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Antioxidant Activity Test with DPPH Method and Identification of Bioactive Compounds of *Kesambi* Stem Bark Extract (*Schleichera oleosa (Lour.) Oken*) Using UPLC-QToF-MS/MS

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Abstract

Free radicals can cause oxidative stress that contributes to various degenerative diseases, so safer natural antioxidant sources are needed. *Kesambi (S. oleosa (Lour.) Oken) stem bark* is traditionally used by the people of Maru Village, Alor Regency, as a herbal remedy for postpartum maternal care, but scientific studies are still limited. This study aimed to determine the antioxidant activity of *kesambi* stem bark extract using the DPPH method, identify bioactive compounds using UPLC-QToF-MS/MS, and evaluate its potential as a traditional herbal ingredient. The study was conducted through maceration extraction using 70% ethanol, followed by phytochemical testing, testing antioxidant activity using the DPPH method at a wavelength of 517 nm, and identification of bioactive compounds using UPLC-QToF-MS/MS. The results of the study showed that the phytochemical test of *kesambi* stem bark extract was positive for alkaloids, flavonoids, tannins, saponins, phenolics, and triterpenoids. The antioxidant activity of the extract was classified as very strong with an IC_{50} value of 6.56 ppm, close to ascorbic acid (IC_{50} 5.68 ppm). UPLC-QToF-MS/MS analysis successfully identified 12 bioactive compounds dominated by phenolics, flavonoid glycosides, condensed tannins, lignans, and steroid saponins that have the potential to act as antioxidants. The conclusion of this study showed that *kesambi* stem bark extract has very strong antioxidant activity and contains secondary metabolites and bioactive compounds that have the potential to support its use as a traditional herbal ingredient.

Keywords: Antioxidants; DPPH; *kesambi* bark; phytochemicals; UPLC-QToF-MS/MS.

INTRODUCTION

Free radicals are highly reactive molecules or atoms due to their unpaired electrons (Botahala, 2024a). These compounds can be produced by normal body metabolism as well as external sources such as pollution, cigarette smoke, and exposure to environmental chemicals. Free radicals can cause oxidative stress that damages cells, proteins, lipids, and DNA. They can also lead to diabetes, heart disease, premature aging, and various other degenerative diseases (Botahala, 2024a). The body needs antioxidants that can neutralize free radicals by donating electrons, stopping the oxidation chain reaction. Antioxidants can come from natural or synthetic sources. However, long-term use of synthetic antioxidants can cause side effects, so using alternative natural antioxidants is safer (Kurang et al., 2020) .

Indonesia is renowned for its abundant natural resources, including a number of medicinal plants that have been traditionally used by local residents to treat various ailments (Botahala & Djasibani, 2025). Knowledge of these medicinal plants is usually passed down traditionally and forms an important part of traditional medicine systems (Botahala et al., 2024). Plants used as medicine are known to contain secondary metabolite compounds such as phenolics, triterpenoids, flavonoids, alkaloids, and saponins which play an important role in health. (Botahala, 2024b).

One type of plant that has great potential for use as a medicine is *kesambi* (*Schleichera oleosa (L.) Oken*). In Maru Village, Pulau Pura District, Alor Regency, local residents use a decoction of kesambi bark as a herbal remedy for postpartum care. This concoction is believed to aid the postpartum recovery process. through its antioxidant properties, which can reduce oxidative stress caused by an imbalance of free radicals in the body. The postpartum period is characterized by the discharge of lochia as part of the uterine healing process (Puspasari and Istiyati, 2024). Therefore, the support of antioxidant and anti-inflammatory compounds is needed to accelerate the body's recovery process. Several studies indicate that various parts of the kesambi plant have

pharmacological potential. *Kesambi* seeds are used to treat skin problems, the bark is used to reduce fever and treat boils, and the leaves function as antibacterials and anthelmintics (Yeti Mariyah, Kholifah Holil, 2020). In addition, in phytochemical tests for *kesambi* bark, ethanol extracts were reported to contain bioactive compounds such as flavonoids, alkaloids, tannins, saponins, steroids/triterpenoids, and phenolics such as 1,2-benzenedicarboxylic acid and squalene (Dewi, 2021). These compounds are known to have various pharmacological activities such as antioxidants, antibacterials, antifungals, and anti-inflammatory. Thus, it is important to explore natural ingredients that are safer and have been used for generations.

Based on these conditions, scientific identification of bioactive compounds is important to strengthen the basis for the use of medicinal plants. Research conducted by Taek *et al.*, (2024) on *Alstonia spectabilis*, which is traditionally used by the people of Nusa Tenggara as an anti- malarial drug, using UPLC-QToF-MS/MS analysis successfully discovered alkaloid bioactive compounds in the bark extract, one of which is *villastonie.* In Rika Widianita's research (2023), this method can identify 38 bioactive compounds in just one analysis, which shows the efficiency of this tool in natural material research.

Although *kesambi* has been extensively researched, scientific studies on the antioxidant activity and bioactive compound profile of kesambi bark are still limited. Furthermore, the content of secondary metabolites in plants can be influenced by environmental factors such as light, temperature, humidity, soil type, and altitude, so the same plant can have different compound contents depending on where it grows. This study aimed to determine the strong antioxidant properties in terms of antioxidant activity to determine the inhibitory power (IC_{50}) against free radicals. (Botahala et al., 2025) , and identified bioactive compounds in *kesambi* stem bark extract from Maru Village, Pulau Pura District, Alor Regency , East Nusa Tenggara Province, Indonesia. It is hoped that the results of this research can be used as an alternative natural herbal ingredient to support postpartum recovery.

MATERIALS AND METHODS

Materials and tools

The materials used in this study were: Bark of *kesambi* tree (*Schleichera oleosa (Lour.) Oken*) (Figure 1) , 70% ethanol solvent, syringe filter (0.22), aluminum foil, distilled water, Mg powder, concentrated HCl, 4 M HCl, Dragendroff reagent, Mayer reagent, 1% $FeCl_3$, glacial acetic acid, concentrated H_2SO_4, 1% NaOH, methanol, DPPH reagent (*2,2-diphenyl1-1 picrylhydrazyl*), ascorbic acid (vitamin C), distilled water, dichloromethane, ethyl acetate, water, acetonitrile. Tools The tools used in this study were: knives, pestles and mortars, slicing boards, analytical scales, Erlenmeyer flasks, measuring flasks, measuring cups, stirring rods, rotary evaporators, No. 60 mesh sieves (0.25 mm), test tubes, test tube racks, dropper pipes, measuring pipettes, beakers, glass funnels, micropipettes, UV-Vis spectrophotometers, UPLC-QTOF-MS/MS.



Figure 1. *Kesambi* bark.

Method

Sample making

The bark of the *kesambi* tree is taken from the second branch and cut into small pieces, washed with running water to remove dust and dirt, then dried at room temperature for several days until the water content is reduced. The drying process is carried out in a shaded place so that the active compounds are not damaged by direct sunlight exposure. Once dry, the kesambi bark is ground using a pestle and mortar to form a simple powder and sieved using a No. 60 (0.25 mm) mesh sieve, in order to obtain a uniform sized powder and facilitate the release of active compounds during the extraction stage . The obtained simplicia powder was macerated using 70% ethanol solvent for 30 minutes. The mixture was then filtered using filter paper to separate the residue and filtrate. The obtained filtrate was concentrated using a rotary evaporator at 50°C until the solvent evaporated for 2 hours, after which it was poured into a cup and aired with a fan until the solvent disappeared until the obtained extract resembled a paste. This extract was used for phytochemical tests, antioxidant activity and metabolite profile analysis with UPLC-QToF-MS/MS.

Quantitative Test (Extract Yield)

A total of 5 g of powdered *kesambi* bark was placed in a maceration container, then 500 mL of ethanol was added. The maceration process was carried out at room temperature with occasional stirring for 30 minutes to ensure even contact between the solvent and the sample. The mixture was then filtered to separate the filtrate from the residue. The filtered residue was then added to fresh 70% ethanol solvent and macerated again. The filtering and solvent replacement process was repeated until the solvent obtained was completely clear.

All the filtrates obtained were collected and then air-dried until all the ethanol solvent evaporated, resulting in a thick extract. The yield of the extract was calculated by comparing the weight of the thick extract obtained with the initial weight of the powdered simplicia. A good yield is 100% (Hapsari, 2023).

The % yield formula as seen in the formula below:

$$\%Yield = \frac{Weight\ of\ the\ extract\ obtained}{Initial\ weight\ of\ the\ crude\ drug\ powder} \times 100$$

Qualitative Test **(** Phytochemical Test **)**

Testing procedure for *kesambi* stem bark was carried out based on the procedures carried out by (Botahala et al., 2020), (Botahala et al., 2024), and (Sanni et al., 2024) with several modifications for this study.

- ***Flavonoid Test***

Weigh 100 mg of the extract, add 2 mL of ethanol, and shake until homogeneous. Add Mg powder and 5 drops of concentrated HCl. The formation of a red, yellow, or orange color indicates the presence of flavonoids.

- ***Alkaloid Test***

Weighed 100 mg of extract dissolved in 5 mL of 4 M HCl. The obtained solution was divided into 2 test tubes. The first tube was added with 3 drops of Dragendorff's reagent and the second tube was added with 3 drops of Mayer's reagent. The formation of an orange precipitate in the first tube and a yellow precipitate in the second tube indicates the presence of alkaloids.

- ***Tannin*** **Test**

Weigh 100 mg of extract, add 2 mL of distilled water, stir, and add 3 drops of 1% $FeCl_3$. The formation of a blue or blackish green color indicates the presence of tannins .

- ***Saponin*** **Test**

Weigh 100 mg of extract, put it into a test tube, add 2 mL of hot, cooled water and shake for 10 seconds. If a stable foam forms for 10 minutes at a height of 1–2 cm, this indicates the presence of saponins.

- ***Steroid*** **/ Triterpenoid Test**

Weigh 100 mg of the extract, add 5 mL of chloroform solution, then add 10 drops of anhydrous acetic acid and 3 drops of concentrated sulfuric acid. A color change of red or brown indicates the presence of terpenoids (triterpenoids), while a color change to blue, purple, or green indicates the presence of steroids.

- ***Phenolic*** **Test**

Weigh 100 mg of extract, dissolve with 70% ethanol to a volume of 2 mL, add 3 drops of 1% $FeCl_3$ reagent, resulting in a color change from light green to blackish, indicating a positive result containing phenolic compounds.

Bioactive Compounds using UPLC-QToF-MS/MS

The procedure for identifying compounds using the UPLC-QToF-MS/MS technique was carried out based on the procedure carried out by Taek *et al.*, (2024) with several modifications for this study.

The analysis was performed by inserting a 5 µL sample into the chromatography system. The compound separation process took place in a column containing a C18 stationary phase (octadecyl silica). The mobile phase consisted of phase A (water with 0.1% formic acid) and phase B (acetonitrile with 0.1% formic acid), using a stepwise elution system. Changes in the composition of the mobile phase during the analysis affected the elution of compounds in the column.

The eluted compounds were then transferred to a mass spectrometry system. At this stage, the sample was ionized using a positive-mode ESI source, which produced positively charged ions. These ions were then separated according to their mass-to-charge ratio (m/z) using a Q-ToF analyzer. The resulting separation was detected and processed using MassLynx software version 4.1 to obtain mass spectral data for each chromatogram peak.

Compound identification was performed based on the mass spectra data obtained. Observed parameters included retention time (Rt), peak height, and area. Each chromatogram peak was considered to represent a single compound. Molecular formula determination was performed using the ChemSpider database, taking into account that the ESI (+) process involves the addition of one proton (H^+), so the measured m/z value was corrected by subtracting the proton mass.

Antioxidant Activity Test using the DPPH method

DPPH solution with a concentration of 0.1 mM was prepared by dissolving 3.94 mg of DPPH powder in 100 mL of methanol pa. The stock solution of *kesambi* bark extract was prepared by dissolving 100 mg of extract in 100 mL of methanol to obtain a concentration of 1000 ppm. Then, the solution was diluted into several concentration variations, namely 1.25; 2.5; 5; and 10 ppm in a 5 mL volumetric flask . From the 1000 ppm stock solution, 6.25 µL; 12.5 µL; 25 µL; and 50 µL were taken, respectively, then methanol pa was added until it reached the 5 mL mark.

A total of 1 mL of sample solution from each concentration was mixed with 3 mL of 0.1 mM DPPH solution, then homogenized and incubated for 30 minutes at room temperature in the dark. The blank was made from a mixture of 1 mL of methanol and 3 mL of 0.1 mM DPPH solution, while the positive control used ascorbic acid solution with the same treatment. The test was carried out three times (triplicate). After incubation, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. The percentage of inhibition was calculated using the equation:

$$\%\ Inhibition = \frac{A_o - A_s}{A_o} \times 100$$

A_o= Is the blank absorbance
A_s= Is the absorbance of the sample.

The percentage inhibition value for each concentration was calculated as the average of three repetitions, which was then analyzed using linear regression with sample concentration (ppm) on the X-axis and percent inhibition on the Y-axis according to the formula:

$$y = bx + a$$

The IC_{50} value is determined by entering y=50 into the linear regression formula. IC_{50} is the sample concentration that has the ability to inhibit 50% of DPPH free radicals. (Botahala, 2024a).

RESULTS

Kesambi bark extract yield test.

Extraction of *kesambi* bark produces 1 gram of extract with a yield of 20%, which shows that the extraction process is quite effective in extracting secondary metabolite compounds.

Table 1. Yield of *Kesambi* Stem Bark Extract.

Sample	Weight of Simple Ingredients	Weight of thick extract	Yield
(*S. oleosa*) Stem Bark	5 grams	1 gram	20%

Phytochemical Test Results

Phytochemical tests were conducted to determine the presence of secondary metabolites in kesambi bark extract. This test was conducted qualitatively to detect color changes using several reagents to detect specific compound classes such as alkaloids, flavonoids, tannins, saponins, phenolics, steroids, and triterpenoids, as shown in Table 2.

Table 2. Results of phytochemical tests on *kesambi* stem bark.

No	Compound	Analysis results
1	Phenolic	+
2	Saponin	+
3	Steroid	-
4	Triterpenoid	+
5	Alkaloid	+
6	flavonoid	+
7	Tannin	+

Bioactive Compounds using UPLC-QtoF MS/MS

Based on the analysis results, the *kesambi* bark extract showed 12 peaks at different retention times , as seen in Figure 2. Each peak indicates the presence of compounds that were successfully separated and detected by the UPLC-QtoF-MS/MS instrument. Differences in peak height indicate differences in intensity or relative abundance of compounds in the sample. The results of the analysis of bioactive compounds in the kesambi bark extract include phenolics, condensed tannins, flavonoid glycosides, lignans, phenolic glycosides, pyrazole derivatives, urea-derived nonsteroidal steroids, lipids, and steroidal saponins. The compound that has the highest peak (3β,25R)-14-Hydroxyspirost-5-en-3-yl6-deoxy-α-L-mannopyranosyl-(1→2)-[β-D xylopyranosyl-(1→4)]-β-D-glucopyranoside, with a retention time of 17.05 minutes and an area of 73.87%, which is included in the steroid saponin group, as listed in Table 3.

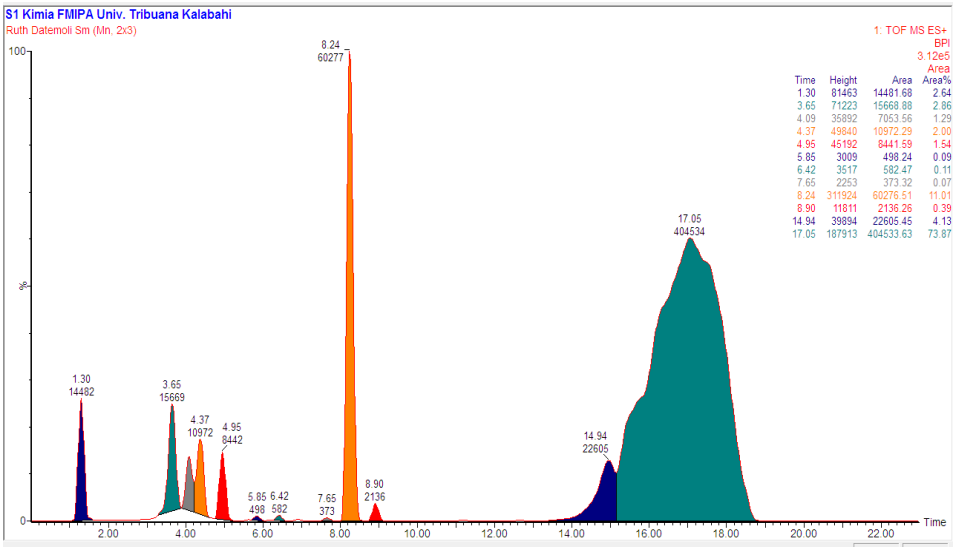


Figure 2. Chromatogram of UPLC QTOF MS/M analysis results.

Table 3. Data from the interpretation results of the UPLC-QToF-MS/MS chromatogram of the *kesambi* stem bark extract compound (S chleichera oleosa (Lour.) Oken)

Rt	%Area	Measured mass/parent ion (m/z)	Product/daunther ion (m/z)	Molecular ion formula (MH)	Name compound	Group Compound
1.30	2.64	381,0822	380,305	$C_{17}H_{16}O_{10}$	9-hydroxy-7-oxo-7H-furo[3,2-g]chromen-4-yl β-D-glucopyranoside)	Phenolic (Furocoumarin glycoside)
3.65	2.86	1153,2614	1152,020	C60H48O24	Cinnamtannin B2	Condensed tannins
4.09	1.29	467,1951	466,395	$C_{21}H_{22}O_{12}$	Plantagoside	Flavonoid Glycosides
4.37	2.00	385,1622	384,428	$C_{22}H_{24}O_{6}$	Schisandrin C	Lignan
4.95	1.54	600,2644	599,630	$C_{28}H_{41}NO_{13}$	Girokolsid	Phenolic glycosides
5.85	0.09	217,1090	216,244	$C_{11}H_{12}N_4O$	(3-(4-Methylphenyl)-1H-pyrazole-5-carbohydrazide	Pyrazole
6.42	0.11	465,490	464,466	$C_{24}H_{32}O_{9}$	16-Glucuronide-estriol	Steroid
7.65	0.07	323,325	322,364	C19H18N2O3	Kebuzone	Non-Steroid
8.24	11.01	325,2284	324,425	$C_{16}H_{28}N_4O_3$	1-Azepanyl[4-(4-morpholinylcarbonyl)-1-piperazinyl]methanone	Urea (Carbamide).
8.90	0.39	348,2746	347,507	C19H33N5O	1-[6-(Dimethylamino)-3-pyridinyl]-3-[3-methyl-2-(4-methyl-1-piperidinyl)butyl]urea	Urea Derivative / Piperidine-Urea Derivative .
14.94	4.13	852,7852	851,439	$C_{57}H_{102}O_4$	Triglycerides (1-linolenoyl-2-oleoyl-3-linoleoyl-glycerol)	Lipids (Triglycerides / Unsaturated Glycerolipids)
17.05	73.87	872,7852	871,027	$C_{44}H_{70}O_{17}$	(3β,25R)-14-Hydroxyspirost-5-en-3-yl 6-deoxy-α-L-mannopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranoside	Steroidal Saponins

Results of *Kesambi* Stem Bark Extract

The results of the antioxidant activity test of *kesambi* stem bark extract (*S. oleosa (L.) Oken) using the DPPH method showed a decrease in the absorbance value of the DPPH* solution after the addition of the sample extract. The decrease in absorbance increased with increasing extract concentration, which indicates that *kesambi* stem bark extract has the ability to reduce DPPH free radicals. Based on the results of the inhibition percentage calculation, it is known that the antioxidant activity of the extract increases at higher concentrations.

Table 4. Results of Antioxidant Activity Test of *Kesambi* Bark Extract (*S. oleosa (L.) Oken*).

Concentration (ppm)	(Average) %Inhibition
1.25	10.09
2.5	17.49
5	37.06
10	77.28

After obtaining the percentage of inhibition from each concentration with the equation y = ax + b, it was determined by linear regression calculation on the IC_{50} value of *kesambi* extract. The determination of the IC_{50} value was carried out using linear regression analysis where x is the concentration (ppm) and y is the percentage of inhibition as seen in Figure 3.

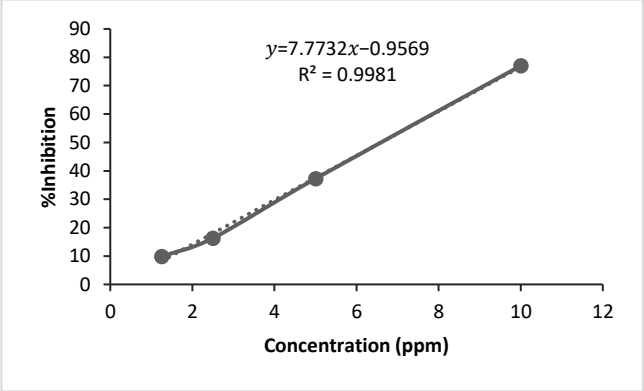


Figure 3. Linear regression curve for determining $IC_{50\ concentration}$.

The test results showed that the inhibition percentage of *kesambi* bark extract increased with increasing concentration, from 10.09% at 1.25 ppm to 77.28% at 10 ppm. Regression analysis produced the equation y = 7.7732x − 0.9569 with an R^2 value of 0.9981, which indicates a very strong relationship between extract concentration and inhibition percentage. The IC_{50} value obtained was 6.56 ppm, so the *kesambi* bark extract is classified as having very strong antioxidant activity (IC_{50} < 50 ppm).

The results of this study also show that the antioxidant activity of kesambi stem bark extract is superior to that of several previous studies. This high antioxidant activity is supported by the content of secondary metabolites such as flavonoids, phenolics, tannins, alkaloids, saponins, and triterpenoids, as well as

bioactive compounds identified through UPLC-QTOF-MS/MS analysis. These findings strengthen the potential of kesambi stem bark as a source of natural antioxidants and support its use in traditional medicine, particularly to aid postpartum maternal recovery.

DISCUSSION

Kesambi bark extract yield test.

S. oleosa stem bark using 70% ethanol produced 1 gram of extract with a yield of 20%. The yield value is an important parameter in the extraction process because it describes the efficiency of the solvent in extracting compounds found in plant materials. The higher the yield value obtained, the greater the number of components successfully extracted from the simplex, although the amount of yield is not always directly proportional to the level of active compounds or biological activity of the extract.

A yield of 20% indicates that the extraction process used was quite effective in dissolving various chemical components found in the bark of the kesambi tree. The high yield is thought to be influenced by several factors, including the size of the simplex particle, the solvent-to-material ratio, the extraction time, the extraction temperature, and the characteristics of the solvent used. A smaller simplex particle size will increase the contact surface area between the material and the solvent, thereby accelerating the diffusion of secondary metabolites into the solvent. Similarly, the use of an adequate solvent ratio and optimal extraction time will increase the efficiency of mass transfer during the extraction process.

The use of 70% ethanol as a solvent offers advantages due to its polar-semipolar properties, which allow it to dissolve various secondary metabolites with a wide range of polarities. The combination of ethanol and water enhances the solvent's penetration into plant tissue, resulting in a more effective release of bioactive compounds. 70% ethanol is known to be highly suitable for extracting phenolic compounds, flavonoids, tannins, saponins, glycosides, and various other secondary metabolites that contribute to the extract's biological activity.

However, the yield cannot be the sole indicator of extract quality. High yields may reflect the extraction of non-active compounds, such as sugars, proteins, or other polar compounds. Therefore, evaluation of extract quality needs to be supported by phytochemical characterization, compound identification using analytical instruments, and biological activity testing. In this study, the high yield, accompanied by the presence of various secondary metabolites and a very strong antioxidant activity value (IC_{50} = 6.56 ppm), indicates that the extraction method applied is not only quantitatively efficient but also effective in producing an extract rich in bioactive compounds.

Overall, the extract yield of 20% indicates that the extraction method using 70% ethanol is an effective method for obtaining secondary metabolite compounds from the bark of *kesambi* (*S. oleosa*). These results provide a strong basis for the development of further research, both in phytochemical tests, identification of bioactive compounds, and testing the antioxidant activity of kesambi bark extract.

Phytochemical test

Phytochemical test results showed that 70% ethanol extract of *kesambi* (*S. oleosa*) stem bark contains various types of secondary metabolites, namely alkaloids, flavonoids, tannins, saponins, phenolics, and triterpenoids, while steroid compounds were not detected. The presence of these secondary metabolites indicates that *kesambi* stem bark extract has a fairly high potential for biological activity, especially as a source of natural antioxidant and anti-inflammatory compounds.

Flavonoids, phenolic compounds, and tannins are the main components responsible for antioxidant activity. These three groups of compounds are able to ward off free radicals through the mechanism of hydrogen atom or electron donation, thereby stopping oxidative chain reactions that can potentially damage cells. Furthermore, the hydroxyl groups found in phenolic and flavonoid compounds can also chelate transition metal ions that act as catalysts in free radical formation, further enhancing the extract's antioxidant capacity.

In addition, alkaloids are known to possess various pharmacological activities, such as antioxidant, antimicrobial, and anti-inflammatory properties. Saponins play a role in increasing cell membrane permeability, exhibit immunomodulatory activity, and help suppress inflammation. Meanwhile, triterpenoids have been widely reported to possess antioxidant, anti-inflammatory, hepatoprotective, and anticancer activities through mechanisms that inhibit oxidative stress and modulate various cell signaling pathways. Thus, the presence of these various secondary metabolites is thought to work synergistically to produce the biological activity of *kesambi* bark extract.

The results of this study align with previous studies that reported that kesambi bark contains various secondary metabolites, particularly flavonoids, tannins, saponins, alkaloids, phenolics, and triterpenoids. However, there were differences in the presence of steroid compounds compared to previous reports. These variations can be influenced by various factors, such as the environmental conditions where the plant grows, the age and maturity of the plant, the harvest season, the plant part used, the type of solvent, the solvent concentration, the extraction method, and the sensitivity of the phytochemical identification method used.

Overall, the phytochemical profile of the 70% ethanol extract of kesambi stem bark indicates that this plant is a potential source of secondary metabolites. The

dominance of phenolic compounds, flavonoids, and tannins, supported by the presence of alkaloids, saponins, and triterpenoids, is thought to contribute to the extract's antioxidant activity. Therefore, kesambi stem bark extract has the potential to be developed as a raw material for herbal and phytopharmaceutical products with antioxidant activity to help prevent cell damage caused by oxidative stress.

Bioactive Compounds Using UPLC-QtoF MS/MS

Analysis using the Ultra Performance Liquid Chromatography–Quadrupole Time-of-Flight Tandem Mass Spectrometry (UPLC-QToF-MS/MS) technique on 70% ethanol extract of *kesambi* (*S. oleosa*) stem bark successfully identified 12 secondary metabolite compounds classified into six main groups, namely phenolic compounds, condensed tannins, flavonoid glycosides, phenolic glycosides, lignans, and steroid saponins. Identification of these compounds provides a more comprehensive picture of the chemical composition of the extract compared to qualitative phytochemical tests, because the UPLC-QToF-MS/MS method is able to detect compounds based on molecular mass, fragmentation patterns, and retention times with a high level of sensitivity and accuracy.

The presence of various groups of secondary metabolites indicates that *kesambi* bark is a source of bioactive compounds that have the potential to be developed as raw materials for phytopharmaceuticals and herbal medicinal products. In general, phenolic compounds, flavonoids, tannins, lignans, and saponins are known to possess various biological activities, including antioxidant, anti-inflammatory, antimicrobial, antitumor, antidiabetic, hepatoprotective, and immunomodulatory properties. These biological activities are primarily related to their ability to inhibit the formation of reactive oxygen species (ROS), suppress oxidative stress, and modulate various cell signaling pathways that play a role in the inflammatory process and the development of degenerative diseases.

One of the compounds successfully identified is *9-hydroxy-7-oxo-7H-furo[3,2-g]chromen-4-yl β-D-glucopyranoside* , which belongs to the phenolic compound group. Phenolic compounds are known to have strong antioxidant activity because the presence of hydroxyl groups in their aromatic structure allows the donation of electrons or hydrogen atoms to free radicals, thereby inhibiting chain oxidation reactions. In addition, phenolic compounds are also reported to be able to inhibit the inflammatory process by reducing the production of pro-inflammatory cytokines and inhibiting the activation of transcription factors such as NF-κB.

Cinnamtannin B2, a compound belonging to the condensed tannin group, is a polyphenol with very high antioxidant activity. This compound has the ability to scavenge free radicals, inhibit lipid peroxidation, and protect cell membranes from oxidative damage. Several studies also report that Cinnamtannin B2 has anti-inflammatory, anticancer, and cardioprotective activities through mechanisms such as inhibiting oxidative stress and regulating various cell signaling pathways.

The flavonoid glycoside group is represented by *Plantagoside* , which is known to have strong antioxidant and anti-inflammatory activity. The presence of sugar groups in the flavonoid structure increases the compound's solubility in polar solvents, thereby affecting its bioavailability. In addition to acting as a free radical scavenger, Plantagoside has also been reported to inhibit the formation of inflammatory mediators such as nitric oxide (NO), prostaglandins, and various pro-inflammatory cytokines.

Schisandrin C, a lignan, is a secondary metabolite that has been extensively studied due to its broad pharmacological activity. Schisandrin C is known to possess high antioxidant activity by enhancing endogenous antioxidant systems, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Furthermore, this compound exhibits hepatoprotective, neuroprotective, anti-inflammatory, and antitumor effects, potentially supporting various therapeutic applications.

The presence of *Girokolsid*, a phenolic glycoside, further enhances the biological potential of *kesambi* bark extract. Phenolic glycoside compounds generally possess antioxidant and antimicrobial activity derived from the combination of phenolic structures with sugar groups, which increases the stability and solubility of the compounds in biological systems.

The analysis results showed that the compound with the highest abundance was *(3β,25R)-14-Hydroxyspirost-5-en-3-yl 6-deoxy-α-L-mannopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranoside*, with an area reaching 73.87% of the total ion chromatogram. This compound belongs to the steroid saponin group which is known to have various biological activities, such as anti-inflammatory, immunomodulatory, antitumor, antimicrobial, hypocholesterolemic, and antioxidant. The dominance of this compound indicates that steroid saponins are likely the main components that contribute greatly to the biological activity of kesambi bark extract. In addition to being able to interact with cell membranes, steroid saponins are also known to regulate immune responses and inhibit the formation of inflammatory mediators through various molecular mechanisms.

In addition to these main secondary metabolites, the analysis also detected the presence of several unsaturated lipid compounds. Unsaturated lipids are known to play a role in maintaining cell membrane integrity and have the potential to provide anti-inflammatory effects by regulating fatty acid metabolism and inhibiting the formation of inflammatory mediators, such as prostaglandins and leukotrienes. Although their concentration is relatively lower than that of steroidal saponins, the presence of these compounds is thought to contribute to the extract's biological activity.

Overall, the results of UPLC-QToF-MS/MS analysis showed that the 70% ethanol extract of kesambi stem bark has a very diverse composition of secondary metabolites with a dominance of steroidal saponins. The combination of phenolic compounds, condensed tannins, flavonoid glycosides, phenolic glycosides, lignans, steroidal saponins, and unsaturated lipids is thought to work synergistically in producing various biological activities, especially as antioxidants and anti-inflammatories. These findings strengthen the potential of kesambi stem bark as a source of natural bioactive compounds that are worthy of further development through pharmacology, toxicity research, and isolation of active compounds to support the development of natural-based phytopharmaceuticals.

Antioxidant Activity Test Results of *Kesambi* Stem Bark Extract

S. oleosa stem bark had excellent ability to capture free radicals. The inhibition percentage increased gradually with increasing extract concentration, namely from 10.09% at a concentration of 1.25 ppm to 77.28% at a concentration of 10 ppm. The increase in the inhibition percentage indicates a dose-response relationship (*dose-dependent*), where the higher the extract concentration, the greater the ability of the bioactive compounds contained therein to neutralize DPPH free radicals.

High antioxidant activity is thought to be closely related to the content of secondary metabolites identified through phytochemical tests and UPLC-QToF-MS/MS analysis. Flavonoids, phenolics, and tannins are known to be effective electron or hydrogen atom donors, thus reducing DPPH free radicals to a more stable form. This mechanism is characterized by a color change in the DPPH solution from purple to yellow due to the reduced number of free radicals. In addition, the presence of saponins, lignans, and various phenolic glycosides is also thought to contribute synergistically to the extract's antioxidant capacity through free radical scavenging mechanisms, inhibition of lipid peroxidation, and enhancement of the endogenous antioxidant defense system.

Linear regression analysis yielded the equation $y = 7.7732x - 0.9569$ with a coefficient of determination ($R^2 = 0.9981$). The R^2 value, which is very close to one, indicates that the relationship between extract concentration and inhibition percentage is very strong and almost all variations in inhibition percentage can be explained by changes in extract concentration. Thus, the increase in antioxidant activity occurs consistently with increasing extract concentration, so that the test results have a very good level of linearity.

Based on the regression equation, the IC_{50} value was obtained at 6.56 ppm. The IC_{50} value is the concentration of extract required to inhibit 50% of DPPH free radicals. The smaller the IC_{50} value, the higher the antioxidant capacity of a sample. Based on the classification of antioxidant activity, kesambi bark extract is included in the very strong antioxidant category because it has an IC_{50} value of less than 50 ppm. This value indicates that the extract only requires a relatively low concentration to provide a 50% free radical inhibitory effect, thus indicating excellent potential as a natural antioxidant source.

This powerful antioxidant activity is thought to be the result of the synergistic action of various secondary metabolites contained in the extract. Phenolic and flavonoid compounds act as *primary antioxidants* through proton or electron donation mechanisms, while tannins inhibit oxidation by chelating transition metal ions that trigger free radical formation. Saponins and lignans, on the other hand, contribute by inhibiting oxidative stress, protecting cell membranes, and modulating the endogenous antioxidant system. The synergy between these various bioactive compounds is thought to be the primary factor contributing to the highly potent antioxidant activity of *kesambi* bark extract.

CONCLUSION

Based on the results of research on antioxidant activity testing and identification of bioactive compounds from *kesambi* bark extract (*S. oleosa* (Lour.) Oken), it can be concluded that:

- The results of phytochemical screening showed that the kesambi bark extract contained several groups of secondary metabolites, namely alkaloids, flavonoids, tannins, saponins, phenolics, and triterpenoids, while the steroid group showed negative results.
- Identification results using UPLC-QToF-MS/MS revealed the presence of 12 bioactive compounds from several secondary metabolite groups, including phenolics, flavonoids, condensed tannins, lignans, steroidal saponins, and natural lipids. These compounds are known to possess biological activity, primarily as antioxidants and anti-inflammatory agents.
- *Kesambi* bark extract has very strong antioxidant activity based on the DPPH method, with an IC_{50} value of 6.56 ppm. This value indicates that the extract is able to effectively scavenge DPPH free radicals.

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REFERENCES

Botahala, L. (2024a). *Jamu Ku-He-Se (Kunyit - Jahe - Serai)* (M. Karbeka & M. Sari (eds.); 1st ed.). Get Press Indonesia. https://www.getpress.co.id/product/jamu-ku-he-se-kunyit-jahe-serai

Botahala, L. (2024b). *Orasi Ilmiah : Jamu KU-HE-SE, Biofarmaka Yang Nyaris Terlupakan*. https://lothbotahala.wordpress.com/wp-content/uploads/2025/12/orasi-ilmiah-jamu-ku-he-se-biofarmaka-yang-nyaris-terlupakan.pdf

Botahala, L., & Djasibani, H. R. (2025). *Manfaat Jamu KU-HE-SE bagi Kesehatan: Solusi Menjaga Sistem Imun* (S. Rohmah (ed.); 1st ed.). Detak Pustaka. https://detakpustakatoko.com/product/buku-manfaat-jamu-ku-he-se-bagi-kesehatan-solusi-menjaga-sistem-imun/?srsltid=AfmBOoqSOsDuFRyvNog-1uHg3mJJHRMg87DKpcplKGBGEyKQyzKLR5Ed

Botahala, L., Djasibani, H. R., Karbeka, M., & Nahak, M. H. (2025). Identification of Jamu Ku-He-Se as an Alternative Health Supporting Ingredient. *Biology, Medicine, & Natural Product Chemistry*, *14*(1), 9–16. https://doi.org/10.14421/biomedich.2025.141.9-16

Botahala, L., R. Djasibani, H., F. Kafolamau, S., & Likma, Y. (2024). Determination of Phytochemicals and Antioxidants Activity in Herbs Spices of Kitchen. *Journal of Science and Technology*, *16*(2), 1–9. https://doi.org/10.30880/jst.2024.16.02.001

Botahala, L., Sukarti, Arifuddin, W., Arif, A. R., Ischaidar, Arafah, M., Kartina, D., Armah, Z., Yasser, M., Pratama, I., Patarru, O., Santi, & Hamsah, H. (2020). *Deteksi Dini Metabolit Sekunder pada Tanaman (Early Detection of Secondary Metabolites in Plants)* (L. Botahala & W. Afrida (eds.); 1st ed.). Mitra Cendekia Media.

Dewi, Y. K. (2021). *Uji Aktivitas Antioksidan dari Ekstrak Kulit Batang Kesambi [Schleichera oleosa (Lour) Oken] Menggunakan Metode Ekstraksi Bertingkat*. *3*(1), 22–31. https://doi.org/10.20414/spin.v3i1.3020

Hapsari, D. (2023). *Uji Toksisitas Fraksi Aquadest, N-Heksana dan Etil Asetat Daging Buah Majapahit (Crescentia Cujete L) Terhadap Artemia Salina Leach dengan Metode BSLT (Brine Shrimp Lethality Test)*.

Kurang, R. Y., Koly, F. V. L., Kafolapada, D. I., Kimia, P. S., & Tribuana, U. (2020). Aktivitas Antioksidan Ekstrak Etil Asetat Daun Kelor (Moringa Oleifera L). *Ournal of Pharmaceutical Care Anwar Medika Artikel*, *3*(1), 13–21.

Puspasari, L., & Istiyati, S. (2024). *Perawatan pada ibu nifas normal*. *2*(September), 484–489.

Rika Widianita, D. (2023). Identifikasi Senyawa Antivirus SARS-COV-2 dari Ekstrak Etanol 96% Daun Jambu Biji (Psidium guajava) dengan Metode UPLC-MS/MS. *AT-TAWASSUTH: Jurnal Ekonomi Islam*, *VIII*(I), 1–19.

Sanni, J. A., Sanni, G. O., Awoniyi, R. R., Osanyinlusi, R., Richards, Y. E., Adesina, G. I., Adenuga, O. O., Apata, S. A., & Ekun, O. E. (2024). Effects of Processing on the Proximate Composition, Mineral Content and the Phytochemical Analysis of Groundnut Seeds (Arachis hypogeae). *Biology, Medicine, & Natural Product Chemistry*, *13*(1), 63–71. https://doi.org/10.14421/biomedich.2024.131.63-71

Taek, M. M., Ma, B., Muslikh, F. A., & Maulina, N. (2024). *Profil Metabolit dari Ekstrak dan Aktivitas Antimalaria Tablet yang Berasal dari Korteks Alstonia spectabilis*. *17*(2).

Yeti Mariyah, Kholifah Holil, dan M. A. A. (2020). *Uji Fitokimia dan Aktivitas Antioksidan Ekstrak Kesambi (Schleichera oleosa (Lour.) Oken) dengan Pelarut Metanol* (Vol. 2507, Issue February). skripsi Program Studi Biologi Fakultas Sains Dan TeknologiUniversitas Islam Negeri Maulana Malik Ibrahim Malang.

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