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# Antioxidant Activity Comparison of Purnajiwa Fruit (*Kopsia arborea Blume*.) Extracted Using Different Solvents And Methods

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**Abstract:** The choice of extraction method can significantly influence the phytochemical profile and antioxidant activity of the obtained extracts. Purnajiwa (Kopsia arborea Blume.) is a potential medicinal plant that can be developed into traditional medicine. Purnajiwa has been the subject of several studies focusing on its phytochemical composition and antioxidant activity. This study was experimental research to compare the antioxidant activity (IC50) of purnajiwa extracted using two different methods (maceration and soxhlet) and solvents (ethanol and methanol). Data were analyzed statistically using one-way and two-way ANOVA to compare and evaluate the differences in the data with a 95% confidence level. Research showed that methanol and soxhlet produced extract with lower IC50 than ethanol. Additionally, using methanol solvent with the soxhlet method produced the lowest IC50, indicating the highest antioxidant activity compared to other groups. In conclusion, using methanol and soxhlet is the optimal method and solvent in purnajiwa extraction.

**Keywords:** ethanol; inhibitory concentration 50% (IC50); maceration; methanol; Soxhlet.

## 1. Introduction

In plant extract analysis, the choice of extraction method can significantly influence the phytochemical profile and antioxidant activity of the obtained extracts. Several studies have delved into comparing the outcomes of different extraction techniques, mainly focusing on maceration and soxhlet extraction methods. The selection of extraction methods and solvents in phytochemical studies significantly influences the composition and yield of bioactive compounds obtained from plant materials. Several studies have underscored the importance of choosing appropriate solvents and extraction techniques to maximize the recovery of phytochemicals with desired properties. For example, Nawaz et al. (2019) investigated the impact of solvent polarity and extraction methods on corn silk's phytochemical composition and antioxidant potential, demonstrating that different extraction methods and solvents influence the extraction efficiency of phytochemicals. Similarly, Ghasemzadeh et al. (2015) emphasized that the extraction procedure, particularly the choice of solvent, is crucial in determining the amount of phytochemicals recovered from samples. Moreover, Sharma et al. (2021) focused on comparing the effects of different solvents on the phytochemical profile and volatile bioactive compounds of Nephrolepis exaltata, illustrating how solvent selection can influence the characterization of phytochemicals. Additionally, Do et al. (2014) as well as Sasadara and Wirawan, (2021) highlighted that the extraction solvent significantly affects the total phenol content, total flavonoid content, and antioxidant activity, emphasizing the importance of solvent choice in phytochemical extraction. Numerous research shows that the selection of extraction methods and solvents is a critical factor in phytochemical studies, directly influencing the composition, yield, and bioactivity of the extracted compounds. Considerations of solvent polarity, extraction techniques, and plant material characteristics are needed to optimize the extraction of phytochemicals for various applications.

Purnajiwa (*Kopsia arborea Blume*.) is a potential medicinal plant that can be developed into traditional medicine. Purnajiwa has been the subject of several studies focusing on its phytochemical composition and antioxidant activity Purwanto et al. (2017) investigated the antioxidant activity of Purnajiwa (Kopsia arborea Blume.) fruit extract using various solvents. Apriliani et al. (2020) conducted a study on the phytochemical analysis and antioxidant activity of Purnajiwa fruit extract. Additionally, a study by Ariati et al., (2022) utilized DNA barcoding to authenticate Purnajiwa, identifying it as Kopsia arborea in certain regions. While there is mention of different taxonomies for Purnajiwa in the literature, the focus remains on the phytochemical characterization and antioxidant potential of Purnajiwa, specifically Kopsia arborea Blume. Kopsia is recognized as a unique and dynamic source of alkaloids. Kopsia species typically possess indole alkaloid chemicals that exhibit high potency and a wide range of biological activities (Chen et al., 2020; Hop & Son, 2022; Wong et al., 2021). The alkaloid compounds found in Kopsia generally possess unique structures and exhibit notable biological activity (Kogure et al., 2012). Multiple investigations were carried out to separate and create indole alkaloid compounds from Kopsia. Considering its potential for development as a medicinal substance, optimizing extraction method and solvent is necessary to extract the drug efficiently. This research evaluated the antioxidant activity (IC50) of purnajiwa (Kopsia arborea Blume.) extracted by methanol and ethanol using maceration and Soxhlet extraction..

## 2. Methodology

### 2.1. Sample preparation and extraction

The mature fruits of purnajiwa were gathered from Denpasar (Bali, Indonesia). The Laboratory of Genetic Resources and Molecular Biology verified the sample with the voucher number SDGBM/07/23/034. The plant components were meticulously separated and cleaned under a continuous flow of water, followed by drying in an oven at a temperature of 45℃ until a consistent weight was achieved. Subsequently, the desiccated substance was finely ground into a uniform powder. The powdered material (100 g) was extracted using maceration (25℃, 1 day) and soxhlet method (8 h) using 96% ethanol and 100% methanol solvent (1000 ml, ration 1: 10). The filtrate was obtained and concentrated using a rotary evaporator to produce a crude extract for further analysis.

### 2.2. Antioxidant activity evaluation

A DPPH method was employed to conduct an antioxidant activity assay. A DPPH solution was produced by dissolving 6 mg of DPPH in 50 mL of methanol. A mixture of extracts at different concentrations (ranging from 60 to 220 μg/ml) was mixed with 2.5 mL of DPPH solution and subsequently kept in the dark at room temperature for 30 minutes. The absorbance measurement was conducted using a UV-Vis spectrophotometer at a specific wavelength of 517 nm. The radical inhibition % was determined using equation (1).

$$Percentage\ of\ inhibition = \frac{A\ control - A\ sample}{A\ control}\ x\ 100\% \quad \text{.....(1)}$$

The control absorbance (A control) refers to the absorbance of the DPPH solution in the absence of any extract, and sample absorbance was the measurement of the absorbance of the sample. A model of linear regression was built for each extract. The IC50, or half-maximal inhibitory concentration, represents the quantity of antioxidants needed to reduce the initial DPPH concentration by 50%. Every sample was analyzed in triplicate.

**2.3. Data Analysis**

The IC50 obtained for each sample was statistically analyzed using IBM SPSS Statistics Ver. 25 with 1 95% confidence level. Data were analyzed using Way ANOVA and Two-Way ANOVA followed by the Bonferroni post-hoc Test to evaluate the influence of method and solvent extraction on antioxidant activity (IC50). Data were shown as average and deviation standard (mean ± SD).

## 3. Results and Discussions

The selection of plant extraction method and solvent significantly influences phytochemicals' extraction efficiency and antioxidant activity. Research has indicated that the polarity of solvents is crucial in this process (Nawaz et al., 2019, 2022), that have emphasized the importance of using a combination of polar and nonpolar solvents to enhance the extraction yield of bioactive phytochemicals with potent antioxidant properties from various plant parts. Thavamoney et al. (2018) have also shown how the choice of solvents, such as hexane, ethyl acetate, butanol, ethanol, and water, can impact the extraction and recovery of phytochemical antioxidants from fruit parts. Moreover, the choice of solvent affects the extraction yield and the extracted phytochemicals' antioxidant activity. Enitan et al. (2021) have highlighted that different solvents used for extraction can lead to variations in both the quantitative phytochemical content and antioxidant activity of plant extracts. Furthermore, plant extracts' phytochemical composition and antioxidant activity are positively correlated with using organic solvents during the extraction process (Idowu et al., 2020; N. Kumar et al., 2023). The choice of solvents with varying polarities can influence the yield of phenolic and flavonoid content and the antioxidant activities of plant extracts (Iloki-Assanga et al., 2015). Therefore, carefully selecting extraction methods and solvents is crucial for maximizing the extraction efficiency and antioxidant potential of phytochemicals from plant materials.. Figure 1 shows $IC_{50}$ of *Kopsia arborea Blume.* extracted with ethanol and methanol using maceration and Soxhlet extraction method.

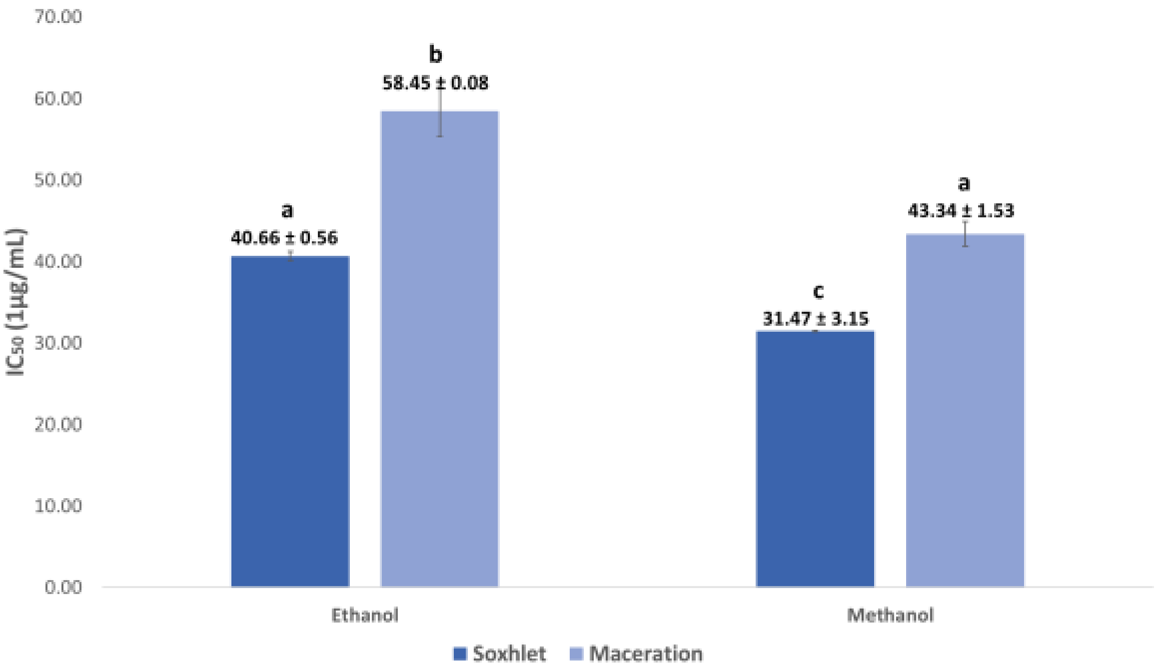


**Figure 1.** $IC_{50}$ of Kopsia arborea Blume. extracted with ethanol and methanol using maceration and Soxhlet extraction method. The values are shown above each bar as mean ± SD. The letters (a,b,c) above the value show a significant difference. A bar with the same letter indicates an indifferent result ($p > 0.05$).

In this study, using methanol as an extraction solvent generally produced a lower IC50, indicating more potent antioxidant activity than ethanol in maceration and soxhlet method extraction (Fig 1.). Statistical analysis showed a significant difference in IC50 using methanol and ethanol solvent ($p<0.05$) in the same extraction method. Studies by Ali et al. (2021) and Shanmugapriya et al. (2017) have recommended methanol as an optimal solvent for obtaining high levels of phytochemical and antioxidant constituents from plant leaves. The choice of solvent for plant extraction significantly impacts the antioxidant activity of the extracts. Various studies have shown that methanol extracts generally exhibit higher antioxidant activity than ethanol extracts (G. Kumar et al., 2019; Munir et al., 2014). Methanolic extracts have also provided more effective antimicrobial activity than extracts obtained using ethanol or other polar substances (Sánchez et al., 2010). Moreover, solvent selection can influence plant extracts' phenolic content and antioxidant potential. Methanol extracts have been reported to have higher phenolic content than ethanolic and aqueous extracts in certain plant species (Malik et al., 2016). Methanol is recognized as an effective solvent for extracting bioactive compounds from plants, leading to vigorous antioxidant activity (Uddin, 2008). In specific plant species, the choice of extraction solvent can impact the extracts' bioactivity. A Dracocephalum polychaete study shows that the plant extract's antioxidant capacity was assessed using the DPPH method, highlighting the importance of solvent selection in determining antioxidant activity (Khodami et al., 2015). Overall, the literature supports methanol as a preferred solvent for extracting bioactive compounds with high antioxidant activity from plant materials. Its effectiveness in extracting phenolic compounds and enhancing antioxidant potential makes it a solvent of choice in various studies.

This study also compared the effect of the extraction method on antioxidant activity. The use of Soxhlet extraction produced lower IC50 both in the use of methanol (31.47 ± 3.15µg/mL) and ethanol solvent (40.66 ± 0.56 µg/mL) as compared to the use of maceration method (58.45 ± 0.08 and 43.34 ± 1.53 µg/mL in ethanol and methanol, respectively), with a significantly different ($p < 0.05$). The selection of the extraction method plays a crucial role in determining the antioxidant activity of plant extracts. Studies have consistently shown that the Soxhlet extraction method yields extracts with higher antioxidant activity than maceration (Chuah et al., 2020; Dotulong et al., 2018; Warnis et al., 2021). Soxhlet extraction has been found to result in extracts with the highest total phenolic and flavonoid content, leading to enhanced antioxidant capacity. This method enables repetitive extraction cycles, facilitating increased contact between the plant material and the solvent, ultimately enhancing the extraction yield and bioactivity of the extracts (Chuah et al., 2020).

Furthermore, the Soxhlet extraction method has been associated with higher extract yield and better phytochemical component preservation than maceration (Dotulong et al., 2018). Extracts obtained through Soxhlet extraction have demonstrated vigorous DPPH radical scavenging activity, indicating their potent antioxidant properties (Taşkın, Yilmaz, et al., 2020). Additionally, Soxhlet extraction has been highlighted as a standard technique that has been used for decades and is often employed as a reference method to evaluate the effectiveness of other extraction methods (Nowak et al., 2021). In various studies, Soxhlet extraction is superior in terms of antioxidant activity compared to maceration, as evidenced by higher inhibition of acetylcholinesterase enzyme and better antioxidant potential (Taşkın, Yilmaz, et al., 2020). The Soxhlet method has also been associated with the highest inhibitory activities against α-amylase and acetylcholinesterase, further emphasizing its efficacy in extracting bioactive compounds with antioxidant properties (Maaiden et al., 2022). The literature supports that the Soxhlet extraction method generally produces plant extracts with higher antioxidant activity than maceration. The repetitive extraction cycles and efficient contact between the plant material and the solvent in Soxhlet extraction contribute to the superior antioxidant potential of the extracts obtained through this method.

Statistical analysis showed the extraction method's and solvent variables' significant effect on the IC50 value (corrected model $p = 0.000$). Without the effect of method and solvent, the IC50 value can significantly change (intercept $p = 0.000$). The method ($p = 0.000$) and solvent ($p = 0.000$) significantly affected the IC50 value. The correlation value generated based on statistical analysis showed a strong correlation between the method and solvent to IC50 (R squared 0.970). Soxhlet extraction is a well-established method for extracting phytochemicals from plant materials, and it is known for its ability to achieve high extraction yields and enhance the phytochemical and antioxidant activity of the extracts. The repetitive extraction cycles in Soxhlet extraction facilitate increased contact between the plant material and the solvent, leading to higher extraction yields than other methods like maceration and ultrasonic extraction (Chuah et al., 2020). The circulation flow of the solvent in Soxhlet extraction allows for the complete depletion of the plant material, resulting in higher total phenolic and flavonoid content in the extracts. Studies have shown that the Soxhlet extraction method, which involves higher temperatures, can favor the extraction of polyphenolic compounds and enhance antioxidant activity (Montiel, 2023). However, it is essential to note that the higher temperatures used in Soxhlet extraction may lead to the degradation of thermolabile phenolic compounds, potentially affecting the antioxidant activity of the extracts (Gajić et al., 2019). Furthermore, Soxhlet extraction effectively extracts a wide range of compounds from various plant sources, producing valuable extraction products (Hanaphi, 2023). Due to its efficiency, the method has been established as a standard technique for extracting valuable compounds from different plant sources (Bimakr et al., 2012). Regarding antioxidant activity, Soxhlet extraction has been associated with producing extracts with higher antioxidant capacity compared to other extraction methods like maceration (Inostroza et al., 2018). Additionally, studies have demonstrated that Soxhlet extracts exhibit strong antioxidant properties, as evidenced by their DPPH radical scavenging activity (Taşkın, Guler, et al., 2020; Taşkın, Yilmaz, et al., 2020). Overall, the literature supports the effectiveness of Soxhlet extraction in increasing extraction yield, phytochemical content, and antioxidant activity of plant extracts. The method's ability to thoroughly extract compounds from plant materials due to repetitive cycles and solvent circulation flow makes it a valuable technique for obtaining bioactive compounds with enhanced antioxidant properties.

## 4. Conclusions

The choice of solvent and extraction method also needs to consider the characteristics of the natural material to be extracted. If the target compound has been characterized as unstable at high temperatures, the selection of extraction methods that require heating must be reconsidered. The polarity of the target compound strongly influences the choice of extraction solvent. Suppose the extraction aims to increase the extraction yield. In that case, polar solvents such as methanol or ethanol can be recommended, considering that polar phenolic and flavonoid compounds are generally distributed the most in a natural material sample. However, if the polarity characteristics of the target compound are known, then the extraction process is carried out using a solvent with a polarity similar to the target compound.

**Author Contributions (Style: Times New Roman, 10 Points, Title Case, Bold)**

Conceptualization, M.M.V.S.; methodology, E.C..; analysis, N.L.K.A.A.D. & P.E.S.K.Y..; data curation, P.E.S.K.Y.; writing—original draft preparation, M.M.V.S.; writing—review and editing, N.L.K.A.A.D. & P.E.S.K.Y.; visualization, M.M.V.S.; project administration, N.L.K.A.A.D.; funding acquisition, M.M.V.S.. All authors have read and agreed to the published version of the manuscript.

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**Data Availability**

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**Conflicts of Interest**

The authors declare no conflict of interest

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**Appendix**