Extractive Content and Antioxidant Activity of Cajuput Bark (*Melaleuca cajuputi* Powell)

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Abstract

Both wood and non-wood industries generate large amounts of residues from processes like bark peeling and removal. In the cajuput oil industry, the waste products include distillation residues and cajuput bark. The diverse extractives and chemical components found in cajuput bark have the potential for enhanced value through various applications. This study aimed to determine the chemical components, extractive content (EC), and percentage inhibition of antioxidant activity (%IAA) of cajuput bark extract to explore more effective uses of this byproduct. Cajuput bark (*Melaleuca cajuputi* Powell) was sourced from a 26-year-old cajuput stand in Paliyan, Gunungkidul. The bark powder was extracted using a methanol-water solvent mixture at concentrations of 40% and 80%. Extraction was conducted in water baths at temperatures of 60°C and 100°C for 120 minutes. GC-MS identified several chemical components in the methanol extract of cajuput bark, including sugars (erythritol, D-arabinose, D-lyxose, adonitol, d-galactose, D-glucitol, and L-rhamnose), fatty acids (lactic acid, glycerol, glyceric acid, malic acid, and palmitic acid), and phenolic derivatives (gallic acid and protocatechuic acid). The average extractive content of the methanol extract of cajuput bark was 4.07%, and the percentage inhibition of antioxidant activity was 40.26%.

Keywords: cajuput, cajuput bark, chemical compounds, GC-MS, extractive content, antioxidant activity

Introduction

Cajuput (*Melaleuca cajuputi* Powell) is a native Indonesian plant utilised primarily for cajuput essential oil production. This plant is also valuable for reforestation and agroforestry due to its economic importance and its ability to thrive in poor environmental conditions (Kartikawati *et al*. 2014). While the leaves, twigs, and branches are used in the essential oil distillation process, other parts of the plant, such as the fruits, flowers, bark, and roots, remain underutilised. In contrast, related species such as gelam bark (*Melaleuca cumingiana* Turcz.) have been studied for various applications, including activated carbon material (Abdullah 2001), antibacterial (Sudiansyah *et al.* 2023), natural dye (Nintasari and Purwanto 2016), and raw material for binderless bark particleboard (Christy *et al.* 2021).

All parts of the plant contain different chemical components. Analysing the chemical composition of the bark of various *Melaleuca* species is crucial to enhance its value as a raw material. Recent research by Zamzami *et al.* (2021) revealed that the methanol extract of gelam bark (*Melaleuca cumingiana* Turcz.) primarily consists of compounds such as polyphenols, flavonoids, alkaloids, tannins, steroids, and terpenoids. Similar findings are anticipated for *M. cajuputi* Powell.

The bark of many species is known to be composed of polyphenols, lignans, and antioxidants (Pietarinen *et al.* 2006). Antioxidants, in particular, are widely used as food additives to protect against oxidative degradation caused by free radicals and combat harmful reactive oxygen species in the human body (Luis *et al.* 2014). Solvent extraction is a common method for isolating various bioactive compounds, including antioxidants, from plant bark. Solvent extraction is the simplest, easiest and fastest method to provide bioactive content with low cost. The most common solvents extraction methods used was methanol and ethanol as extractants on water bath. The biggest advantage of water bath are all factor can be accurately controlled and the reactant are heated evenly (Han *et al*., 2020). In this study, extractive content and antioxidant activity of cajuput bark extract were investigated under different conditions. Chemical components composition was identified to explore potential methods for enhancing the utilisation of cajuput bark.

Materials and Methods

Sample preparation

Cajuput bark (*Melaleuca cajuputi* Powell.) was sourced from a 26-year-old cajuput stand, planted in 1998, in Paliyan, Gunungkidul. The bark was collected from the trunks of several trees after their leaves had been harvested for essential oil production. Using a machete, the bark was removed from the ground surface to a height of 1.3 m, revealing the inner bark. The bark was homogenised by mixing material from different trees and cutting it into small pieces. Finally, the cajuput bark was ground to a powder with a mesh size of 40–60 for the extraction process.

Extraction of cajuput bark

Two grams of cajuput bark powder were dissolved in a methanol-water solvent (40% and 80% v/v) in a 500 mL Erlenmeyer flask. Extraction was conducted in water baths for 120 minutes at 60°C and 100°C. After extraction, the

solution was filtered, and the filtrate was evaporated on a heating plate. The extractive content of cajuput bark was calculated by comparing the oven-dry weight of the extract with the dry weight of the initial powder.

Chemical Component Analysis

The chemical compounds in cajuput bark extract were analysed using GC-MS with the derivatisation method (Moldeveanu and David 2019). The derivatisation reagents consisted of trimethylchlorosilane (TMCS), N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA), and pyridine. A 5 mg sample of cajuput bark methanol extract was diluted with 100 µL of pyridine in an injection vial and sonicated. Then, 1 µL of TMCS solution and 99 µL of BSTFA solution were added to the vial, which was sonicated again. The vial was heated for 60 minutes at a temperature of 103 ± 2°C. For GC-MS analysis, three different solvents were used: methanol, *n*-hexane, and acetone. The sample was injected into the GC-MS system with a RTX-5MS capillary column (30 m × 0.25 mm × 0.25 µm; GL Sciences, Tokyo, Japan). The temperature was programmed to rise at a rate of 6°C per minute from 70°C to 290°C. The system was run with helium serving as the carrier gas, a separation ratio of 1:9.5, and a total retention time of 60.83 minutes. The analysis was carried out using the relative peak area method. Compounds were identified using the NIST 20 library (National Institute of Standards Technology).

Determination of DPPH inhibition antioxidant activity (IAA)

Antioxidant activity was determined using the DPPH (1 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay (Gao *et al.* 2006). Cajuput bark extract was diluted in several concentrations (1000, 500, 250, and 125 ppm). Then, 3 mL of 4% (w/v) DPPH solution was mixed with 0.1 mL of each extract solution (A1). A_0 consisted of 0.1 mL of methanol and 3 mL of DPPH (blank absorbance). Methanol served as the absorbance blank. All samples were incubated in a dark at room temperature for 30 minutes. Absorbance was measured at 517 nm with a WPA 800+ spectrophotometer (A1). The test was carried out in duplicate. Catechin and tannic acid were used as positive controls for comparison. The percentage inhibition of antioxidant activity (%IAA) was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Results and Discussion

Extractive content of cajuput bark

As shown in Table 1, the average extractive content (EC) of the cajuput bark methanol extract in this study was 4.07%, which was greater than the values reported by Arisandi *et al.* (2024), ranging from 1.00% to 1.03%, and by Batubara *et al.* (2012), which was 1.20%. In comparison, the average EC of the methanol extract from *E. globulus*, which belongs to the same family as cajuput, was reported to be 9.92% by Luis *et al.* (2014) and 2.7% by Vazquez *et al.* (2008).

Increasing the concentration of the methanol solvent from 40% to 80% at 60°C and 100°C resulted in increases in the extractive content from 1.65–2.15% to 5.45–6.77%, respectively. At the same methanol solvent concentration, the extraction temperature did not affect the EC value. This contradicted with Vergana-salinas *et al.* (2012) showed that the use of high temperature on extraction process did not damage the chemical compound but provided better extractive yield and antioxidant activity. Low methanol concentration (40%) at temperatures of 60°C and 100°C yielded EC values in the range of 1.65–2.06%, while high methanol concentration (80%) at the same temperatures increased the EC values to 5.45–6.77%. This suggests that the concentration of the methanol solvent had a considerable effect on the efficiency of the methanol extraction of cajuput bark. This finding confirmed the work of Arisandi *et al*. (2024), who observed that methanol solvents provided higher EC values for cajuput bark extraction than water solvents.

Several studies have reported higher EC values from bark extraction using methanol than other solvents, such as *n*-hexane, acetone, ethyl acetate, and ethanol (Vazquez *et al.* 2008; Luis *et al.* 2014; Arisandi *et al.* 2024). Additionally, the high EC value of the bark indicates that extractives in this part are more abundant than in the stem (Pereira *et al.* 2004). According to Lukmandaru (2011), extractives soluble in methanol solvents include highly polar compounds such as starch, sugars, and dissolved carbohydrates.

As explained by Piwowarska and González-Alvarez (2012), increasing the solvent concentration enhances the solubility of polyhydrogen bonds, hydrophobic interactions with polysaccharides, and protein bonds in cell walls, facilitating the dissolution of extractive components. Consequently, higher amounts of dissolved substances lead to a higher EC value of the extracted sample.

Table 1. Extractive content and antioxidant activity of cajuput bark extract

Samples	Temperatures (°C)	Duration (minutes)	Methanol Conc. (%)	Dry weight extract (g)	EC (%)	IAA at 1000 ppm (%)
A1(1)	60	120	40	0.04	*1.65*	35.13
A1(2)	60	120	40	0.05	2.15	*26.41*
A2(1)	60	120	80	0.13	5.77	**45.04**
A2(2)	60	120	80	0.15	**6.77**	43.90

B1(1)	100	120	40	0.04	2.06	43.60
B1(2)	100	120	40	0.04	2.06	44.50
B2(1)	100	120	80	0.15	6.64	41.00
B2(2)	100	120	80	0.12	5.45	42.52
		Min		0.04	1.65	26.41
		Max		0.15	6.77	45.04
		Average		0.09	4.07	40.26
		Standard deviation		0.05	2.23	7.04

Note: EC = extractive content; %IAA = percentage inhibition of antioxidant activity; bold = the highest value; italic = the lowest value.

Cajuput Bark Chemical Component Analysis

GC-MS analysis of the cajuput bark methanol extract was carried out using three different solvents. The injection with methanol as a solvent did not identify the chemical components in the cajuput bark sample and instead indicated impurities as evidenced by a similarity index value of less than 40%. Consequently, the derivatisation method involving TMCS was employed, with *n*-hexane and acetone serving as solvents, to improve the results of the chemical composition identification.

Table 2. GC-MS result of Cajuput Bark Methanol Extract (TMCS Derivative)

No	Name	SI (%)	Formula	Concentration (%)	
				Acetone	Hexane
1	Lactic acid	91	$C_9H_{22}O_3$	-	6.33
2	Boric acid	82	$C_9H_{27}BO_3$	6.21	4.02
3	Glycerol	94	$C_{12}H_{32}O_3$	15.06	19.01
4	Glyceric acid	92	$C_{12}H_{30}O_4$	-	2.86
5	Erythritol	84	$C_{16}H_{42}O_4$	3.23	2.42
6	D-Arabinose	85	$C_{17}H_{42}O_5$	4.03	-
7	Malic acid	88	$C_{22}H_{48}O_5$	3.07	-
8	D-Lyxose	84	$C_{17}H_{42}O_5$	4.76	-
9	Adonitol	94	$C_{20}H_{52}O_5$	7	6.33
10	Protocatechoic acid	85	$C_{16}H_{30}O_4$	-	2.42
11	d-Galactose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1Z)-	91	$C_{22}H_{55}NO_6$	8.76	5.51
12	D-Glucitol	91	$C_{24}H_{62}O_6$	45.67	32.45
13	Gallic acid	92	$C_{19}H_{38}O_5$	-	3.64
14	L-Rhamnose	85	$C_{18}H_{44}O_5$	2.22	-
15	Palmitic acid	90	$C_{19}H_{40}O_2$	-	15.01
	Total (%)			100	100

Note: SI= Similarity Index (%)

Table 2 presents the results of the GC-MS analysis of the cajuput bark methanol extract using *n*-hexane and acetone as solvents. According to these results, cajuput bark consisted of two main groups of compounds: fatty acid and sugar groups. The analysis with both solvents identified glucitol (32.45–45.67%) and glycerol (15.06–19.01%) as the compounds with the highest concentrations in the cajuput bark extract. The *n*-hexane-soluble extract was identified to be richer in fatty acid compounds, while the acetone-soluble extract is richer in sugar compounds. Additionally, several simple phenolic compounds, such as protocatechuic acid and gallic acid, were detected only in the *n*-hexane-soluble extract.

Arisandi *et al.* (2024) studied the *n*-hexane extract of cajuput bark extracted by soxhlet extraction and identified several lipophilic components, including hydrocarbons (13%), fatty acids (21.3%), fatty alcohols (9.63%), sterols and steroids (31.4%), triterpenoids (21.9%), and aldehyde compounds (1.7%). This study found similar results for fatty acid fractions. A clear difference was seen on the abundant of aldehyde, triterpenoid, sterols and steroids compound that appears when cajuput bark was extracted by n-hexane as extraction solvent. Other acid compounds were found as simple phenolic acids. Additionally, other studies have reported some phenolic derivatives in methanol extracts from plant bark. Yazaki and Hillis (1967) found that methanol extracts from the bark of several *Eucalyptus* species contained ellagic acid and gallic acid derivatives such as ellagitannin, methyl, and glycosyl. Similarly, methanol extracts from the bark of several plants in the *Myrtaceae*

family, such as *E. maidenii* and *E. urograndis*, contained quinic acid, gallic acid, protocatechuic acid, catechin, and ellagic acid (Santos *et al.* 2012). Those findings confirmed the presence of phenolic compounds and their derivatives in cajuput bark.

GC-MS analysis detected several sugar compounds in the cajuput bark methanol extract. The same sugar components were also identified in the bark of other species within the *Myrtacae* family, to which cajuput belongs. Vazquez *et al.* (2008) reported that 60.96% of the *E. globulus* bark content was composed of sugars, including glucose, xylose, arabinose, galactose, and mannose. Miranda *et al.* (2016) similarly found that the bark extract of *E. sideroxylon* contained sugar groups, such as monosaccharides, glucose, xylose, galactose, arabinose, mannose, and rhamnose. The considerable amounts of sugar components identified in this study might be attributed to the use of polar solvents, such as methanol, which are effective in dissolving starch and sugars in plant extracts (Arisandi *et al.* 2024).

GC-MS analysis was able to identify only a few polar components of the cajuput bark methanol extract, suggesting that this method might not be fully effective for such identification. For more accurate identification, it is recommended to employ other tools, such as LC-MS. In addition, the presence of numerous impurities in the methanol extract solution might have contributed to this challenge, suggesting the need for pre-screening or purification of the methanol extract before injection into the GC-MS system.

Antioxidant activity of cajuput bark

The average percentage inhibition of antioxidant activity (%IAA) for the cajuput bark methanol extract at 1000 ppm in this study was 40.26%. Catechin compounds and tannic acid have standard percentage inhibition values at 1000 ppm of 85.65% and 90.30%, respectively, which are considerably higher than the value obtained in this study. This confirms that the cajuput bark methanol extract was weaker than the standards.

According to Table 1, increasing the methanol concentration from 40% to 80% at 60°C resulted in an increase in the %IAA value from 26.41–35.13% to 43.90–45.04%. However, at a higher temperature of 100°C, the %IAA value slightly declined from 43.60–44.50% to 41.00–42.52%. Meanwhile, at a methanol concentration of 40%, raising the temperature from 60°C to 100°C gave a boost to the %IAA value from 35.13–26.41% to 43.60–44.50%. On the other hand, at a methanol concentration of 80%, increasing the temperature in the same range caused the %IAA value to fall from 43.91–45.04% to 41.00–42.52%. These results show that both temperature and methanol concentration did affect the %IAA value. Specifically, at low solvent concentrations, an increase in extraction temperature increased the %IAA value. At high solvent concentrations, however, a rise in temperature lowered the %IAA value.

Several studies reported effective %IAA levels exceeding 70%. For instance, Hou *et al.* (2016) reported 86.0% IAA value for *M. braceteta* ethanol extract, which was higher than the IAA value observed in this study. Sulaiman *et al.* (2017) obtained an optimum IAA value of 72.95% for *Clinacanthus nutans* ethanol extract by using an ethanol solvent concentration of 10% for 120 minutes, whereas Yim *et al.* (2013) achieved a IAA value of 84.70% for *Schizophyllum commune* aqueous extract at a temperature of 35.7°C.

In this study, extraction was conducted within the temperature range of 60–100°C. The increase in antioxidant activity obtained at high temperatures most likely due to an increase in the total yield of the extract (Vergara-Salinas et al, 2012). As shown in Table 1 it appears that the samples with high extractive content provided better antioxidant value. Research has shown that effective antioxidant activity is generally reached in the extraction temperature range of 35–75°C with low solvent concentrations. It has been proven that the IAA value increased with the increasing temperature up to a certain point but decreased when the concentrations were high. Similarly, Sulaiman *et al.* (2017) reported that increasing the solvent concentration caused a slight decrease in the DPPH antioxidant activity. They also noted that pH and the chemical structure of DPPH also contributed to this decrease at high solvent concentrations. Therefore, the decrease in the IAA value in this study was likely caused by the interaction of high temperature and high solvent concentration.

The antioxidant activity can be affected by its chemical composition. The results of GC-MS identification showed that the antioxidant activity can be affected by the chemical composition of the extract. GC-MS analysis revealed that the cajuput bark methanol extract in this study was primarily composed of sugars and fatty acids, with small amounts of simple phenolic compounds such as gallic acid (3.64%) and protocatechuic acid (2.42%), which are known to exhibit antioxidant activity. Plants with high phenol and polyphenol contents typically exhibit strong antioxidant properties (Luis *et al.* 2014). These compounds possess redox molecular structures that act as reducing agents, hydrogen donors, and singlet oxygen quenchers, targeting free radicals. Therefore, the low antioxidant activity of the cajuput bark methanol extract observed in this study was likely due to the extract's low phenolic content. Furthermore, the presensce of numerous inactive antioxidant compounds as well as active antioxidant compounds that were not fully dissolved in methanol as the extraction solvent might have contributed to low antioxidant activity. In addition, the method used might have influenced the results, suggesting the need for comparisons with other antioxidant activity test methods to have a better understanding of the antioxidant potential of cajuput bark.

Conclusion

Extraction of cajuput bark with methanol solvent using a simple extraction method revealed the presence of various chemical compounds, including sugars (erythritol, D-arabinose, D-lyxose, adonitol, d-galactose, D-glucitol, and L-rhamnose), fatty acids (lactic acid, glycerol, glyceric acid, malic acid, and palmitic acid), and phenolic derivatives (gallic acid and protocatechuic acid). The resulting methanol extract of cajuput bark had an extractive content of 6.77% and percentage inhibition of antioxidant activity of 45.04%. The low antioxidant activity value was likely due to the low phenolic content in the cajuput bark methanol extract, a high amount of impurities, and the presence of numerous inactive antioxidant compounds. Future research is suggested to quantify the phenolic compounds and their derivatives in cajuput bark extracts to better understand their antioxidant potential.

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