Antioxidant Activity of *Inocarpus fagifer* Seed Extract Using Non-Polar Solvents

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**Abstract.** The Gayam plant (*Inocarpus fagifer*) is a crucial part of tropical ecosystem conservation. This plant, a member of the legume family, can grow up to 30 meters tall, and its fruit is commonly known as Gatep. Scientific studies have shown that the leaves, bark, and seeds of the Gayam plant contain compounds with both antioxidant and antimicrobial properties. The maceration process produced a thick extract of Gayam seeds with a distinctive aroma and a dark brown color in DCM solvent and orange in n-hexane solvent. In addition, the yield was 2.97% in DCM solvent and 2.01% in n-hexane solvent. Inhibition (%) of Gayam seed extract was 14.16% in DCM solvent and 6.13% in n-hexane solvent.

# INTRODUCTION

Tropical ecosystems are among the world's richest sources of biodiversity, providing terrestrial habitats that play a crucial role in maintaining Earth's highly diverse and productive biological variety. Through the significant contributions of their flora and fauna, these ecosystems help sustain global ecological balance. However, Indonesia, as an integral part of tropical ecosystems, faces serious environmental threats, particularly in relation to species extinction and habitat degradation, which lead to a decline in biodiversity. Rapid population growth has driven an increase in basic needs, triggering land-use changes involving forests, rice fields, and community gardens, carried out by both the government and private sector. These impacts are highly detrimental, causing simultaneous declines in the populations of various plant, animal, and microbial species. As a result, Indonesia has been designated as one of the global priority areas for biodiversity conservation [1].

One key component of this conservation strategy is the Gayam plant (*Inocarpus fagifer*), which plays a strategic role in maintaining the balance of tropical ecosystems. This plant contributes significantly to addressing the environmental and economic challenges faced by Indonesia. As the sustainability of tropical ecosystems becomes increasingly threatened, the preservation of the Gayam plant stands out as a fundamental pillar of conservation efforts.

The Gayam fruit, also known as Gatep, belongs to the legume family (Fabaceae) and can grow up to 20–30 meters in height. The fruit is flat and kidney-shaped, with a tough outer skin and a smooth surface. Gayam trees are classified as perennials, and their fruits have a relatively hard texture and lack ovaries, unlike most other fruits. The seeds of the Gayam contain sap and are encased in a hard outer shell. When unripe, the fruit is green, turning yellow or brown as it ripens. Gayam trees can grow as tall as 30 meters, with trunk diameters reaching up to 65 cm. The trunks often feature irregular grooves and may develop buttress roots, with drooping branches that offer various potential benefits. Scientific studies have reported that Gayam leaves, bark, and seeds all have antioxidant and antimicrobial properties [2].

Antioxidants are compounds that prevent or slow down the oxidation of lipids and other biomolecules. They do this by stopping the start or spread of oxidative chain reactions. These compounds are crucial for protecting cells from free radical damage, as they balance the creation and removal of these radicals. By neutralizing them, antioxidants prevent further cellular damage and can lower the risk of various diseases. Ultimately, antioxidants are important for their ability to suppress free radical activity in both food and biological systems [3].

In the food industry, antioxidants are used to prevent food from going rancid. While synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are effective, their use has raised concerns due to potential side effects, including toxicity and even links to cancer. Because of this, plants are now seen as a safer and more effective alternative to these synthetic options [4-6].

Antioxidant activity is a key metric for characterizing compounds and food samples, whether natural or synthetic. It measures their ability to neutralize or scavenge free radicals, which are harmful to biological systems. The presence of these protective compounds is directly related to a substance's antioxidant power. Various methods exist to test for this activity, with the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays being among the most common [7, 8].

# MATERIALS AND METHOD

**Materials**

The primary material used was Gayam seeds, which were collected from Bantul, Special Region of Yogyakarta. Furthermore, other materials used for extraction include n-hexane (Merck, Germany), DCM (Merck, Germany), gallic acid, and DPPH reagent (Rey’s Laboratory).

# Maceration Process

The Gayam seeds (1 kg) were collected, sorted, and cleaned. The seeds were then cut into smaller pieces and air-dried at room temperature. They were ground into a fine powder using a grinder with 60-mesh. Finely ground Gayam seed powder was weighed at 100 grams and macerated in a jar with the addition of 500 mL each of *n*-hexane and DCM (dichloromethane) for 48 hours on the first day. The *n*-hexane and DCM macerates were each filtered using filter paper. The obtained filtrates were measured using a measuring cylinder, followed by remaceration by adding 500 mL each of *n*-hexane and DCM on the second day to obtain macerates I and II. All the macerates were combined into a single container, and evaporation was performed using a rotary vacuum evaporator, yielding a thick extract.

# Antioxidant Assay

The ability of Gayamseed extract to scavenge DPPH radicals was followed by a slightly adjusted version of the method from Lestari et al. [7]. The test solution was prepared by dissolving the extract in methanol at a concentration of 10 mg/mL. For the reaction, 33 µL of this extract was added to 1 mL of DPPH solution. The mixture was then incubated at 37°C for 20 minutes before its absorbance was read at 517 nm on a UV-Vis spectrophotometer. A blank was prepared with methanol instead of the extract, and gallic acid was used for comparison as a positive control. The percentage of DPPH radical scavenging activity was calculated using Equation 1:

DPPH radical scavenging activity (%) = (Eq. 1)

Extracts showing high DPPH scavenging activity were further analyzed to determine their IC₅₀ values (μg/mL). The IC₅₀ was calculated using linear regression from a plot of extract concentrations versus the average percentage of antioxidant activity, based on triplicate measurements. The IC₅₀ value shows the concentration of the extract needed to neutralize half of the DPPH radicals, which is a measure of its antioxidant effectiveness [8].

# RESULT AND DISCUSSION

**Extraction of Gayam Seed**

The extraction process was carried out using the maceration method. An interaction occurs between the solvent and the simplicial during maceration, leading to a pressure difference between the inside and outside of the cells. This causes groups of secondary metabolite compounds present in the cytoplasm to dissolve in the solvent and be extracted homogeneously from the Gayam seeds. This method of extraction is beneficial because it's simple, uses inexpensive equipment, and is effective at pulling out natural compounds [7, 9]. The maceration process produced a thick extract of Gayam seeds with a distinctive aroma and a dark brown color in DCM solvent and orange in *n*-hexane solvent (Figure 1). In addition, the yield was 2.97% in DCM solvent and 2.01% in n-hexane solvent (Table 1). The yield of the Gayam seed extract was significantly affected by the extraction temperature, type of solvent, extraction time, and the ratio of solvent to material [10].

A close-up of a scale

AI-generated content may be incorrect.A scale with a blue screen

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**FIGURE 1**. Gayam seed extract using different solvent

**TABLE 1**. Color and % Yield of Gayam Seed Extract

|  |  |  |
| --- | --- | --- |
| **Sample in solvent** | **Color** | **Yield (%)** |
| DCM | Brown | 2.97 |
| *n*-hexane | Orange | 2.01 |

# Inhibition of Gayam Seed Extract

The antioxidant activity test of each extract with different solvents was carried out using the DPPH method at a maximum wavelength of 517 nm, using several concentration variations, namely 10,000 ppm, with gallic acid as a comparison solution (control). The use of a comparison solution was intended to determine the absorbance of DPPH (radical) in reducing the sample. DPPH compound is a stable free radical that is purple in color. When reduced, the free radical will turn yellow (diphenyl picryl hydrazine), functioning to measure single electron transfer activity as well as to measure radical scavenging activity.

The sample solution and DPPH reagent mixture were incubated at 37°C for 30 minutes. DPPH is an enzyme with an active site that binds substrates in reactions to produce a product, while antioxidants are inhibitors that can bind to the enzyme, thereby stabilizing it. Therefore, an optimum temperature is required for the enzyme to function optimally. The reaction's absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer, where the UV-Vis spectrophotometer is an instrument using spectrophotometric techniques in the ultraviolet and visible light regions. This instrument is used to measure the absorption of ultraviolet or visible light by a substance in solution form. The brighter the color of the dissolved particles, the lower the absorbance value obtained [10].

The interaction between antioxidants and DPPH occurs when the antioxidants in the sample can neutralize the free radicals of DPPH used. The antioxidant compounds will act by donating their hydrogen atom to DPPH radicals. This process changes the test solution's color from purple to yellow, which indicates the antioxidant's effectiveness at neutralizing the DPPH free radicals through electron pairing [11].

A graph of a number of blue rectangular objects

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**FIGURE 2. Inhibition (%) of Gayam Seed Extract**

The DPPH inhibition of Gayam seed extract reached 14.16% in DCM solvent and 6.13% in *n*-hexane solvent, suggesting that the active compounds in Gayam seeds are more soluble in polar solvents. Based on existing studies, the dichloromethane extract showed an IC50 value of 230 ppm, which is also considered moderate antioxidant activity. In another study, the *n*-hexane extract from Gayam seeds has an IC50 value of 156 ppm, indicating a moderate level of antioxidant activity. Gallic acid has an IC50 value of 0.39 ppm as a positive control.

Dichloromethane (DCM) is a semi-polar solvent that's capable of pulling out a broader range of substances from Gayam seeds than non-polar n-hexane. A GS/MS analysis of the DCM extract would probably show all the compounds from the n-hexane extract, plus additional compounds that are slightly more polar. These would likely include phenolic compounds, alkaloids, flavonoids, and various other semi-polar substances like phthalates. The use of n-hexane, a non-polar solvent, for extracting Gayam seeds is expected to yield primarily non-polar compounds. Consequently, GS/MS data from this extract would probably show a significant presence of fatty acids and their derivatives—a common feature in seeds rich in fats and oils. Other likely components include plant sterols (like beta-sitosterol), waxes, long-chain hydrocarbons, and non-polar terpenoids such as squalene.

According to Mazza et.al, the factor that causes the low antioxidant activity of flavonoid compounds in DCM extract is that the flavonoid compounds in the extract are not in a pure form, so the flavonoid compounds present in the extract may still be bound to glycoside groups. This is because glycoside groups bound to flavonoids can reduce antioxidant activity. In addition, the low antioxidant activity in the extract can be seen from the phytochemical content of each organ [12]. The seed extract of the Gayam plant contains flavonoids, alkaloids, and carbohydrates ranging from 75.79% to 77.70%, making them a potential alternative food source. In addition to carbohydrates, Gayam seeds also contain 7% fat, 10% albumin, and 2.5% ash, as well as protein ranging from 10.54% to 11.64%, moisture content of 4.09% to 6.53%, fat content of 2.26% to 2.50%, ash content of 2.95% to 4.04%, and crude fiber ranging from 0.83% to 1.13% [13].

The ethanol extract from Gayam seeds exhibits strong antioxidant activity with an IC50 value of 66.03 ppm. Meanwhile, the methanol extract from Gayam seeds also shows very strong activity with an IC50 value of 31.24 ppm. Methanol is more polar than ethanol. It is generally more effective at dissolving and extracting the highest concentration of the most potent polar antioxidant compounds. The GC/MS data for Gayam seed extracts using methanol and ethanol would primarily show a high concentration of phenolic acids and flavonoids, which are the main contributors to their strong antioxidant properties. The exact profile and relative abundance of these compounds would differ slightly between the two solvents, reflecting their different polarities. The extract is composed of thirteen different components: one flavonoid (homopterocarpine), eight fatty acids and their esters (including palmitic acid, linoleic acid, and ethyl oleic), and four compounds that have not yet been identified [11].

# CONCLUSION

A thick extract of Gayam seeds with a distinctive aroma and a dark brown color in DCM solvent and orange in n-hexane solvent. In addition, the yield was 2.97% in DCM solvent and 2.01% in *n*-hexane solvent. Inhibition (%) of Gayam seed extract was 14.16% in DCM solvent and 6.13% in *n*-hexane solvent. The factor that causes the low antioxidant activity of flavonoid compounds in DCM extract is that the flavonoid compounds in the extract are not in a pure form, so the flavonoid compounds present in the extract may still be bound to glycoside groups.

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