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Phytochemical analysis and antioxidant activity of *Araucaria* spp. from Taman Bunga Nusantara, Cianjur, West Java, Indonesia

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Abstract. *Sugita P, Ustati MN, Kurniawanti, Syahbirin G, Dianhar H, Rahayu DUC. 2025. Phytochemical analysis and antioxidant activity of* Araucaria *spp. from Taman Bunga Nusantara, Cianjur, West Java, Indonesia. Biodiversitas 26: 118-126. Araucaria columnaris* (AC) and *Araucaria cunninghamii* (ACu) are members of the *Araucaria* genus known for their various pharmacological activities. This study aims to analyze the phytochemical properties, Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and antioxidant activities of twigs from AC and ACu, using different solvents (i) acetone (A); (ii) ethanol (E); and (iii) Ethyl acetate (Ea). Quantitative measurements for TPC, TFC, and antioxidant activity were conducted using the Folin-Ciocalteu method, aluminum chloride colorimetric method, and the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, respectively. Phytochemical screening indicated the presence of phenolics, flavonoids, steroids, and triterpenoids in all extracts. Saponins were found exclusively in acetone extracts, while none contained alkaloids. The TPC is expressed as mg of gallic acid equivalent (GAE) per g of extract, while TFC is presented as mg of quercetin equivalent (QE) per g of extract. The TPC of AC twigs in acetone (ACA) was the highest, followed by ethanol (ACE) and ethyl acetate (ACEa). Similarly, the highest TPC was also observed in acetone (ACuA) for ACu twigs, followed by ethanol (ACuE) and ethyl acetate (ACuEa). Regarding TFC, the order for AC twigs was ACA > ACEa > ACE, while for ACu twigs, it was ACuEa > ACuE > ACuA. ACuE exhibited strong antioxidant activity with an IC_{50} value of 29.57 ± 1.11 µg/mL, whereas the other extracts displayed moderate antioxidant activity. The results of this research provide preliminary data that can serve as a foundation for future studies.

Keywords: Antioxidant activity, *Araucaria columnaris*, *Araucaria cunninghamii*, TFC, TPC, twigs

INTRODUCTION

Herbal remedies are considered a rich source of components with a wide range of biological actions. Due to safety concerns, especially regarding oxidative stress, interest in medicinal plants as an alternative to synthetic medications has increased. Oxidative stress arises when the generation of ROS (Reactive Oxygen Species) and cellular antioxidant activity are imbalanced (Iqbal et al. 2015). ROS plays an important role in the pathogenesis of various human diseases (Prasad et al. 2017). Numerous human medical conditions, such as cancer, heart disease, aging, and neurological disorders, are linked to oxidative stress. Consuming adequate antioxidants can help mitigate oxidative stress caused by free radicals. Various plant materials have been identified as potential sources of antioxidant compounds.

The Araucaria genus, part of the Araucariaceae family, comprises evergreen coniferous trees with nineteen species, many of which have medicinal uses. The Araucariaceae family is rich in biflavanoid, diterpene, phenyl propanoid, and lignans (Aslam et al. 2013). Tungmunnithum et al. (2018) highlighted that phenolic and flavonoid compounda, secondary metabolites with hydroxyl (-OH) groups attached to an aromatic ring, directly enhance antioxidant activity (Côté et al. 2010; Bendary et al. 2013). Numerous studies have shown that phenolic compounds reduce free radicals, degrade peroxides, and prevent oxidative diseases (Tumilaar et al. 2024). The mechanism behind antioxidant activity involves interactions with the -OH group present in phenolics (Lu et al. 2014).

Gautam et al. (2014) reported that methanol crude extract of *A. cunninghamii* leaves exhibits significant DPPH radical scavenging activity, with an IC_{50} of 181.897 µg/mL. The total of phenolic compounds (TPC) and flavonoid compounds (TFC) in the extract were 36.111 mg gallic acid equivalents (GAE)/g extract and 42.9 mg rutin equivalents (RE)/g extract, respectively. Michael et al. (2010) demonstrated that the antioxidant activity of *A. excelsa* leaf extracts using 70% aqueous methanol, methanol, acetone, and ethyl acetate achieved SC_{50} values of 73 ± 12, 138 ± 5, 147 ± 4, and 297 ± 6 µg/mL, respectively. The corresponding TPC values were 37 ± 2, 24 ± 3, 32 ± 2, and 25 ± 3 mg GAE/g extract. Similarly, Patial and Cannoo (2020) observed that methanolic extracts of *A. columnaris* leaves, contained TPC and TFC of 117.69 ± 0.49 mg GAE/g dried plant extract (DPE) and 79.73 ± 0.75 mg RE/g DPE, respectively.

Phytochemicals in the leaves of the *Araucaria* genus have been extensively studied, identified compunds include

terpenoids, phenylpropanoids, flavonoids, protein, furan, and polysaccharides (Frezza et al. 2020). Frezza et al. (2024) identified six compounds from *A. cunninghamii* leaves, including shikimic acid, pheophytin A, methyl-(E)-communate, iso-cupressic acid, ladanein, and 7,4′,7″,4‴-tetra-*O*-methylcupressuflavone (**1**). The polar fraction of *A. cunninghamii* leaves contained several biflavonoids, such as **1**, 7,7″-di-*O*-methylcupressuflavone (**2**), 7,4′,7″-tri-*O*-methyl-cupressuflavone (**3**), 4′,4″,7,7″-tetra-*O*-methyl-amentoflavone (**4**), 4′,7″-di-*O*-methylamentoflavone (**5**), and 4′,4‴,7″-tri-*O*-methylamentoflavone (**6**) (Ilyas et al. 1968; Rahman and Bhatnagar 1968; Khan et al. 1971; Chen et al. 2013).

Kurniawanti et al. (2023) isolated three biflavonoids from the acetone extract of *A. columnaris* leaves: **1, 3** and 4'",7-di-*O*-methylcupressuflavone (**7**). Similarly, compounds identified from *A. hunsteinii* include **1**, **3**, **7**; 7,7″,4‴-tri-*O*-methylagathisflavone (**8**), 7-*O*-methylcupressuflavone (**9**), 4′,4‴-di-*O*-methylamentoflavone (isoginkgetin) (**10**), 7,7″-di-*O*-methylagathisflavone (**11**) (Agusta et al. 2022; Sugita et al. 2023). Additionally, eight biflavonoids have been identified from *A. excelsa* leaf extracts, such as **1, 4**, **11**, 7''-*O*-methylamentoflavone (**12**), 4′-*O*-methylcupressuflavone (**13**), 7,7''-di-*O*-methylamento-flavone (**14**), 4', 7-di-*O*-methylcupressuflavone (**15**), 7,7'',4'-tri-*O*-methylagathis-flavone (**16**), and 7,4′,7″-tri-*O*-methylamentoflavone (**17**) (Ilyas et al. 1978). While research on the Araucaria genus has largely focused on leaf extracts, studies on twig extracts remain limited. Biflavonoids such as procyanidin, fukugetin, amentoflavone, and podocarpus flavone are recognized for their potent antioxidant properties, including DPPH radical scavenging (Arwa et al. 2015). Venditti et al. (2018) noted that cupressuflavone exhibits strong ABTS and DPPH radical scavenging activity. Amentoflavone and agathisflavone also demonstrated significant antioxidant effects by scavenging various radicals, including DPPH, ABTS, nitric oxide (NO), superoxide, and hydroxyl radicals (Andrade et al. 2018; Bajpai et al. 2019).

Recent phytochemical studies have underscored the potential global significance of ethnomedicinal plants with notable biological properties. The objectives of this study are to: (i) identify and analyze phytochemical profiling; (ii) estimate phenolic and flavonoid content; (iii) evaluate the antioxidant activity of *A. columnaris* and *A. cunninghamii* twigs extracts using DPPH in vitro methods; and (iv) provide valuable insights for sustainable utilization and pharmaceutical applications of Araucaria twigs while promoting biodiversity awareness. Sample selection was influenced by proximity to IPB University campus, the abundance of Araucaria plant collections, and the lack of previous phytochemical studies on the samples.

MATERIALS AND METHODS

Collection and authentication of plant material

Twigs of *A. columnaris* and *A. cunninghamii* were collected from individual trees on 1 December 2023, from Taman Bunga Nusantara Cianjur, West Java, Indonesia (6.7276° S, 107.0794° E). The samples were identified as *A. columnaris* (G.Forst.) Hook (FIPIA-DEP63) and *A. cunninghamii* (FIPIA-DEP64) by Herbarium Bandungense Sekolah Ilmu dan Teknologi Hayati Institut Teknologi Bandung (SITH ITB), Bandung, Indonesia. After air-drying, the twigs are ground into a fine powder using a medical grinder.

Procedures

Extraction

Forty-five grams of the twigs of *A. cunninghamii* and 50 g of *A. columnaris* were macerated in ethanol at a 1:3 ratio for 24 hours thrice, followed by maceration in acetone and ethyl acetate thrice. The yield of crude extracts of the ethanol, acetone, and ethyl acetate of *A. cunninghamii* twigs were 2.43%, 6.01%, and 2.36%, respectively. In comparison, the yields of *A. columnaris* twigs were 4.78%, 12.14%, and 2.07%, respectively. The crude extracts were kept at -20°C until further use.

Thin Layer Chromatography (TLC) analysis of A. columnaris *and* A. cunninghamii *twig extracts using different solvents*

This study conducted thin-layer chromatography (TLC) analysis on the twig extracts of *A. columnaris* and *A. cunninghamii*. The extracts were developed using a solvent system of chloroform and methanol in a 19:1 ratio. Spot detection was performed under UV light at 254 nm and 366 nm, with cerium sulfate as a visualizing agent.

Preliminary phytochemical analysis

Qualitative phytochemical analysis of the crude extracts was determined using the method of Shaikh and Patil (2020). The plant constituents identified include alkaloids, triterpenoids, steroids, phenolics, flavonoids, and saponins.

Alkaloids. 300 mg of each extract in the test tube was added with 10 mL of chloroform and a small amount of 10% ammonia. The mixture was agitated to ensure homogeneity before being filtered. Then, the filtrate was added with H_2SO_4 2M with a ratio of 1:1, and it agitated until two layers formed. The top layer was divided into three equal amount portions. One portion was treated with a few drops of Mayer's reagent and two others with a few drops of Dragendorf's and Wagner's reagents, respectively. The appearance of a yellow precipitate, a reddish-brown precipitate, and a brown/reddish precipitate indicated the presence of alkaloids, respectively. Meyer's reagent was prepared by mixing 1.3 g of mercuric chloride and 5.0 g of KI (potassium iodide) and added with distilled water made up to 100 mL. Dragendorff reagents A and B were mixed and diluted to 100 mL with water. Solution A is bismuth nitrate (0.17 g) in acetic acid (2 mL) and water (8 mL). Solution B is KI (4 g) in acetic acid (10 mL) and water (20 mL). Wagner's reagent was prepared by dissolving 1.27 g I_2 (sublimed) and 2 g KI and added with distilled water made up to 20 mL, and then the solution was made up to 100 mL with adding more water.

Phenolics and flavonoids. A mixture of chloroform and water (1:1) was added to a test tube containing 100 mg of extract, then agitated and allowed to stand until two layers formed. The top layer was divided into two equal

portions and was put into a test tube, respectively. One portion was added with a few drops of ferric chloride 5% solution for phenolics testing. The presence of phenolics was indicated by color changes to dark green/bluish-black. The other portion was added with 100 mg magnesium powder, 1 mL concentrated HCl, and 1 mL *n*-amyl alcohol for flavonoid testing. After a few minutes, the pink, scarlet color indicates the presence of flavonoids.

Steroid and triterpenoid. Fifty grams of crude extract was dissolved in 2 mL acetic anhydride, then 1-2 drops of concentrated H_2SO_4 (along the side of the test tube). The presence of steroids was indicated by a color change to green or blue, while the presence of triterpenoids was indicated by red color.

Saponins. 100 mg of crude extract was mixed with 5 mL of distilled water in a test tube and shaken vigorously. The formation of stable foam indicated the presence of saponins.

Total phenolic content determination

Folin-Ciocalteu method determined the content of total phenolic compounds (TPC) of the extract (Kefayati et al. 2017). 20 µL of the extract was mixed with 50% Folin-Ciocalteu reagent (100 µL) in a 96-well microplate and incubated for 5 min. Then, the mixture was added with 7.5% Na_2CO_3 (80 µL) and incubated in the dark for 2 h at room temperature. After incubation, the absorbance of clear solutions was read at wavelength (λ) of 750 nm. The calibration curve was prepared using different concentrations of gallic acid solution (0, 50, 75, 100, 150, 200, and 225 µg/mL), then mixed with the abovementioned reagents. The TPC was expressed as gallic acid equivalent (GAE) in mg per g DPE.

Total flavonoid content determination

The total flavonoid content (TFC) of crude extracts was determined using the $AlCl_3$ colorimetric method (Kefayati et al. 2017). 2.5 mL of extract, previously dissolved in 90% ethanol, was mixed with 10 µL $AlCl_3$ 10% solution in 90% ethanol, 10 µL potassium acetate 1M, and 120 µL distilled water. The mixture was shaken and then stabilized at room temperature for 30 minutes. After 30 min, the absorbance of the yellow solution was measured at wavelength (λ) of 415 nm. Quercetin was used as standard. The calibration curve was prepared using various concentrations of quercetin solution (0, 100, 200, 300, 500, 400, and 600 µg/m), then mixed with the abovementioned reagents. The TFC was expressed as mg QE/g DPE.

DPPH radical scavenging assay

The DPPH radical scavenging activity was measured as explained by Koehnlein et al. (2012) with modification. Briefly, DPPH (125 µM) in ethanol was used in the experiment. The extract was dissolved in ethanol at 32.25, 62.5, 125, 250, and 500 µg/mL. 100 µL extract was mixed with DPPH (100 µL) and then incubated at room temperature (dark) for 30 min. After 30 min, the absorbance values were measured at 515 nm and converted into a percentage of antioxidant activity. Ascorbic acid was used as a positive control. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{Inhibition of DPPH \%} = \frac{Acontrol - Asample}{Acontrol} \times 100\%$$

$A_{control}$ was the absorbance of the reaction in the presence of water, and A_{sample} was the absorbance of the reaction in the presence of the extract. The extract concentration that inhibits 50% DPPH (IC_{50}) was calculated from the graph of the DPPH scavenging of percentage inhibitions plotted against the respective concentration.

RESULTS AND DISCUSSION

Phytochemical screening

A maceration procedure was used to extract the chemical compounds of *A. columnaris* and *A. cunninghamii* twigs. Phytochemical studies detected alkaloids, flavonoids, phenolics, saponins, steroids, and triterpenoids. The results of the study are shown in Table 1. The chromatogram profile of *A. columnaris* and *A. cunninghamii* twigs in various solvents is shown in Figure 1.

Total phenolics and flavonoids Content of *A. columnaris* and *A. cunninghamii* twigs extract in varying solvents.

The total phenolic content of *A. columnaris* and A. *cunninghamii* twig extracts in varying solvents is shown in Table 2. Gallic acid is commonly used as the standard for calculating total phenolics. The standard curve of gallic acid is shown in Figure 2. Since plant phenolics constitute a significant class of compounds that serve as key antioxidants or free radical scavengers, the Folin-Ciocalteu method was employed to determine the total phenolic content.

Table 1. Phytochemical content of the crude extracts of *Araucaria columnaris* and *A. cunninghamii* in various solvents

Samples	Alkaloids	Flavonoids	Phenolics	Saponins	Steroids	Triterpenoids
ACA	-	+	+	+	+	+
ACE	-	+	+	-	+	+
ACEa	-	+	+	-	+	+
AcuA	-	+	+	+	+	+
AcuE	-	+	+	-	+	+
AcuEa	-	+	+	-	+	+

Notes: *A. columnaris* ACA: twig acetone extract; ACE: twig ethanol extract; ACEa: twig ethyl acetate extract. *A. cunninghamii* ACuA: twig acetone extract; ACuE: twig ethanol extract; ACuEa: twig ethyl acetate extract; and - : negative, + : positive

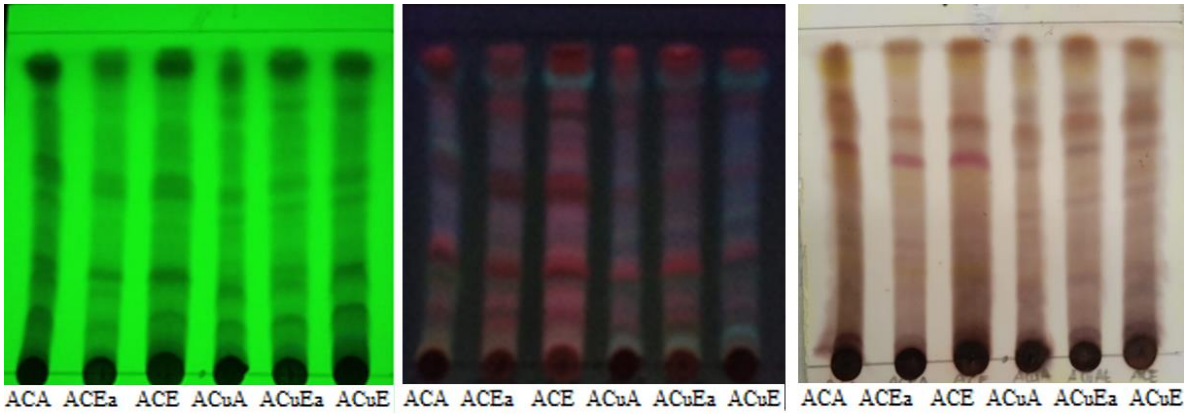

Figure 1. Chromatograms profile of *Araucaria columnaris* and *A. cunninghamii* in varying solvents developed using chloroform: methanol (19:1). A. Observed under UV light 254 nm; B. Observed under UV light 365 nm; C. After spraying with cerium sulfate

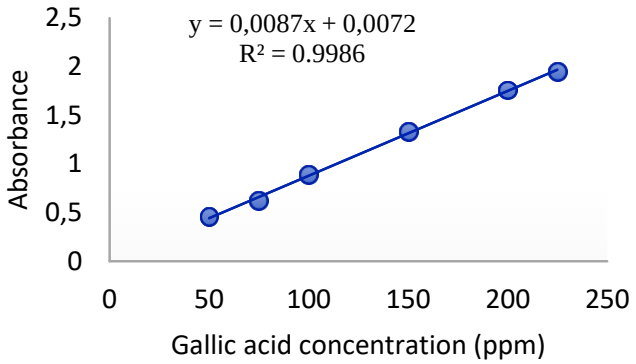

Figure 2. The standard curve of gallic acid

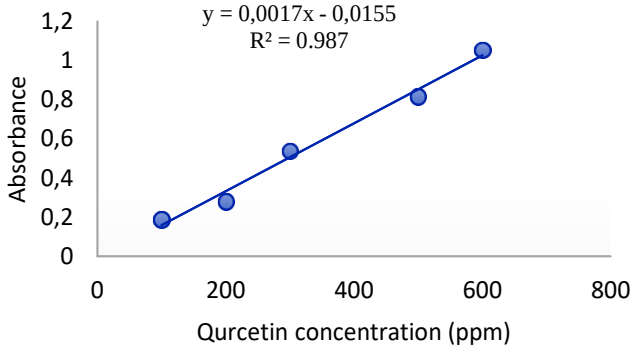

Figure 3. The standard curve of quercetin

Table 2. Total phenolics and flavonoid contents of *Araucaria columnaris* and *A. cunninghamii* twigs extract in varying solvent

Plants extract	Total phenolics (mg GAE/g DPE)	Total flavonoid (mg QE/g DPE)
ACA	129.48 ± 9.85	33.82 ± 9.92
ACE	81.93 ± 7.37	16.18 ± 10.95
ACEa	64.00 ± 8.97	26.96 ± 7.13
ACuA	127.10 ± 7.32	13.63 ± 5.14
ACuE	106.49 ± 14.03	19.12 ± 7.09
ACuEa	96.72 ± 7.32	26.77 ± 10.93

The calibration curve from gallic acid showed maximum absorbances at 750 nm wavelength (equation y = 0,0087x+0,0072, R^2 = 0.9986) (Figure 2). The highest total phenolic content of *A. columnaris* twigs was in acetone (ACA)extract, followed by ethanol (ACE) and ethyl acetate (ACEa) extracts, i.e., 64.00 ± 8.97 and 129.48 ± 9.85 mg GAE/g DPE, respectively. ACuA of *A. cunninghamii* twigs exhibited the highest total phenolic content followed by ACuE and AcuEa, i.e., 96.72 ± 7.32 and 127.10 ± 7.32 mg GAE/g DPE. The highest phenolic content of both samples was in acetone (ACA), followed by ACuA and ACuE.

The result of the total flavonoid contents of the six crude extracts is presented in Table 2. The quercetin standard calibration curve equation was y = 0,0017x-0,0155 R^2 = 0.987 (Figure 3). The order of flavonoid content of A. columnaris twigs was ACA > ACEa > ACE, varied from 16.18 ± 10.95 to 33.82 ± 9.92 mg QE/g DPE. In contrast, the order of flavonoid content of *A. cunninghamii* twig extracts was ACuEa > AcuE > ACuA (13.63 ± 5.14 and 26.77 ± 10.93 mg QE/g DPE, respectively). The highest flavonoid content of both samples in varying solvents was ACA, followed by ACEa and ACuEa.

Antioxidant activity

The ability of the extract to scavenge DPPH radicals is shown in Table 3. The ACuE has the highest ability to scavenge free radicals compared to other extracts. Table 3 presents the IC_{50} values for DPPH radical scavenging activity, which indicates the antioxidant capacity of twig extracts from *A. columnaris* and *A. cunninghamii* in various solvents. The antioxidant capacity of these extracts was evaluated at different concentrations, resulting in linear curves that depict the relationship between concentration and % inhibition for each extract. The IC_{50} value, representing the concentration required to scavenge 50% DPPH free radicals, was calculated using the linear regression equation.

Discussion

The phytochemical analysis of *A. columnaris* and *A. cunninghamii* twigs (Table 1) in different solvents revealed that saponins were present only in the acetone extracts. Phenolics, triterpenoids, steroids, and flavonoids were found in all extracts. No alkaloids were detected in any of the extracts. These findings suggest that the specific solvents used for extraction play a crucial role in the solubility and extraction efficiency of different phytochemicals. The ability of acetone extract to isolate saponins could be due to its polarity, which is more suitable for extracting these compounds than other solvents. The presence of phenolics, triterpenoids, steroids, and flavonoids in all extracts highlights their relatively higher solubility in various solvents. As Aslam et al. (2014) reported, the aerial parts of *A. columnaris* extracted with methanol and chloroform revealed the presence of tannins and cardiac glycosides, indicating that different plant parts and solvents affect the composition of phytochemical compounds.

Variations in geographic origins and environmental factors may also play a role in the observed differences in antioxidant potency. The qualitative results of the phytochemical screening of hexane, chloroform, and methanol extracts of leaves of *A. columnaris* showed the presence of steroids, diterpenoids, triterpenoids, saponins, polyphenols, flavonoids, coumarins, glycosides, and carbohydrates (Patial and Cannoo 2020). On the other hand, the methanol and aqueous extracts of *A. columnaris* bark peel revealed the presence of phenolic compounds, flavonoids, and tannins (Jadav and Gowda 2017).

Phenolics and flavonoids in plants help the plant grow and protect it from harmful environmental conditions (Kumar et al. 2023). Moreover, flavonoids and phenolic substances are well-known for their antioxidant properties (Abeysinghe et al. 2021). The polyphenolic constituents are also a good source of antioxidants that can reduce power, metal chelators, radical scavengers, and other functions (Patial and Cannoo 2020). On the other hand, triterpenoids and steroids are also present in the two plant samples. Terpenoids protect plants from abiotic and biotic stress and possess antioxidant, anticancer, anti-inflammatory, antiseptic, astringent, antiplasmodial, diuretic, and digestive properties. Steroids, a subset of terpenoids, also function as growth hormones (Abeysinghe et al. 2021).

Thin Layer Chromatography (TLC) is used for analysis due to its excellent analytical technique for identifying various compounds. Figure 1. shows that phenolic compounds appear as black spots or bands on TLC plates under UV light at 254 nm (Figure 1.A) (Burman et al. 2019). All extracts had a reddish-brown stain on the TLC plates after being sprayed with cerium sulfate, indicating the presence of polyphenols like phenolic acids, tannins, and flavonoids (Figure 1.C) (Gwatidzo et al. 2018). Moreover, the purplish-blue patches (Figure 1.B) demonstrate blue fluorescence in UV 365 nm, verifying the presence of terpenoids in the extract.

These phytochemical compounds identified in the *A. columnaris* and *A. cunninghamii* twigs extracts may be responsible for the biological activities. In previous research, three biflavonoids were isolated from the dried leaves of A. columnaris collected from the Botanical Garden in Bogor, West Java, Indonesia. These compounds were identified as (i) 4',4''',7,7''-tetra-*O*-methylcupressuflavone; (ii) 4',7,7''-tri-*O*-methylcupressuflavone and; (iii) 4''',7-di-*O*-methylcupressuflavone (Kurniawanti et al. 2023). On the other hand, acetone extract of *A. cunninghamii* dried leaves contained compound (i), (iv) 7,4',4'''-tri-*O*-methylrobustaflavone; and (v) 7,7''-di-*O*-methylamentoflavone. It is the first report of compounds iv and v isolated from the species (data not shown). Five known compounds were isolated from *A. columnaris* and *A. cunninghamii* dried leaves, as shown in Figure 4.

Table 3. IC_{50} values of DPPH radical scavenging activity of *Araucaria columnaris* and *A. cunninghamii* twig extracts with different solvents.

Plants extract	IC_{50}* (µg/mL)	Antioxidant activity
ACA	57.85 ± 3.20	Moderately active
ACE	54.31 ± 1.72	Moderately active
ACEa	81.53 ± 1.79	Moderately active
ACuA	68.30 ± 3.28	Moderately active
ACuE	29.57 ± 1.11	Strongly active
ACuEa	53.62 ± 1.81	Moderately active
Ascorbic acid	14.74 ± 0.36	Strongly active

Notes: *The category of DPPH scavenging activity very strong activity: IC_{50} < 10 µg/mL; strong activity: 10 µg/mL < IC_{50} < 50 µg/mL; moderate activity: 50 µg/mL < IC_{50} < 100 µg/mL; weak activity: 100 µg/mL < IC_{50} < 250 µg/mL; and inactive IC_{50} > 250 µg/mL (Phongpaichit et al. 2007)

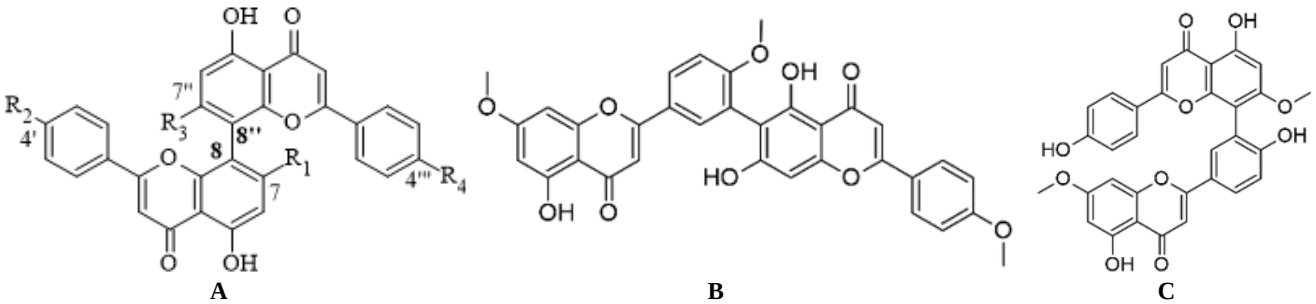

Figure 4. Structures of the biflavonoids identified in *Araucaria columnaris* and *A. cunninghamii* leaves. A. (i) R_1=R_2=R_3=R_4=CH_3; (ii) R_1=R_2=R_3=CH_3; R_4=H; (iii) R_1=R_4=CH_3; R_2=R_3=H; B. (iv); C. (v)

Plants with high phenol and flavonoid contents mostly have significant antioxidants. The Folin-Ciocalteu reagent for determining phenolic consists of phosphomolybdic acid ($H_3PMo_{12}O_{40}$) and phosphotungstic acid ($H_3PW_{12}O_{40}$), which reduced by the polyphenol compounds in the sample to a blue molybdenum-tungsten oxides complex (W_8O_{23} and Mo_8O_{23}). The intensity of molybdenum-tungsten oxides depends on the number of phenolic groups. The higher concentration of phenolic compounds caused an increased amount of phenolic ions, which reduced the heteropoly acid (phosphomolybdate-phosphotungstate) to the molybdenum-tungsten complex, resulting in blue hue (Michael et al. 2010; Fachriyah et al. 2020).

The $AlCl_3$ colorimetric method measured the total flavonoids, with quercetin serving as a standard. The principle of the $AICI_3$ colorimetric is forming a stable complex between C-4 carbonyl groups, followed by the reaction of C-3 or C-5 hydroxyl groups from flavones and flavonols with $AlCl_3$ reagent. $AlCl_3$ forms a stable complex with ortho-dihydroxy groups on flavonoids' A or B rings (Makuasa and Ningsih 2020). The standard curve of quercetin is presented in Figure 3. Table 2 summarizes that both plants' phenolics (TPC) and flavonoids (TFC) content are extracted in varying solvents. The results showed that the TPC of acetone extract of *A. columnaris* twigs (ACA) is the highest, followed by ethanol extract (ACE) and ethyl acetate extract (ACEa). The highest phenolic content of *A. cunninghamii* twigs was obtained in ACuA, followed by ACuE and AcuEa.

Previous studies have highlighted the importance of the extraction solvent, particularly its polarity, in determining the total phenolic and flavonoid concentrations in an extract. Variations in flavonoid content can also result from differences in plant varieties, growing conditions, soil nutrients, and climate. Furthermore, the phenolic and flavonoid content may vary depending on the plant part used and other factors influencing the plant's growth and development (Shanmugavel et al. 2018).

DPPH, a stable free radical that accepts an electron or a hydrogen radical to form a stable diamagnetic molecule, is widely used in radical-scavenging assay. In the DPPH radical scavenging experiment, antioxidants react with DPPH to produce yellow-colored 2,2-diphenyl-1-picrylhydrazine (Ionita 2021). Lu et al. (2014) stated that the level of discoloration indicates the capacity of antioxidants to scavenge radicals. Ascorbic acid was used as a standard to determine the percentage of DPPH inhibition because of its high antioxidant activity. The percentage of DPPH inhibition against the ascorbic acid concentration is presented in Figure 5. The lower the IC_{50} value of the samples, the higher the antioxidant activity.

The present study revealed that the acetone, ethanol, and ethyl acetate extract of *A. columnaris* and *A. cunninghamii* twigs contained steroids, triterpenoids, saponins phenolics, and flavonoids, which might correlate with the antioxidant activities. In this test, the ethanol extract of *A. cunninghamii* twigs (ACuEa) exhibited profound antioxidant activity (IC_{50} 29.57 ± 1.11 µg/mL). Meanwhile, other extracts showed moderate antioxidant activity. However, the antioxidant activity was lower than ascorbic acid (IC_{50} 14.737 ± 0.36 µg/mL), often used as a positive control because of its high antioxidant activity (Table 1). In other studies, the methanol extract of *A. cunninghamii* leaves from Cairo, Egypt, exhibited moderate antioxidant activity with an IC_{50} value of 53.7 µg/mL (El-Hawary et al. 2021), while the methanol extract of *A. cunninghamii* inner barks from the Himalayas, India, showed an IC_{50} of 53.52 ± 2.4 µg/mL (Kumar et al. 2020). Metabolic profiling of *A. cunninghamii* leaves from Cairo revealed the presence of phenolic acids, biflavonoids, and lignans (El-Hawary et al. 2021).

The ethanol extract of *A. columnaris* twigs derived from Taman Bunga Nusantara, Cianjur, Indonesia, has better antioxidant activity than the acetone extract of *A. excelsa* leaves from the National Research Centre Garden Cairo, Egypt, *A. cunninghamii* leaves from Cairo, Egypt and *A. cunninghamii* inner barks derived from Himalayan, India. This difference in antioxidant activity can be attributed to variations in the chemical composition of the extracts, such as the types and concentrations of bioactive compounds and the solvents used for extraction, which can influence the solubility and availability of antioxidant compounds. Furthermore, environmental factors and the plant's geographic origin may contribute to differences in metabolite profiles, affecting the overall potency of the antioxidant properties (Akhter et al. 2024).

The antioxidant activities of ACA, ACE, ACEa, ACuA, and ACuEa were similar to the methanol extract of *A. cunninghamii* barks from the Himalayas, India (IC_{50} of 53.52 ± 2.4 µg/mL) (Kumar et al. 2020) and the methanol extract of *A. cunninghamii* leaves from Egypt (IC_{50} of 53.7 µg/mL) (El-Hawary et al. 2021). However, all of these extracts, including ACuE, exhibited stronger antioxidant activity than the methanol, ethanol, and ethyl acetate extracts of *A. columnaris* leaves from Pakistan, which had IC_{50} values of 120.65, 150.9 and 170.65 µg/mL, respectively (Zaffar et al. 2014). Additionally, Gautam et al. (2014) reported that the antioxidant activity of an 80% methanol extract of *A. cunninghamii* leaves from the Botanical Garden of Guru Nanak Dev University, Amritsar, India, had an IC_{50} value of 181.897 µg/mL. This difference in antioxidant activity could be attributed to variations in the types and concentrations of bioactive compounds present in the extracts. Different geographic origins and environmental factors may also contribute to the observed differences in antioxidant potency (Figueiredo et al. 2008).

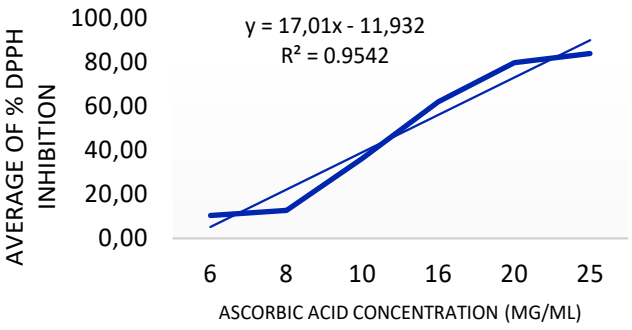

Figure 5. The curve of DPPH inhibition (%) against ascorbic acid

A previous study by Michael et al. (2010) showed that *A. excelsa* shoot methanol extract revealed the presence of seven phenolic compounds namely orientin, iso-orientin, vitexin, isovitexin, taxifolin, taxifolin 3-O-glucopyranoside, and gallic acid. Furthermore, the computational analysis indicated that the antioxidant potential of *A. columnaris* might also be attributed to the presence of hydrocarbons, diterpenes, phytosterol, phenolic acid, and flavonoids such as quercetin, myricetin, rutin, β-sitosterol acetate, abietic acid, agathic acid dimethyl ester, vanillic acid, palmitic acid, and methyl communate (Patial and Cannoo 2019). The presence of these compounds contributes to the antioxidant activity of both species, as these classes of compounds are known for their ability to neutralize free radicals and reduce oxidative stress. Free radicals are linked to several chronic illnesses, including diabetes, asthma, atherosclerosis, cancer, aging, and Alzheimer's and Parkinson's disease (Pham-Huy et al. 2008).

It has been established that the presence of flavonoids and phenolics in ethanol extract contributes to its higher potential to scavenge radicals than other extracts (Mehmood et al. 2022). In examining radical scavenging capacity, a trend can be observed that the more polar protic solvents were more effective at extracting the antioxidant components in the plant extract.

A previous study showed that the phenolic compounds are responsible for the antioxidant activity (Gautam et al. 2014). The antioxidant potential of the extract may also be affected by its flavonoid level. The OH group position significantly affects the flavonoids' capacity to inhibit DPPH free radicals. Flavonoids with catechol groups in their structure, such as quercetin, rutin, and flavanol group members, exhibit potent inhibitory effects. There is a significant correlation between antioxidant activity and the levels of tannic acid, flavonoids, and total polyphenols. (Patial and Cannoo 2019). Similarly, phenolic compounds and flavonoids in the twig extracts of *A. columnaris* and *A. cunninghamii* contribute to their antioxidant properties.

In conclusion, Phytochemical screening of *A. columnaris* and *A. cunninghamii* twig extracts using different solvents revealed the presence of phenolics, flavonoids, steroids, and triterpenoids in all extracts. Saponins were found exclusively in acetone extracts, while none contained alkaloids. The highest TPC of AC twigs was obtained in acetone (ACA), similar to that of ACu twigs. Regarding TFC, the order for AC twigs was ACA > ACEa > ACE, while for ACu twigs, it was ACuEa > ACuE > ACuA. ACuE exhibited strong antioxidant activity with an IC_{50} value of 29.57 ± 1.11 µg/mL, whereas the other extracts displayed moderate antioxidant activity.

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