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Phytochemical Screening and Antioxidant Activity of Phenolic Compound From *Anacardium Occidentale* Leaf Extract

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Abstract. *Indonesia is known for its high biodiversity, particularly in herbal medicine, with cashew trees being one of the most used plants. Cashew leaves contain proteins, fats, carbohydrates, calcium, and vitamins B1 and B2. They are used as herbal medicine in various parts of Indonesia, including kidney disease treatment, mouthwash, and kanker disease drugs. The ethanol extract from cashew leaves contains secondary metabolite compounds such as phenolic, flavonoids, and tannins. The bioactivity of cashews includes antiviral, antifungal, antibacterial, and anti-inflammation properties. The cardanol compound from cashew leaves has the highest antioxidant activity compared to cardol and anacardic acid, with IC50 values of 3,22±0.07 µg/ml and 0,06±0.01 µg/mL, respectively. This research aims to isolate phenolic compounds from cashew leaves, identify their structure, and measure antioxidant activity.*
The study analyzed the phytochemical screening of A. occidentale leaf extracts, revealing variations in their components. Hexane extracts were positive for tannin and terpenoid compounds, while dichloromethane extracts were positive for all compounds except saponin and cardiac glycoside. Ethyl acetate extracts were positive for terpenoids and flavonoids. Methanol extracts were positive in nearly all assays except for anthraquinone. The isolated compound from A. occidentale leaf yielded a yellow powder with a melting point of 247-248°C. The crude extract had an IC50 value of 59.19 ± 2.38 µg/mL, while the phenolic separated element had a value of 191.815 ± 1.07 µg/mL. Flavonoids are a group of antioxidants that act as chelators and scavengers of free radicals, targeting hydroxyl and peroxyl radicals, superoxide anions, and peroxynitrites. The Anacardiaceae family species contain numerous phenolic compounds with potent antioxidant properties, which can help manage elevated free radical generation conditions. Further research is needed to determine the specific phenolic chemicals responsible for the antioxidant activity of the species and evaluate their contribution to this activity. Anacardium occidentalale L. leaves, seeds, and stem bark extracts have been found to have antioxidant and antimicrobial properties. The traditional herbal medicine of Indonesia, Jamu, is a valuable source of antioxidants and prooxidants.

Keywords: *Anacardium occidentale, cashew, antioxidant, DPPH*

INTRODUCTION

Indonesia has the highest biodiversity, notably in herbal medicine (Aracelli et al., 2016). Indonesia boasts rich and distinctive vegetation since it is located near the equator, between two large continents, and comprises hundreds of islands with varying temperatures. Indonesian rainforest encompasses 143 hectares and is "home" to about 80% of herbal vegetation (Huang et al., 2021). Meanwhile, Huang (2021) discovered 1845 species of Indonesian plants with promise for herbal medicine. The cashew tree (Anacardium occidentale) is one of the plants used in herbal medicine. Cashews grow in eastern Indonesia, including Nusa Tenggara Barat, Nusa Tenggara Timur, Sulawesi Selatan, and Sulawesi Tenggara (Ambarwulan et al., n.d.). Cashews include proteins, lipids, carbs, calcium, and vitamins B1 and B2 (Okoliko Victor et al., 2013). Almost every component of the cashew may be utilized as a herbal remedy. People in Ogan Komering Ulu, South Sumatra, utilize cashew leaves to treat renal problems

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(Ambarwulan et al., n.d.). Indonesians use cashew stem bark as a mouthwash and a treatment for kanker disease.

Cashew leaf ethanol extract includes secondary metabolite components such as phenols (Pereira et al., 2020), flavonoids (Aracelli et al., 2016), and tannins (Khayruzamri et al., 2023). Cashews have been shown to have antiviral, antifungal, antibacterial, and anti-inflammatory properties (Andrade et al., 2023; Costa et al., 2020; da Silva et al., 2016; Tan & Chan, 2014). Da Silva (2016) discovered that ethanol extracted from cashew stem bark inhibits Streptococcus mutans. Cashews' antioxidant activity is primarily derived from nut extract (da Silva et al., 2016) and shell liquid (Sudjaroen et al., n.d.).

Aly (2022) showed that the n-hexane extract of cashew nuts possesses antioxidant activity with an IC50 of 0.60 mM (Aly et al., 2022). Tan and Chan (2014) measured antioxidant activity in a cashew nut shell using the 2,2'-azino-bis (3-ethyl-benzhithiazoline-6-sulfonic acid) (ABTS) and 1,1-diphenyl-2-picrythydrazyl (DPPH) techniques. The cardanol compound exhibits the most robust antioxidant activity compared to cardol and anacardic acid, with IC50 values of 3,22±0,07 µg/ml from the DPPH technique and 0,06±0,01 µg/mL from the ABTS method.

Many illnesses are caused by increased reactive oxygen species (ROS) in the human body; hence, research into antioxidant activity is necessary. ROS is a radical species produced in the human body. ROS results from cell metabolism that has positive and negative effects depending on its concentration. In normal conditions, ROS generation and intracellular activity are balanced. According to Khayruzamri et al. (2023), oxidative stress is an equilibrium of pro-oxidants and antioxidants, outnumbering antioxidants. This study attempts to extract phytochemicals from cashew leaves and assess antioxidant activity.

RESEARCH METHOD

All sample studies are conducted using an aluminum silica gel 60 GF254 thin-layer chromatography plate from MERCK—sigma-Aldrich silica gel 60 G and 1,1-diphenyl-2-picrythydrazyl (DPPH) powder. The chemical was evaluated using an infrared spectrophotometer and UV-Vis spectrophotometer. The plant specimens were Purchased from a cashew plantation in Pare-Kediri. The plants were desiccated and ground into powder. Phytochemical screening techniques modify the sample preparation as Khayruzamri et al. (2023) described. Four tubes, each holding 200 g of powdered material, were extracted with 500 mL of different solvents: n-hexane, dichloromethane, ethyl acetate, and methanol. The extraction process will occur at room temperature for 72 hours, split into three intervals of 24

hours each. The extract underwent filtration and concentration to its initial volume using a rotary evaporator at 40 degrees Celsius. The samples contained n-hexane, dichloromethane, ethyl acetate, and methanol.

Phytochemical screening fehling's test every different extract weighing 0.5 g was dissolved using a water bath. Two millimeters of the solution were added to 1 mL of each of Fehling's solutions in individual test tubes. The mixture was stirred and heated in a water bath for 10 minutes. The brick-red precipitate that happens indicates the existence of a reducing sugar. Anthraquinones examination 0.5 grams of each sample were placed in a 5 mL chloroform tube. The mixture was stirred for 5 min. Each extract was strained and combined with the same amount of 10% ammonia solution volume. The presence of A pink-violet or red hue in an ammoniacal layer showed the presence of anthraquinone.

Flavonoids examination, acetone eliminated tannins from every 0.5 g extract. After evaporating the acetone in a water bath, The residual material was dissolved in warm water. Every mixture was filtered while it was still hot. The filter was cooled, and 5 mL of a 20% NaOH solution was added. A yellow liquid indicated the presence of flavonoids. Analysis of tannins 0.5 grams of each extract were heated in ten milliliters of water in a test tube and afterward filtered. 15% ferric chloride was added and observed for a blue-black color, which signifies the presence of hydrolyzable tannins. The Salkowski test identifies the existence of terpenoids. Each 0.5 g extract was mixed with two cc of chloroform. 3 ml of high-concentration sulfuric acid (H2SO4) was added carefully to create a separate layer. The reddish-brown hue of the interface shows the existence of terpenoids. Cardiac glycosides evaluation with the Keller-Killiani test aach 0.5 g sample was combined with 5 ml of glacial acetic acid and one drop of ferric chloride solution. A milliliter of solid sulfuric acid was dispensed underneath. A brown ring at the interface signified the existence of a deoxysugar specific to cardenolides.

The powdered substance was extracted at ambient temperature for 72 hours in 20 liters of methanol. The extract was concentrated back to its original volume by filtering and using a rotary evaporator at 40 degrees Celsius. The plant's crude extract produced a yield of 7.6%. Antioxidant testThe sample's ability to scavenge radicals was tested against 2,2-Diphenyl-1-picryl hydroxyl radical (Sigma-Aldrich) using UV spectrophotometry at 517 nm. The solution contains a 10 mg sample dissolved in 1 mL of methanol. 33 microliters of the sample solution was combined with 1 milliliter of DPPH solution. The mixture was incubated at 37ºC for 20 minutes. Antioxidant activity was evaluated using a UV-Vis spectrophotometer. The solution contains 33 µL of methanol combined with 1 mL of DPPH solution. Ascorbic acid functioned

as the antioxidant benchmark. The inhibition percentage is determined using the following formula:

$$Inhibition, = \frac{Ab - As}{As} \times 100\%$$

Ab = blanko absorbance

As = sample absorbance

RESULT AND DISCUSSION

Phytochemical screening of plant materials

Table 1 displays the results of the phytochemical examination on the plants under investigation. Differences in phytochemical components were observed among the four extracts analyzed. Hexane extracts indicated the existence of tannin and terpenoid components. Dichloromethane extracts test positive for all compounds except saponin and cardiac glycoside. The ethyl acetate extract showed significant positive outcomes, specifically for terpenoids and flavonoids. The methanol extracts showed positive results in nearly all assays except for anthraquinone. As mentioned, flavonoids and tannins found in all plants are probably accountable for their capacity to eliminate free radicals. Flavonoids and tannins are phenolic chemicals in plant phenolics, important antioxidants that scavenge free radicals.

Table 1. Phytochemical screening of n-hexane extract, dichloromethane extract, ethyl acetate extract, and methanol extracts of *A. occidentale* leaf.

Phytochemical constituents	**Results**			
	n-Hexane extracts	**Dichloromethane extracts**	**Ethyl acetate extracts**	**Methanol extracts**
Tannin	+	+	-	+
Saponin	-	-	-	+
Cardiac glycoside	-	-	-	+
Reducing sugar	-	+	-	+
Terpenoid	+	+	+	+
Flavonoid	-	+	++	++
Anthraquinon	-	+	-	-

++ = present (strong), + = present, - = absent

Antioxidant activities

The DPPH test evaluates the responsiveness of the test compounds to a stable free radical. DPPH is a nitrogen-free radical that can be deactivated by antioxidants that donate

hydrogen, transforming it into a non-radical form (Prior, Wu, & Schaich, 2005). DPPH shows a significant absorption peak at 517 nm in the visible spectrum. When a scavenger causes the unpaired electron in a free radical to pair up, the absorbance declines, resulting in the DPPH solution changing color from deep violet to light yellow as it loses its hue. The reduction in absorbance measurement indicates the antioxidant capacity of the extract in neutralizing radicals (Ajileye et al., 2014). The antioxidant capabilities of the raw extract and each component were measured using a DPPH test. The crude extract's IC50 value is 59.19 ± 2.38 µg/mL, the phenolic isolated compound has a value of 191.815 ± 1.07 µg/mL, and the positive control (ascorbic acid) has a value of 5.26 ± 0.74 µg/mL (Juliet et al., n.d.; Sassi et al., 2022; Togola et al., 2020).

Phenolic chemicals are predominantly present in plants and fruits. They are commonly found in plant material and can sometimes be present as glycosides. They have antioxidant properties by acting as chelators and scavengers of free radicals, particularly targeting hydroxyl and peroxyl radicals, superoxide anions, and peroxynitrites. Flavonoids are a group of antioxidants consisting of flavonols, anthocyanins, isoflavonoids, flavanones, and flavones. The chemical sub-groups share a common diphenyl propane (C6C3C6) structure as described by Andrade et al. (2023) and Pereira et al. (2020).

Flavanones and flavones are frequently co-occurring in fruits as a result of certain enzymes, although flavones and flavonols do not exhibit this relationship and are seldom seen together. Plants high in flavanones do not contain anthocyanins. Flavonoids gain antioxidant abilities by utilizing phenolic hydroxyl groups connected to ring structures, functioning as reducing agents, hydrogen donors, singlet oxygen quenchers, superoxide radical scavengers, and metal chelators. They stimulate antioxidant enzymes, decrease a-tocopherol radicals, block oxidases, lessen nitrosative stress, and elevate uric acid levels and low molecular weight molecules. Key flavonoids include catechin, catechin-gallate, quercetin, and kaempferol (Andrade et al., 2023; Huang et al., 2021).

CONCLUSIONS

Anacardiaceae family species contain many phenolic compounds and exhibit strong antioxidant properties. Thus, they can manage various conditions characterized by elevated free radical generation. Additional research is required to determine the specific phenolic chemicals responsible for the antioxidant activity of the species and to evaluate their contribution to this activity.

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