Physico-Chemical Properties and Bioactivity of Resinous *Araucaria cunninghamii* extract

Aishah Agustian, Ganis Lukmandaru, Rizki Arisandi

Abstract

Resin has been identified as a promising non-timber forest product with the potential to generate high economic value. *Araucaria cunninghamii* is recognized as one of the most prominent sources of resin in Indonesia. This study aimed to evaluate the potential of resin from *A. cunninghamii*, focusing on its extractive properties. A total of 20 g of resin was extracted by separation and successive extraction methods using *n*-hexane, ethyl acetate, and methanol solvents. Subsequently, the *n*-hexane extract was fractionated into neutral and acidic fractions. Subsequently, the acidic fraction underwent a saponification process, resulting in the isolation of an unsaponifiable fraction and a phenolic fraction. The components of analysis were identified through the utilization of a GC-MS analysis. The physicochemical test of the resin was conducted in accordance with the Indonesian National Standard (SNI 7636:2020), while the quantification of the total phenolic content was performed through the implementation of the Folin-Ciolceteu method. Furthermore, the measurement of antioxidant activity was conducted through *1,1-diphenyl-2-picryl-hydrazyl* (DPPH) test (inhibition percentage). The results showed that the ethyl acetate extract exhibited the highest solubility. *Araucaria cunninghamii* contained a more dominant neutral fraction. Moreover, the highest level of total phenolic content was obtained in the *n*-hexane soluble fraction (6.84 mg GAE/g). A GC-MS analysis revealed the presence of oxygenated sesquiterpenes (31.5%), sesquiterpenes hydrocarbons (23.8%), and oxygenated diterpenes (19.9%) within the sample. Furthermore, the physicochemical tests exhibited ash content, acid number, and toluene insoluble content of 0.02%, 83.90 and 0.5%, respectively. *Araucaria cunninghamii* had relatively low antioxidant activity. In the phenolic fraction, antioxidant activity increased after partitioning of the *n*-hexane solubles. The phenolic fraction exhibited an increase in its inhibitory effect, with an increase from 19.5% to 28.8%. Further studies are necessary to detect phenolic and non-phenolic components affected by the intensity of antioxidant activity.

Keywords: Total phenolics, antioxidant activity, gum resin, fractionation, GC-MS

Introduction

Sustainable forest practices yield a dual benefit to forest ecosystems, yielding both direct and indirect benefits. These practices facilitate the production of timber. Gum is generally divided into three categories: resin, rubber, and natural adhesive (gum). Resin is an exudate from plants that usually manifests in response to injury and undergoes a hardening process when exposed to air. A wide array of plant families has resins that are utilized widely. These include Araucariaceae, Pinaceae, Dipterocarpaceae, Burseraceae, and Styracaceae (Muhaimin *et al.* 2018). The Araucariaceae family is classified in the coniferous group. One of the genus in the family, Araucaria, is a well-known source of resin that produces various types of extractives. The natural diversity of plant exudates is classified into three different categories: resin, gum, or a mixture of gum and resin, which is usually referred to as gum resin (Seyfullah 2022). In Indonesia, coniferous plants belonging to the Araucariaceae family are renowned for their commercial resin production. One notable species within this family, A. cunninghamii, is a well-documented source of resin, but its utilization has not been widely developed (Marsusi *et al.* 1970).

Previous studies conducted in various countries have showed differences in the composition of resin in *A. cunninghamii*. The oleoresin of *A. cunninghamii* from Australia has been found to contain a high concentration of diterpene components (Franich *et al.* 1999). In addition, the resin of *A. cunninghamii* from India is characterized by higher sesquiterpene hydrocarbons (Verma *et al.* 2014). Arachidonic acid components were identified as the major constituents in *A. cunninghamii* resin from South Africa (Matsabisa *et al.* 2019). Furthermore, in other species of the same genus, *A. heterophylla*, antioxidant activity has been detected in the gum (Samrot *et al.* 2020). As demonstrated in previous studies, there is considerable potential for further research to be conducted on the composition and utilization of *A. cunninghhamii* resin. However, there was a paucity of data concerning the chemical characteristics and potential utilization of *A. cunninghhamii* resin in Indonesia, with existing reports being general in nature. Therefore, this study aims to evaluate the extractive composition, acid-neutral fraction separation, physicochemical properties, and antioxidant activity of *A. cunninghamii* resin.

Materials and Methods

Resin Samples

The material used in the present study was resin extracted from a *A. cunninghamii* tree (with a diameter of

approximately 57 cm and annual ring number 42), which was located in the Faculty of Forestry at Universitas Gadjah Mada, Yogyakarta. Approximately 10–20 g of *A. cunninghamii* resin was dissolved in *n*-hexane, ethyl acetate, and methanol solvents, with the ratio of solvent to resin was set at1:3 (60 mL of solvent). Extraction was conducted using a separation method and successive extraction method, with a hot plate stirrer employed for a duration of one hour. Subsequent to extraction, the solution underwent filtration, desiccation, and heating at approximately 100°C until the solvent had evaporated. The dried extract was then weighed, and the solubility was expressed as a percentage of the initial weight of the resin.

Acid-Neutral Fractionation

A total of 1 g of the *n*-hexane extract of *A. cunninghamii*, extracted using the successive extraction method, was dissolved in dichloromethane and transferred to a separatory funnel. To this mixture, approximately 10% Na_2CO_3 solution was added and shaken to produce two layers. The dichloromethane soluble fraction was washed with distilled water, followed by the addition of a sodium sulfide solution and a 24-hour standing period. The solvent was then evaporated to obtain the neutral fraction. Subsequently, the 10% Na_2CO_3 soluble fraction was acidified using hydrochloric acid to achieve a pH of 3, and dichloromethane solvent was added in the same proportion until two layers were obtained. The dichloromethane-soluble layer was identified as the acidic fraction. The method similar to the one employed to obtain the neutral fraction was conducted. The solubility of the extract was calculated based on the initial weight of the *n*-hexane extract.

Separation of Neutral Fraction by Saponification

Samples of the acidic fraction of *A. cunninghamii* were dissolved into 0.5 M KOH using heating for 1 hour. Subsequently, the solution was subjected to evaporation, after which the filtrate was added to a separatory funnel containing 100 mL of distilled water and 100 mL of dichloromethane. Furthermore, the separatory funnel was shaken and the chloromethane soluble fraction was transferred into an Erlenmeyer flask. This was subsequently combined with an adequate quantity of sodium sulfide solution, allowing for a-24 hour period of incubation. Thereafter, the solvent underwent evaporation in order to collect unsaponifiables fraction. Concurrently, the 0.5 M KOH soluble fraction was subjected to acidification with hydrochloric acid to achieve a pH of 3. This fraction was then combined with toluene solvent, resulting in the formation of two layers. The layer that was dissolved by the toluene solvent was subsequently transferred into an Erlenmeyer flask and dried (phenolic fraction).

Component Identification Using GC-MS Analysis

The dried *n*-hexane extracts of the samples were subjected to gas chromatography-mass spectrometry (GC-MS) analysis using GC-MS-QP 2010 (Shimadzu, Japan). The gas chromatography-mass spectrometry (GC-MS) analysis was performed under the following conditions: the RTX-5MS capillary column (30 m × 0.25 mm I.D. and 0.25 µm; GL Sciences, Tokyo, Japan); a column temperature ranging from 70°C (2 min) to 290°C at a rate of 5°C/min. The injection temperature was set at 200°C, while the detection temperature was fixed at 285 °C. Compounds were identified by comparing experimental GC-MS data with the NIST-MS library (NIST 2011) and relevant studies. The quantification of lipophilic constituents was performed by calculating the relative area percentage, which was obtained through a computerized integrator based on peak areas in the total ion chromatography (TIC).

Total Phenolic Content and Physico-Chemical Properties

The total phenolic content (TPC) was measured using the Folin-Ciocalteu method. Absorbance for standard solution testing was conducted against the blank at a wavelength of 765 nm using a visible spectrophotometer (model VIS-WPA S800+). The standard calibration curve was constructed at varying concentrations of gallic acid according to the absorbance of the sample (Arisandi *et al.* 2019; Arisandi *et al.* 2024). The total phenolic content was calculated based on the equivalent of milligrams of gallic acid per gram of dried extract (mg GAE/g). The analysis was conducted with two replicates, and the results were averaged. Furthermore, the physico-chemical property parameters tested included acid number, toluene insoluble material, and ash content, in accordance with the Indonesian National Standard (SNI) 7636-2020.

Antioxidant Analysis

The antioxidant activity test was conducted by means of two measurements of all samples obtained by successive extraction methods. In addition, the fraction obtained from the separation of the *n*-hexane extract was tested. Each sample was added to a 4000 ppm DPPH solution (8 mg:2 mL), prepared in ethanol, and left to react at room temperature. After 30 minutes, the absorbance value was measured at a wavelength of 517 nm using a UV-VIS spectrophotometer. The results were then averaged based on the percent inhibition.

Results and Discussion

Resin Solubility

In the present study, the results of the successive extraction method are presented in Figure 1. The *A. cunninghamii* resin exhibited a high residual value of 55% of the total initial weight. The three solvents used in this study were found to be incapable of dissolving more than half of the initial weight of the resin. The ethyl acetate solvent was found to be the most effective, with a percentage of 27%, indicating a significant presence of semi-polar components.

The high percentage (44.2%) of ethyl acetate solubility was obtained through the separation method (Figure 2). Furthermore, the use of an *n*-hexane as a solvent did not yield a distinct solubility level compared to the levels produced by the other two solvents (10% vs. 9.71%). This outcome was due to the extraction process starting with *n*-hexane as the initial solvent. The low value of the extract with *n*-hexane solvent indicates the low solubility of the non-polar component. The use of methanol in conjunction with the separation method yielded a solubility percentage of 24.9% for the initial weight of the resin. The methanol as a solvent produced a low percentage of extract value, thereby indicating that methanol has yet to demonstrate effectiveness in dissolving resin of *A. cunninghamii*. A comparison of the two methods revealed methanol solubility percentage resulting from the implementation of the separation method was three times higher than that achieved by the successive method.

This finding aligns with a previous study that reported the insolubility of *A. heterophylla* gum in methanol (Divvela *et al.* 2016). Conversely, the *n*-hexane extract of the oleoresin from *P. merkusii, P. oocarpa,* and *P. insularis* exhibited the highest yield when subjected to the same method and solvent (Sari *et al.* 2018). These results contrast with the findings of this study, which demonstrated the inability of non-polar solvents to dissolve *A. cunninghamii*. A similar trend was observed in copal resin, wherein non-polar solvents proved ineffective in facilitatig dissolution (Lukmandaru 2014).

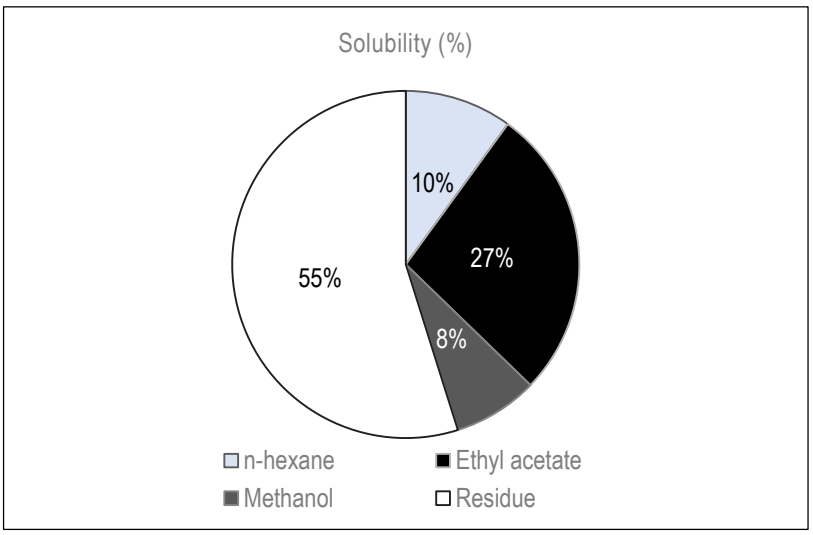

Figure 1. Solubility of *A. cunninghamii* resin through the application of successive extraction methods

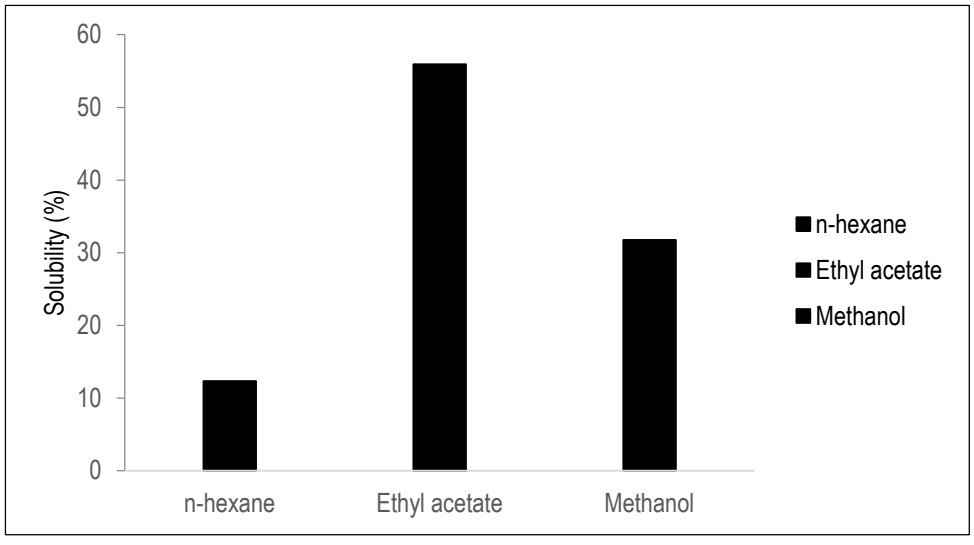

Figure 2. Solubility of *A. cunninghamii* resin through the application of speration method

Yield of Acid Fraction and Neutral Fraction

As demonstrated in Table 1, *A. cunninghamii* exhibited a more pronounced neutral fraction value compared to its acid fraction value. In principle, the neutral fraction generally comprises unsaponifiables substances in alkali, glycerol, and a mixture of acids (fats and resins), while the acid fraction consists of fatty acids and resin acids (Baeza and Freer 2001; Lukmandaru 2012). In a previous study, copal from resin trees (*Agathis* sp.) of the *Araucariaceae* family exhibited a more dominant acid fraction (56 to 80%) compared to the neutral fraction (20 to 44%) (Lukmandaru 2017).

Separation of Neutral Fraction by Saponification

A comparison of the unsaponifiables fraction (67.7%) of *A. cunninghamii* with the phenolic fraction (15.6%) revealed a significant difference in the relative dominance of each fraction. The fraction dissolved in dichloromethane is a non-polar substance that has not been dissolved in *n*-hexane due to differences in polarity (Lukmandaru 2012). A previous study by EFSA Panel (2010) on gum resin from *Pinus oocarpa* Schiede species obtained an acidic fraction with a percentage ranging from 75.7% to 78.2% and an unsaponifiable fraction ranging from 19% to 20.1%. When evaluated in conjunction with the *softwood* group, the percentage of the unsaponifiable fraction of *A. cunninghamii* exhibited a more dominant value. Theoretically, the higher the unsaponifiable content, the greater the potential for pitch problems in the pulp and paper process. This phenomenon can be attributed to the low solubility of lipophilic components in water, which complicates their removal. Consequently, this may result in pitch-related issues during the pulping and papermaking processes (del Rio *et al.* 2009).

Table 1. Composition of neutral fraction, acid fraction, unsaponifiables fraction, and phenolics fraction

Fraction	Solubility (%)
n-hexane solubes	
- Neutral	73.7
- Acid	7.16
Neutral fraction	
- Unsaponifiables	67.7
- Phenolics	15.6

Identification of Chemical Components of *A. cunninghamii* resin

The GC-MS analysis detected the presence of six main chemical compound categories: oxygenated sesquiterpenes (31.5%), sesquiterpene hydrocarbons (23.8%), oxygenated diterpenes (19.9%), diterpenes (13.5%), resin acids (7.5%), and other compounds (3.8%) (see Figure 3, Table 2). In the oxygenated sesquiterpenes category, viridiflorol was the most abudant component, representing 22.3% of the category. Alpha-cadinol was the least abundant, with a percentage of 1.7%. Additionally, androstenediol was identified with a minimal percentage (1.8%), obtained from another compound category, namely, steroids (3.8%).

As reported in the study by Verma *et al*. (2014), *A. cunninghamii* resin was found to contain a higher amount of sesquiterpenes. The present study found similar results, with the highest group being oxygenated sesquiterpenes. The abundance of diterpenes, followed by sesquiterpenes, in *A. cunninghamii* has also been observed in various pine species. This finding suggests that diterpenes are more abundant than monoterpenes and sesquiterpenes in these species.

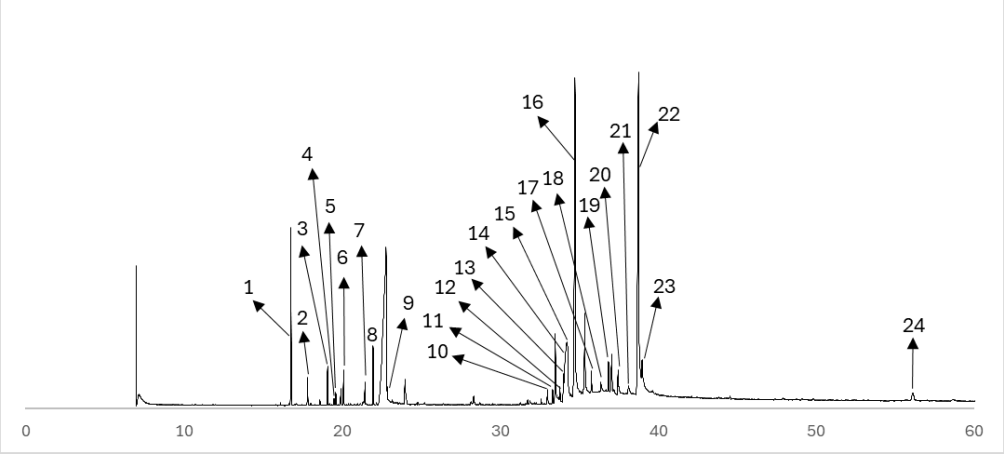

Figure 3. GC-MS chromatogram of the *n*-hexane solubles from *A. cunninghamii*

For example, labdane diterpenes are primarily present in pine species. In addition, it has been reported that β-caryophyllene and germacrene are the most abundant sesquiterpenes in certain coniferous species (Mofikoya 2020). The presence of resin acids, including isopimaric and pimaric acids, was also identified in the resin of *A. cunninghamii*. Furthermore, isopimaric acid has been detected in copal resin (Lukmandaru 2017). A study undertaken previously has reported the presence of arachidonic acid, which is the main component of *A. cunninghamii* resin (Matsabisa *et al.* 2019).

Table 2. Chemical component of *n*-hexane soluble from *A. cunninghamii* resin

Peak	Retention Time (Minutes)	Compound	Formula	Similarity Index (%)	Concentration (%)
		Sesquiterpene Hydrocarbons			23.8
1	16.79	Copaene	$C_{15}H_{24}$	96	6.14
2	17.84	Caryophyllene	$C_{15}H_{24}$	97	2.12
3	19.11	Copaene	$C_{15}H_{24}$	91	2.36
4	19.63	(-)-Aristolene	$C_{15}H_{24}$	88	1.67
5	19.94	.gamma.-Muurolene	$C_{15}H_{24}$	94	1.81
6	20.12	isoledene	$C_{15}H_{24}$	88	2.26
12	33.81	.gamma.-Elemene	$C_{15}H_{24}$	78	1.71
13	34.03	Longifolene	$C_{15}H_{24}$	82	2.50
19	37.09	.beta.-Elemene	$C_{15}H_{24}$	82	3.20
		Oxygenated Sesquiterpenes			31.5
7	21.47	Humulenol-II	$C_{15}H_{24}O$	85	1.87
8	21.99	Caryophyllene oxide	$C_{15}H_{24}O$	77	3.08
9	22.88	.alpha.-Cadinol	$C_{15}H_{26}O$	91	1.73
20	37.48	Ageratriol	$C_{15}H_{24}O_3$	83	2.50
22	38.77	Viridiflorol	$C_{15}H_{26}O$	77	22.3
		Diterpene			13.5
11	33.34	Thunbergen	$C_{20}H_3$	84	1.76
15	34.24	Hibaene	$C_{20}H_{32}$	81	7.42
23	39.01	Thunbergen	$C_{20}H_{32}$	82	4.28
		Oxygenated Diterpenes			19.9
16	34.76	Verticiol	$C_{20}H_{34}O$	83	17.9
17	35.80	Thunbergol	$C_{20}H_{34}O$	84	2.02
		Resin acids			7.50
10	33.02	Pimaric acid, TMS	$C_{23}H_{38}O_2Si$	88	1.81
14	34.14	Isopimaric acid, TMS	$C_{23}H_{38}O_2Si$	83	3.77
21	38.14	Agathic acid 15-methyl ester, TMS	$C_{24}H_{40}O_4Si$	89	1.92
		Other compounds			3.80
18	36.40	Androstenediol	$C_{19}H_{30}O_2$	72	1.83
24	56.13	Lupenone	$C_{30}H_{48}O$	78	2.01

Physico-Chemical Analysis

The ash content of a material is directly related to its mineral content (Amalia *et al.* 2018). The Indonesian National Standard (SNI) for super quality stipulates an ash content requirement of less than 0.02%. As demonstrated in Table 3, the resin satisfied these criteria. The resin produced a blackish residue, which was attributable to impurities. The acid number did not meet the SNI requirements (160-200). A direct relationship was observed between acid numbers (Table 3) and acid fraction percentage (Table 1). The acidic fraction had a low level of acid number value. Moreover, the level of insolubility in toluene did not comply with the stipulated SNI requirements. As demonstrated in Figure 1 and Figure 2, the resin was found to be insoluble in *n*-hexane and toluene solvents, based on successive extraction or separation. It can be thus be concluded that the high level of insolubility in toluene is due to the polymerized component rather than the impurities. Another study reported the insoluble content of toluene in copal resin to be 35.89% (Lukmandaru 2017).

Table 3. Physico-chemical analysis of *A. cunninghamii* resin

No	Parameters	Physico-chemical properties *of. A. cunninghamii* resin	*Grade* specification of SNI 7636:2020		
			I	**II**	**III**
1	Ash content (%)	0.02	≤ 0.04	≤ 0.05	≤0.08
2	Acid numbers	83.9	160- 200 (General required)		>30
3	Toluene insoluble content (%)	0.5	≤ 0.05	≤ 0.07	≤ 0.1

Total Phenolic Content (TPC)

The total phenolic content (TPC) of the fractions derived from *A. cunninghamii* resin is presented in Table 4. The highest TPC level was found in the *n*- hexane soluble extract. This pattern is in line with the amount of phenolic fraction (see Table 1). The Folin-Ciocalteu method is not specific to phenolic compounds, as it can also react with other non-phenolic reducing compounds, potentially leading to an overestimation of the total phenolic content (van Alstyne 1995). The resin had a lower percentage of phenolic fraction and had a more dominant unsaponifiable fraction. In an earlier study (Lukmandaru 2017), the TPC of an ethanol soluble extract from copal resin was found to be higher than the results of the present study. Theoretically, the higher TPC in plants indicates a strong antioxidant therapeutic potential. Phenolic components function as free radical acceptors and chain bond splitters. Phenolics have been shown to interfere with the rapid fractionation of lipids and other molecules by donating hydrogen atoms to radical components (Salim *et al.* 2020).

Table 4. The results of total phenolic content, antioxidant activity, and antioxidant activity of *n*-hexane fractionation separation from *A. Cunninghamii*

Fraction	TPC (mg GAE/ g)	AAO (%) Inhibition
n-Hexane	6.84	19.6
- Neutral	-	-
- Acid	-	-
- Phenolic	-	28.8
Ethyl acetate	5.53	13.3
Methanol	4.21	4.88

Antioxidant Activity of *A. cunninghamii*

The results of the antioxidant activity measurement through the DPPH method are shown in Table 4. In this study, the concentrations that were determined for calculating the percentage of inhibition were 4,000 ppm, 2,000 ppm, 1,000 ppm, and 500 ppm, respectively. The capacity for high antioxidant activity is characterized by low concentrations but high percentages of inhibition. The highest percentage of inhibition was obtained observed in the *n*-hexane soluble, with an inhibition of percentage of 19.6%. This was followed by the ethyl acetate soluble, which exhibited an inhibition percentage of 13.3%. The methanol soluble demonstrated the lowest percentage of inhibition, with a result of 4.88%. This low inhibition percentage level can be explained by the low TPC level (Table 4). Theoretically, the greater the content of phenolic components in plants, the more significant the antioxidant potential (Salim *et al.* 2020). Meanwhile, the inhibition percentage of gallic acid, used as the control was 89.69%. The low percentage of antioxidant activity might be attributable to the presence of more neutral components that lack the potential for DPPH inhibition as an antioxidant activity.

The *n*-hexane soluble exhibited the most potent antioxidant activity. The subsequent stage involved the test of the fractions derived from *n*- hexane soluble resin. It was found that the phenolic fraction was the only constituent that showed the observed inhibitory effect, while the other fractions demonstrated no inhibition, even at high concentrations. The inhibition percentage increased from 19.5% to 28.8% (phenolic fraction). Furthermore, the detection of terpenoid compounds (see Table 2) and antioxidant activity in the non-polar fraction (e.g., *n*-hexane) demonstrates that non-polar compounds can also contribute to antioxidant activity (Isnindar *et al.* 2024).

A previous study has examined the antioxidant properties of frankincense resin, revealing more potent antioxidant activity (Hacini *et al.* 2018). In addition, the antioxidant properties of the methanol soluble gums of *A. heterophylla* and *P. chilensis* exhibited a notable capacity to capture free radicals, with an enhancement in antioxidant potency observed as the concentration of the extract increased (Samrot *et al.* 2020). A previous study also observed low antioxidant activity values in copal resin due to the small amount of phenolic components (Lukmandaru 2017). Further studies are necessary to detect phenolic and non-phenolic components that affect the antioxidant activity. In addition, the employment of other instrumentation, such as HP-LC (high-performance liquid chromatography) or LC-MS

(liquid chromatography-mass spectrometry), is required to identify phenolic components.

Conclusion

The use of ethyl acetate solvent resulted in the highest percentage of solubility in resin. The neutral fraction constituted the predominant fraction in the *n*-hexane solubles. The main compound categories of resin were identified as oxygenated sesquiterpenes, sesquiterpene hydrocarbons, and oxygenated diterpenes. The ash content (0.02%) met the Indonesian National Standard (SNI). Furthermore, the *n*-hexane soluble extract had the highest TPC level. The *n*-hexane solubles demonstrated the highest antioxidant activity. An increase in antioxidant activity was observed in phenolic fraction. In general, *A. cunninghamii* fractions exhibited relatively low antioxidant activity in comparison to gallic acid.

References

Amalia, A.; I. Sari; R. Nursanty. 2018. Antibacterial activity of ethyl acetate extract of sembung leaves (*Blumea balsamifera* (L.) DC.) against the growth of *Methicillin Resistant Staphylococcus aureus* (MRSA) bacteria. In Proceedings of the National Seminar on Biology, Technology and Education 5(1): 387-391.

Arisandi, R.; S.N. Marsoem.; G. Lukmandaru.; T. Ashitani.; K. Takahashi. 2019. The contents of phenolics and cell wall component of *Eucalyptus pellita* F. Muell stemwood and bark. Wood Research, 64(3):411-422.

Arisandi, R.; A. N. Jihad; A. H. S Paleva; F. A. Kurniawan; G. Lukmandaru; N. K Kartikawati; A. Rimbawanto; S. Sunarti; T. Herawan; L. Haryjanto; A. I . Putri ; F. Lestari; A. Nirsatmanto. 2024. Characterization of extractive composition in the wood and bark of cajuputi (*Melaleuca cajuputi subsp. Cajuputi powell*.) grown in Gunungkidul, Indonesia. Wood Research 69(1): 103–119.

del Río, J.C.; G. Marques; I. M. Rodríguez; A. Gutiérrez. 2009. Chemical composition of lipophilic extractives from jute (*Corchorus capsularis*) fibers used for manufacturing of high-quality paper pulps. Industrial Crops and Products 30(2): 241–249.

Divvela, H.N.D.; L. Duppala; V. R. M Kolapalli. 2016. Isolation and acute oral toxicity studies of *Araucaria heterophylla* novel natural polysaccharide gum in albino mice. World Journal of Pharmacy and Pharmaceutical Sciences 5(10):702-711.

EFSA Panel. 2010. Scientific Opinion on the safety of glycerol esters of gum rosin for the proposed uses as a food additive. EFSA Journal 8(7): 1-31.

Franich, R.A.; A.G. McDonald; D. Steward. 1999. Essential oil of the wood of *A. cunninghamii Aiton ex* D. Don. Journal Essential Oil Research 11: 38–40.

Hacini, Z.; F. Khedja; I. Habib; Z. Kendour; Z. Debba. 2018. Evaluation of antibacterial and antioxidant activities of three types of benzoin resin. European Journal of Chemistry 9(4): 408-411.

Isnindar, S. Luliana.; M. Zahid. 2024. Terpenoid dari fraksi n-heksan dan uji aktivitas antioksidan daun singkil (*Premna serratifolia* Linn). Indonesian Journal of Pharmaceutical Education (e-Journal) 4 (2): 286– 295.

Lukmandaru, G. 2012. Extractive composition of mangium (*Acacia mangium*) wood. Journal of Tropical Wood Science and Technology 10(2): 150-158.

Lukmandaru, G. 2014. Fractionation of copal with various organic solvents. In: Proceedings of the National Seminar on the Role and Policy Strategies for Utilization of Non-Timber Forest Products (NTFPs) in Improving the Use of (Forest) Areas. pp. 382-386.

Lukmandaru, G. 2017. Chemical components and antioxidant properties of copal. Journal of Tropical Wood Science and Technology 15(1): 38-47.

Marsusi, M.; C. Mukti; Y. Setiawan; S. Kholidah; A. Viviat. 2001. A study of the epiphytic orchids in Jobolarangan Forest. Biodiversitas Journal of Biological Diversity 2(2): 150-155.

Matsabisa, M.G.; C.I. Chukwuma; C.U. Ibeji; S.K. Chaudhary. 2019. Stem bark exudate (resin) of *A. cunninghamii Aiton ex* D. Don (*hoop pine*) abates glycation, α-glucosidase and DPP-IV activity and modulates glucose utilization in Chang liver cells and 3T3-L1 adipocytes. South African Journal of Botany 121: 193-1999.

Mofikoya, O.O.; M. Mäkinen.; J. Jänis. 2020. Chemical fingerprinting of conifer needle essential oils and solvent extracts by ultrahigh-resolution fourier transform ion cyclotron resonance mass spectrometry. ACS Omega 5(18):10543-10552.

Muhaimin, M.; N. Yati. 2018. Plant species in the Cibodas Botanical Garden collection as fractionation producers and their potential utilization. Proceedings of the National Seminar of the Indonesian Biodiversity Society 2(4): 151-157.

Salim, S.A.; F.A. Saputri.; N.M. Saptarini; Levita, J. 2020. Advantages and limitations of Folin-Ciocalteu reagent in determining total phenol content in plants. Farmaka 18(1): 46-57.

Samrot, A. V.; T. Kudaiyappan; U. Bisyarah; A. Mirarmandi; E. Faradjeva; A. Abubakar; S. Kumar. 2020. Extraction, purification, and characterization of polysaccharides of *Araucaria heterophylla* L and *Prosopis chilensis L* and utilization of polysaccharides in nanocarrier synthesis. International Journal of Nanomedicine 40(8): 7097-7115.

Sari, R. K.; I. Batubara; M. Tillah; D.Tohir. 2018. Antibacterial activity of pine resin against *Staphylococcus aureus*. Journal of Tropical Wood Science and Technology 16(1): 15-22.

Seyfullah, L. J.; E.A. Roberts; P. E. Jardine; J. Rikkinen; A. R. Schmidt. 2022. Uncovering the natural variability of *araucariacean* exudates from ex situ and in situ tree populations in New Caledonia using FTIR spectroscopy. PeerJ Analytical Chemistry 4(17): 1-25.

SNI 7636-2020. Gondorukem. National Standardization Agency: 1-10.

van Alstyne, K.L.1995. Comparison of three methods for quantifying brown algal polyphenolic compounds. Journal of Chemical Ecology 21(1): 45-58.

Verma, R. S.; R.C. Padalia; P. V. Goswami; K. C. Sajendra; D. M. P. Amit. 2014. Chemical composition and antibacterial activity of foliage and resin essential oils of *A. cunninghamii Aiton ex D.Don* and *Araucaria heterophylla (Salisb.)* Franco from India. Industrial Crops and Products 61: 410-416.

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