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Original Research Article



Phytochemical screening and preliminary evaluation of antioxidant activity of three Indonesian *Araucaria* leaves extracts

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Abstract

Araucaria is the largest genus in Araucariaceae and is well known as an evergreen coniferous tree. Several species of *Araucaria* have been used as traditional medicinal plants. This study aims to determine the phytochemical constituents, and preliminary antioxidant activities of three Indonesian *Araucaria*, i.e. *A. hunsteinii*, *A. columnaris*, and *A. cunninghamii* leaves extracts. All the leaves of Indonesian *Araucaria* were macerated in acetone to give acetone extracts. A qualitative phytochemical screening method was used to determine the class of secondary metabolites in the three leaves extracts, including alkaloids, flavonoids, steroids, terpenoids, saponins, and tannins. In addition, the preliminary antioxidant activity of the corresponding extract was determined by spraying DPPH (2,2-diphenyl-1-picrylhydrazyl) to the TLC plate, which will turn a yellow spot as a positive result. The yield of acetone extract of *A. columnaris*, and *A. cunninghamii*, *A. hunsteinii* were 0.85%, 1.64%, and 3.07%, respectively. Phytochemical analysis showed that all leaves acetone extracts contained flavonoids, steroids, terpenoids, phenolics, and tannins. Furthermore, the strongest to the weakest antioxidant activities were summarized as follows *A. hunsteinii*, *A. columnaris*, and *A. cunninghamii*, sequentially. These preliminary results reveal that all Indonesian *Araucaria* leaves extracts are potential for further study.

Keywords *Araucaria*, *Araucariaceae*, phytochemical, antioxidant, DPPH

Introduction

Natural ingredients have been used as traditional medicine in Indonesia for hundreds of years. One of the natural resources used as medicinal ingredients is found in some plant's tissues. Plants on earth there are 374.000 species (Christenhusz and Bying 2016). In 2002, the Food and Agriculture Organization estimated that over 50,000 medicinal plants were utilized around the world (Schippmann et al., 2002). Furthermore, according to the WHO, traditional medicine is used by about 80% of the world's population, and medicinal plants are used by about two billion people (Smith-Hall et al., 2012). Plants are used in medications because they contain useful

compounds and most of the compounds contained in them are not known. These active compounds can function independently or together with other compounds that provide pharmacological effects.

Araucaria is one of the largest genera in the family Araucariaceae and is known as an evergreen coniferous tree. The genus *Araucaria* has 19 species of plants that are commonly used as ornamental plants. *Araucaria* is widespread in the southern hemisphere, including New Zealand, New Caledonia, South America, Southeast Asia, Australia, and the islands of the Southern Pacific (Kunzmann, 2007). In addition, several species of *Araucaria* have been used as traditional medicinal plants such as for emollients, antiseptics, and

treatment of various diseases such as kidney disease, respiratory infections, rheumatism, toothache, contusions, ulcers, and amenorrhoea, as well as for cicatrization of skin wounds (Aslam et al., 2013). In addition, *Araucaria* exhibits pharmacological activities as anti-inflammatory, antiviral, antibacterial, antifungal, antidepressant, anti-ulcer, anticancer, and antitumor (Aslam et al., 2013; Frezza et al., 2020).

There are 3 species of *Araucaria* in Indonesia, namely *Araucaria hunsteinii* K. Schum., *A. columnaris* (Frost. F.) Hook, and *A. cunninghamii*. *A. hunsteinii* is one of the endemic floras of Papua New Guinea (Kosay and Maturbongs 2019). *A. columnaris* is found in New Caledonia (Aslam et al., 2013). In comparison, *A. cunninghamii* grows and spreads naturally in Papua New Guinea, Australia, Queensland, and Papua (Setiadi and Susanto 2012). Several studies have reported the pharmacological effects of this *Araucaria* plant from various regions of the world. Extracts of twigs, leaves, and bark of *A. columnaris* have pharmacological activity as antioxidants (Michael et al., 2010; Patial and Cannoo 2019), antibacterial (Joshi et al., 2016; Verma et al., 2013; Zaffar et al., 2014), anticancer against human kidney cancer cells (Saranya et al., 2015). In addition, leaves extract, stem bark resin, and essential oil of *A. cunninghamii* have pharmacological activities as an antifungal (Sati and Joshi 2013), antioxidant (Gautam et al., 2014), antibacterial (Verma et al., 2014), and anticancer against Chang liver cells. However, studies on the pharmacological effects of *A. hunsteinii* have not been reported.

Various pharmacological activities are influenced by the content of secondary metabolites in it. The differences in the place of growth, soil conditions, temperature, light, and climate, are things that can affect the content of compounds in each plant. The active compounds resulting from secondary metabolites in plants are very diverse and can be classified into several groups, namely saponins, steroids, tannins, terpenoids, flavonoids, and alkaloids. There is no available scientific report on the chemical constituents and pharmacological effects of *Araucaria* species grow in Indonesia, especially the studies on leaves extract are still few so that they can be developed. As a result, the goal of this study was to investigate the secondary metabolite composition and antioxidant activity of the acetone extract of the leaves of three Indonesian *Araucaria* plants.

Materials and Methods

Plant Material Collection

The leaves of *A. hunsteinii*, *A. columnaris*, and *A. cunninghamii* were collected from the Bogor Botanical Garden, West Java, Indonesia.

Plant Extract Preparation

The leaves of *A. hunsteinii*, *A. columnaris*, and *A. cunninghamii* were taken and cut into small pieces, dried and later made as powder.

Preparation of Extract

Dried powdered leaves of Indonesian *Araucaria* (100 g) were extracted with acetone (500 mL, three times) for three days at room temperature, then filtered and concentrated to crude extract.

Qualitative Phytochemical Screening

Test for Alkaloids

Four drops of concentrated ammoniac were added to 50 mg of extract in 10 mL chloroform and agitated vigorously before filtering. To make a two-layer, add 2 M H₂SO₄ to the filtrate and shake thoroughly. The presence of alkaloids was then determined in the top layer (Shaikh and Patil 2020).

The Mayer Test

Two drops of Mayer's reagent were applied to 0,5 mL of filtrate by the sidewalls of the test tube; a white precipitate indicated the presence of alkaloids.

Wagner's Test

Two drops of Wagner's reagent were added to 0,5 mL of filtrate by the sides of the test tube, resulting in a reddish-brown precipitate, indicating the presence of alkaloids.

Dragendroff's Test

Two drops of Dragendroff's reagent were added by the sidewalls of the test tube to 0,5 mL of filtrate. The presence of alkaloids is indicated by a red precipitate.

Test for Steroid and Terpenoids

Liebermann-Burchard Test

Two drops of Liebermann-Burchard reagent were applied to 50 mg extract in 2 mL chloroform; a green to blue color shows the presence of steroid (Yadav and Agarwala 2011).

Salkowski Test

Two drops of strong sulfuric acid were added to 50 mg extract in 2 mL chloroform and mixed well. The presence of terpenoids is indicated by a red-brown color (Yadav and Agarwala 2011).

Test for Phenolic compounds and Tannins (Ferric Chloride test)

50 mg extract in 2 mL distilled water was heated, then two drops of 5% Ferric chloride solution were added, a dark blue or greenish-black color indicates the presence of phenols and tannins (Egbuna et al., 2019).

Test for Flavonoids (Shibata's test)

50 mg extract was boiled in 2 mL distilled water with a few magnesium granules and 0.05 mL concentrated HCl acid. The

solution was agitated, and then 5 mL of amyl alcohol was added. Flavonoids are identified by their red, yellow, or orange color (Shaikh and Patil 2020).

Test for Saponins

50 mg extract is heated in 2 mL distilled water, then shaken vertically for one minute in a test tube. It resulted in the creation of a 1 cm layer of foam, indicating that saponins were present (Yadav and Agarwala 2011).

Preliminary Antioxidant Activities

The preliminary antioxidant activity of each extract was determined by spraying DPPH (2,2-diphenyl-1-picrylhydrazyl) to the TLC plate. The extract was spotted on a silica plate, then put into a chamber containing the solvent $\text{CHCl}_3:\text{MeOH}$ (19:1), $\text{CHCl}_3:\text{MeOH}:\text{H}_2\text{O}$ (6:4:1), and $\text{CHCl}_3:\text{MeOH}:\text{H}_2\text{O}$ (7:3:1). The chromatogram was then dried and sprayed with a 0.002% DPPH solution. The chromatogram was examined after 30 minutes, and the compound, which was an active antioxidant, showed a yellow color (Ahmad et al., 2017)

Results and Discussion

The extraction technique that was used in this experiment was maceration. It is an easy operation and required some simple equipment. In addition, the maceration process could be performed without heating so that there was no damage to the secondary metabolite in the analyte (Meigaria et al., 2016). Acetone was chosen as a solvent in the maceration process because it is an aprotic polar organic solvent which suitable for extracting phenols, flavonoids, and terpenoids. Secondary metabolites are readily soluble in acetone solvents such as chlorophyll and some polyphenolic compounds that function as antioxidants (Taroreh et al., 2015). Acetone extracted less tannin than polar-protic solvents like methanol or ethanol (Haryono et al., 2012). Acetone removes fewer metabolites but is more selective than other polar solvents, making separation easier (Egbuna et al., 2019). The percentage yield of the three Indonesian *Araucaria* leaves acetone extracts is shown in Fig. 1. The extract of *A. hunsteinii* had the highest percentage of yield when compared to *A. cunninghamii* and *A. columnaris*.

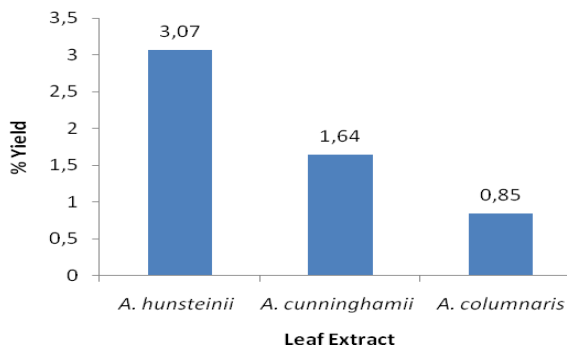


Fig. 1 Graph of the third yield of Indonesian *Araucaria* leaves acetone extract.

Based on Fig. 2, The TLC profile (using 10% H_2SO_4 spray reagent) showed that *A. hunsteinii* had more spots when the more polar eluent was used, followed by *A. cunninghamii* and *A. columnaris*. A high yield value indicates many bioactive components (Dewatisari et al., 2017). These results also align with the results of this study that the higher yield showed more compounds on the TLC plate. Therefore, it could be assumed that the bioactive components in *A. hunsteinii* were more than the other two extracts.

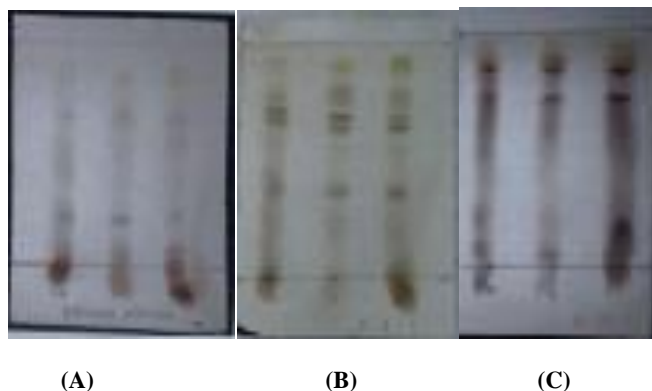


Fig. 2 TLC profile by spraying 10% H_2SO_4 using eluent (A) $\text{CHCl}_3:\text{MeOH}$ (19:1), (B) $\text{CHCl}_3:\text{MeOH}:\text{H}_2\text{O}$ (7:3:1), and (C) $\text{CHCl}_3:\text{MeOH}:\text{H}_2\text{O}$ (6:4:1)

Phytochemical screening is a preliminary step in determining the content of chemicals in the plants being studied. Table 1. shows the results of phytochemical testing on *A. hunsteinii*, *A. cunninghamii*, and *A. columnaris* leaves extracts. Based on Table 1, the alkaloid and saponin test showed negative results on the three plant extracts. It was indicated by the results of the test using Mayer's reagent, which did not show a white precipitate of $[\text{alkaloid}^+]_n[\text{HgI}_4]_n$ (Sugita et al., 2020), no brown precipitate of potassium-alkaloid by Wagner's reagent (Pardede et al., 2013), and no red precipitate of $[\text{alkaloid}^+]_n[\text{BiI}_4]_n$ on the Dragendorff's test (Raal et al., 2020). Moreover, the three extracts showed negative results in the saponin test by producing no foam, indicating compound. The foam indicates glycosides, making a foamy solution in water after hydrolyzed into glucose and its aglycon (Pardede et al., 2013). The flavonoid test of those three plant extracts results in an orange solution on the amyl alcohol layer, indicating a positive result. The orange color indicates the formation of flavilium salt due to the reduction of the benzopyron ring in the flavonoid structure by Mg and HCl as shown in Fig. 3 (Ergina et al., 2014). Steroids and terpenoids test showed positive results, with the green and brown solution for steroids and terpenoids, respectively. Steroids and terpenoids can be dehydrated by adding strong acids and form salts that give some colour reactions (Agust et al., 2014). The reaction between extract of those three plants with FeCl_3 produced a dark green colour indicating the presence of phenols and tannins. The formation of color and precipitate was caused by the complex formation of Fe^{3+} ions with phenol group (Ergina et al., 2014).

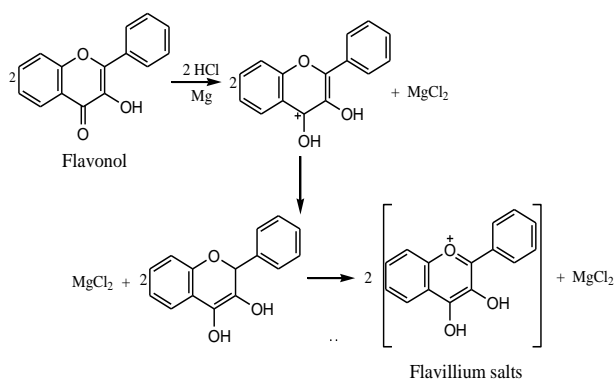


Fig. 3 The reaction mechanism of the flavonoid test

Compared to Table 1, previous studies on the phytochemical contents of *Araucaria* plants from several regions have been reported. For example, *A. cunninghamii* leaves oil extract from Amritsar (India) contains flavonoids, phenols, saponins, and tannins (Gautam et al., 2016), while *A. cunninghamii* leaves from Nancy, France, contain terpenoids, which are sesquiterpenoids and diterpenoids (Lu et al., 2013). Previous phytochemical studies have shown that the methanolic extract of *A. columnaris* leaves from Tamil Nadu, India, contain alkaloids, phenols, flavonoids, and tannins, while the chloroform extract contains flavonoids, tannins and, alkaloids (Banerjee et al., 2014). In addition, the acetone extract of *A. columnaris* leaves contains flavonoids, steroids and tannins antibacterial (Verma et al., 2014). Scientific studies of *A. hunsteinii* are still rare. A previous study reported that the leaves extract of *A. hunsteinii* contains terpenoid compounds (Frezza et al., 2020). Based on the phytochemical results of this study, it was found that the acetone extract of the leaves of *A. hunsteinii* had flavonoids, steroids, terpenoids, and tannins.

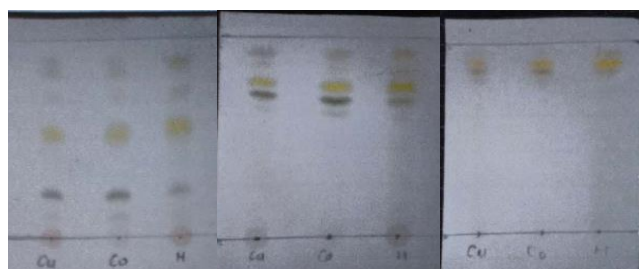


Fig. 4 TLC profile of the preliminary antioxidant activity test by spraying DPPH, using eluent (A) CHCl₃:MeOH (19:1), (B) CHCl₃:MeOH:H₂O (7:3:1), and (C) CHCl₃:MeOH:H₂O (6:4:1)

Antioxidants are compounds that can stop free radical propagation reactions, both from metabolic byproducts that occur in the body and those from the environment, such as cigarette smoke, air pollution, radiation, and certain drugs (Meigaria et al., 2016). In addition, antioxidants are also defined as inhibitors of oxidation by reacting with reactive free radicals to form relatively stable unreactive free radicals.

DPPH is a reagent used to test antioxidant activity and is a purple stable free radical compound. When a purple DPPH solution reacts with an electron donor compound, the DPPH will be reduced, causing the purple colour to fade and turn yellow from the picryl group (Prayoga, 2013). So that when compounds that are antioxidants react with DPPH, the yellow colour will appear due to free radical inhibition. The stronger the intensity of the yellow color, the more compounds that act as antioxidants. Fig. 4 shows that among the three Indonesian *Araucaria* leaves acetone extracts, *A. hunsteinii* produced a strong yellow spot intensity, followed by *A. columnaris* and *A. cunninghamii*. This result shows that the antioxidant compounds of *A. hunsteinii* are higher than the other two *Araucaria* plant extracts.

Table 1. The three acetone extracts of Indonesia *Araucaria* were subjected to a qualitative test for preliminary phytochemical analysis.

| Test | Hu | Cu | Co |
|------------|----|----|----|
| Alkaloids | - | - | - |
| Flavonoids | + | + | + |
| Steroids | + | + | + |
| Terpenoids | + | + | + |
| Saponins | - | - | - |
| Phenolics | + | + | + |
| Tannins | + | + | + |

Hu: *A. hunsteinii*; Cu: *A. cunninghamii*; Co: *A. columnaris*

One of the secondary metabolites that act as antioxidant compounds is phenolic compounds (Gautam et al., 2016). Several studies have reported the total phenolic content of the *Araucaria* plant. For example, the total phenolic compound in 80% methanol extract of *A. cunninghamii* leaves was 36.111 mg GAE/g DPE (Gautam et al., 2014), while the methanol extract of *A. columnaris* leaves was 79.73 ± 0.75 mg GAE/g DPE (Patial and Canoo 2020). Based on the study of the total phenol content in both *Araucaria* plants, the number of antioxidant compounds possessed by *A. columnaris* was more than that of *A. cunninghamii*. In line with the analysis results, the antioxidant potential of *A. columnaris* was more potent than that of *A. cunninghamii*. While the total phenol of *A. hunsteinii* has not been reported yet, the results of the initial analysis of antioxidant activity indicate that *A. hunsteinii* has the potential to have the strongest antioxidant activity.

Conclusion

Phytochemical analysis showed that all leaves acetone extracts contained flavonoids, steroids, terpenoids, phenolics, and tannins. Furthermore, the strongest to the weakest antioxidant activities were summarized as follows *A. hunsteinii*, *A. columnaris*, and *A. cunninghamii*, sequentially. These preliminary results reveal that all Indonesian *Araucaria* leaves extracts are potential for further study.

Conflict of Interest

There are no conflicts of interest among the authors in this inquiry.

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