). The application of the Soxhlet technique for extraction can maintain higher temperatures and continuous solvent circulation, which improves the extraction efficiency of polar and heat-sensitive compounds. Maaiden *et al*. showed that Soxhlet extraction resulted in a higher polyphenol extract than maceration (Maaiden *et al*., 2022).

The difference in extraction efficiency between the maceration and Soxhlet extraction methods is estimated to be influenced by several factors, such as solvent dynamics, temperature control, and the continuous extraction process. In Soxhlet extraction, a solvent circulation mechanism allows the thorough dissolution of bioactive compounds from the plant matrix until it is completely saturated, thus facilitating a more efficient extraction process (Horozić *et al*., 2023). This is in contrast to maceration, which relies on soaking plant material

in solvents at room temperature, often leading to incomplete extraction (Rachmaniah *et al*., 2021). In addition, heating during Soxhlet extraction can increase the solubility and diffusion of phenolic compounds into solvents (Iftikhar *et al*., 2022). However, it should be noted that heating can also degrade phytochemical compounds. In addition, the special design of the Soxhlet apparatus allows repeated contact between plant material and fresh solvents, thereby improving the mass transfer and dissolution of bioactive molecules from the plant matrix (Ghanimi *et al*., 2022; Raghu & Velayudhannair, 2023).

Plants of the genus *Kopsia* are known to produce alkaloid compounds. In this study, alkaloids in both extracts were specifically identified using GC-MS. Table II lists the alkaloid compounds identified in both extracts. There were 13 compounds in the Soxhlet extraction and 12 compounds in the maceration extract. However, the percent area under the curve (AUC) value of these compounds showed that maceration extracts (14.85%) had a larger area value of alkaloid compounds than Soxhlet extraction (7.05%).

In this study, Soxhlet extraction yielded a higher total alkaloid content in the quantitative assays, whereas GC-MS analysis indicated a larger percentage area of alkaloids in the macerated extract. This discrepancy can be explained by the inherent limitations of GC-MS, which primarily detects volatile and thermally stable compounds. Certain alkaloids present in the Soxhlet extract may not have been detected because they are less volatile or decompose upon heating, even though their total concentrations are higher. Moreover, the area under the curve (AUC) values generated by GC-MS were calculated relative to the total number of peaks detected.

Aspidospermidine is an alkaloid compound with the largest area value (5.80%) that appears in the maceration extraction. Aspidospermin is an alkaloid compound widely found in the plant genus *Aspidosperma*. This compound has a distinctive structural framework. Some studies have shown the various pharmacological activities of aspidospermidin, such as aspidospermidin-17-ol from *Monascus purpureus*, with bioactive activities, including antiviral, anti-inflammatory, immunomodulatory, and anticancer activities (Teiba *et al*., 2024).

Table II. Alkaloid Compounds Identified on Chromatograms (GC-MS) in Macerated and Soxhlet Extraction Extracts of the Ripe Fruit (*Kopsia arborea* Blume.)

No.	Compound Name	Extraction Method			
		Soxhletasi		Maceration	
		RT	AUC	RT	AUC
1.	*Pyridine, 3-ethyl-*	4.223	0.92	3.956	0.64
2.	*2H-Pyrazole-3-carboxylic acid, 2-methyl-*	-	-	7.838	0.09
3.	*Ethanone, 1-(1-ethyl-1H-pyrazol-yl)-*	12.765	0.76	-	-
4.	*1H-Indole-5-ol*	-	-	13.762	0.09
5.	*[1,2,5]Oxadiazolo[3,4-b][1,4]diazocin-5,7(4H,6H)-dione, 8,9 dihydro*	14.223	0.11	-	-
6.	*Aspidospermidine, 1,2-didehydro-,(5.alpha.,12.beta.,19.alpha.)-*	-	-	21.726	1.87
7.	*3,5-Dimethyl-1-[4-(1H-pyrrol-1-yl)phenyl]-1H-pyrazole*	23.019	0.12	-	-
8.	*Quebrachamine*	-	-	23.939	1.71
9.	*Aspidospermidine-3-carboxylic acid, 2,3-didehydro-, methyl ester, (5.alpha.,12.beta.,19.alpha.)-*	-	-	25.003	5.80
10.	*Aspidofractinine-3-carboxylic acid , methyl ester, (2.alpha.,3.alpha. ,5.alpha.)-*	25.030	1.08	-	-
11.	*4,6 -Dimethyl -2-pyrimidone*	25.290	0.53	-	-

No.	Compound Name	Extraction Method			
		Soxhletasi		Maceration	
		RT	AUC	RT	AUC
12.	*Indeno[1,2-b]quinoline, 11-(4-pyridylmethylene)-*	25.487	1.90	-	-
13.	*11-Benzyl-2-methyl-2,3,4,5,6,11-hexahydro-azocino[3,4-b]indole-1-one*	25.792	0.26	-	-
14.	*1H-Indole, 3-(5-methyl-2-(pyridin-3-yl)thiazol-4-yl-*	26.032	0.11	-	-
15.	*1,2-Benzisothiazole,3-(hexahydro- 1H-azepin-1-yl)-, 1,1-dioxide*	26.589	0.35	-	-
16.	*Aspidofractinine-3-carboxylic acid, methyl ester, (2.alpha.,3.alpha.,5.alpha.)-*	26.828	0.39	25.071	1.49
17.	*11-Butyl-8-methoxy-11H-indolo[3,2-c]quinoline*	-	-	25.209	1.42
18.	*Aspidofractinine-3-carboxylic acid 1-formyl-17-methoxy-, methyl ester, (2.alpha.,3.beta.,5.alpha.)-*	-	-	26.843	0.73
19.	*1-[3-Amino-4-(pyridin-4-yl)-5H,6H1-[3-Amino-4-(pyridin-4-yl)-5H,6Hthanone*	-	-	27.501	0.41
20.	*Benzenamine, 4,4'-[2-(4-methoxyphenyl)-1H-imidazole-4,5-diyl]bis[N,N-dimethyl-*	-	-	27.690	0.60
21.	*2-Phenyl-3-pyridin-4-yl-7-(pyridin-4 ylmethylidene)-3,3a,4,5,6,7-hexahydro-2H-indazole*	28.219	0.52	-	-
22.	*Oxayohimban-16-carboxylic acid, 16,17-didehydro-19-methyl-, methyl e ster,(19.alpha.,20.alpha.)-*	28.966	0.22	29.168	0.97

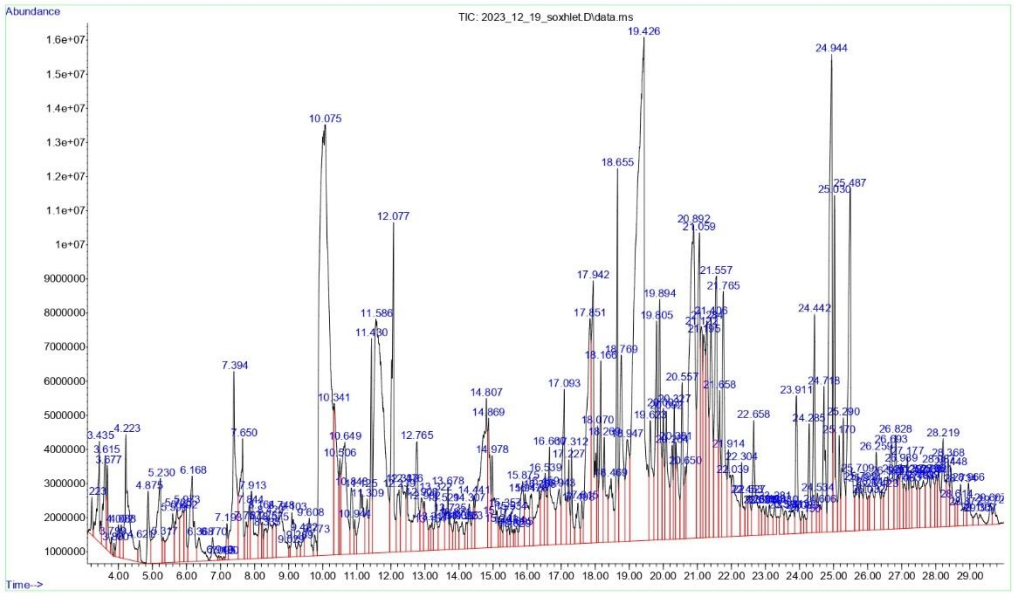

Figure 2. Chromatogram (GC-MS) on a soxhletation extract of the deceased fruit (*Kopsia arborea* Blume.)

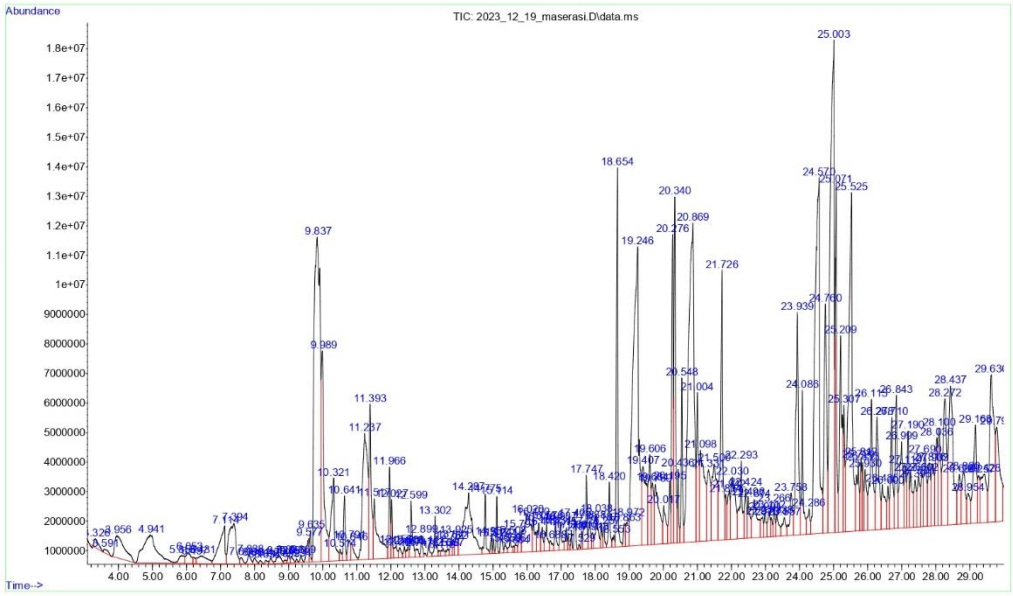

Figure 3. Chromatogram (GC-MS) on the macerated extract of the deceased fruit (*Kopsia arborea* Blume.)

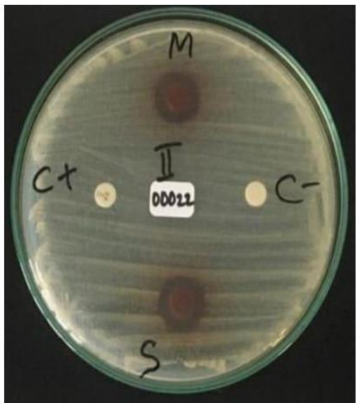

Figure 4. Results of antibacterial assay soxhletation (S) and maceration (M) extract of purnajiwa (*Kopsia arborea* Blume) fruit

The results of the measurement of the diameter of the inhibition zone from the extract of the purnajiwa fruit (*Kopsia arborea* Blume.) against the growth of MRSA (Methicillin-Resistant *Staphylococcus aureus*) bacteria are presented in Figure 4 and Table III. The antibacterial activity test showed that the macerated extract produced a larger inhibition zone than Soxhlet extraction. The results of this study show that the high total content of alkaloids, phenolics, and flavonoids in Soxhlet extract is not in line with the increase in antibacterial activity produced. It is estimated that certain compounds with antibacterial activity are degraded when the Soxhlet method is used, which changes their chemical structure and decreases their sensitivity to bacteria. In contrast, maceration at room temperature results in extracts with a lower total phytochemical content but a stable and intact composition of active compounds. Minor compounds or secondary metabolites with MRSA-inhibiting activity are likely to be better preserved during maceration, resulting in higher antibacterial effectiveness, even though the total number of compounds extracted is lower. In addition, maceration can maintain a natural balance of phytochemicals that favors synergistic effects.

Table III. Measurement Results of Inhibition Zone Diameter

Replication	Inhibitory Zone Diameter (mm)		
	Soxhlet	Maceration	C-
1	11.43	12.74	0
2	11.98	12.28	0
3	11.66	12.26	0
Average ± SD	11.69±0.28	12.61±0.27*	0

Remarks: SD = standard deviation ; C- = Negative Control (5% DMSO Solution)
The asterisk (*) indicates a significant difference ($p < 0.05$) between the Soxhlet and maceration extract.

In this study, the inhibitory activity of purnajiwa (*Kopsia arborea B*lume.) extracts was classified as strong, with inhibition zone diameters ranging from 10–20 mm. These results indicate that the purnajiwa fruit extract demonstrates promising potential as a natural antibacterial agent, warranting further investigation to support its application in addressing antibiotic resistance. A recent comprehensive review highlighted that both flavonoids and alkaloids are among the most effective phytochemicals against MRSA, acting through mechanisms such as membrane disruption and efflux pump inhibition (Liang *et al*., 2022).

Alkaloids, phenolics, and flavonoids contribute to antibacterial activity against MRSA through distinct but complementary mechanisms. Alkaloids disrupt bacterial cell membrane integrity and interfere with essential metabolic pathways, such as peptidoglycan synthesis, thereby weakening their structural stability (Prakoso *et al*., 2020). Phenolic compounds exert broad-spectrum effects by forming complexes with bacterial components, altering membrane permeability, and disrupting the biosynthesis of structural components (Elmaidomy *et al*., 2023; Rahayu *et al*., 2020). Flavonoids further enhance anti-MRSA efficacy by modulating key target proteins, such as PBP2a, increasing susceptibility to β-lactam antibiotics, reducing membrane fluidity, and impairing bacterial energy production (Jieputra *et al*., 2024; Rahayu *et al*., 2020).

CONCLUSION

This study demonstrates that the extraction method significantly affects the yield, phytochemical content, and antibacterial activity of the Purnajiwa (*Kopsia arborea* Blume) fruit extract. The Soxhlet method resulted in a higher yield and total content of alkaloids, phenolics, and flavonoids than maceration, supported by the identification of 13 alkaloid compounds through GC-MS analysis. However, macerated extracts consistently showed larger diameters of inhibition zones against Methicillin-Resistant *Staphylococcus aureus* (MRSA), indicating better antibacterial activity.

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