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Differences In The Antioxidant Activity Of 70% Ethanol Extract From Young And Old Leaves Of The Cajeput Plant (*Melaleuca cajuputi*)

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Abstract

Cajuput leaves (Melaleuca cajuputi) are a natural source of phenolic compounds with potential as antioxidants to combat free radicals. The content of active compounds in the extract is highly dependent on the maturity of the leaves used. This study aimed to determine the difference in antioxidant activity between 70% ethanol extracts of young and old cajuput leaves. This quasi-experimental study compared the antioxidant activity of young and old leaf extracts. The leaves were extracted using 70% ethanol via maceration. Phytochemical screening was conducted to identify secondary metabolites. Antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method with a UV-Vis spectrophotometer, and the results were expressed as IC~50~ and Antioxidant Activity Index (AAI) values. Data were analyzed using an Independent Sample T-Test. Phytochemical screening revealed the presence of phenols, tannins, and saponins in young leaves, while old leaves contained alkaloids, phenols, tannins, and saponins. The young leaf extract showed higher antioxidant activity with an IC~50~ value of 14.46 ppm and an AAI of 2.76, compared to the old leaf extract with an IC~50~ of 17.82 ppm and an AAI of 2.24. Both extracts are classified as having very strong antioxidant activity. However, statistical analysis showed no significant difference in antioxidant activity between the two extracts ($p > 0.05$). Although the young leaf extract exhibited a slightly higher antioxidant activity, there is no statistically significant difference between the antioxidant activity of 70% ethanol extracts from young and old cajuput leaves. Both can be utilized as effective natural antioxidant sources.

Keywords: Antioxidant, Cajuput Leaves, DPPH, Extract, Melaleuca cajuputi

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INTRODUCTION

The high level of free radical exposure in the body can trigger degenerative diseases such as cancer, diabetes, and cardiovascular diseases, which are leading causes of death worldwide. An effective way to reduce the negative impact of free radicals is by using antioxidants. Natural antioxidants from plants are increasingly in demand because they are considered safer and more effective than synthetic ones. Cajuput leaves (Melaleuca cajuputi) are one of the Indonesian herbal plants that contain bioactive compounds such as flavonoids, alkaloids, triterpenoids, and phenolics, which have potential as antioxidants (Pamungkas et al., 2023). The active compounds in plant extracts depend on the maturity of the leaves used in the extraction process. Research by Leviana et al., (2023) on durian leaves showed that old leaves had higher antioxidant activity, while research by Maleke et al., (2024) on soyogik leaves indicated that young leaves had higher activity. This suggests that the effect of leaf maturity on antioxidant activity varies between plant species. To date, a scientific comparison of the antioxidant content in young and old cajuput leaves is still minimal. Therefore, this study aims to determine the difference in antioxidant activity between 70% ethanol extracts of young and old cajuput leaves, which is expected to provide valuable information for the herbal and pharmaceutical industries in selecting the optimal leaf part for development.

METHOD

This study used a quasi-experimental design. Young and old cajuput leaves were collected from Baler Bale Agung Village, Jembrana, Bali. The leaves were sorted, washed, air-dried, and ground into a powder. Extraction was performed using the maceration method with 70% ethanol as the solvent for 7 days with remaceration. The filtrate was concentrated using a rotary evaporator at 50°C to obtain a thick extract. Phytochemical screening was conducted to identify alkaloids, flavonoids, terpenoids, saponins, phenols, tannins, and steroids. Antioxidant activity testing was carried out using the DPPH method. A series of extract concentrations (5, 10, 15, 20, 25, and 30 ppm) were reacted with a 40 ppm DPPH solution. After 30 minutes of incubation in a dark room, the absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. The percentage inhibition was calculated, and the IC~50~ value

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was determined from the linear regression equation of the concentration versus % inhibition curve. The AAI value was calculated using the formula: AAI = [DPPH concentration] / IC~50~. Data were analyzed for normality with the Shapiro-Wilk test, homogeneity with Levene's Test, and hypothesis testing with the Independent Sample T-Test using statistical software. This study used a quasi-experimental design. Young and old cajuput leaves were collected from Baler Bale Agung Village, Jembrana, Bali. The leaves were sorted, washed, air-dried, and ground into a powder. Extraction was performed using the maceration method with 70% ethanol as the solvent for 7 days with remaceration. The filtrate was concentrated using a rotary evaporator at 50°C to obtain a thick extract. Phytochemical screening was conducted to identify alkaloids, flavonoids, terpenoids, saponins, phenols, tannins, and steroids. Antioxidant activity testing was carried out using the DPPH method. A series of extract concentrations (5, 10, 15, 20, 25, and 30 ppm) were reacted with a 40 ppm DPPH solution. After 30 minutes of incubation in a dark room, the absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. The percentage inhibition was calculated, and the IC~50~ value was determined from the linear regression equation of the concentration versus % inhibition curve. The AAI value was calculated using the formula: AAI = [DPPH concentration] / IC~50~. Data were analyzed for normality with the Shapiro-Wilk test, homogeneity with Levene's Test, and hypothesis testing with the Independent Sample T-Test using statistical software.

RESULTS

The yield of the young leaf extract was 12.2%, while the old leaf extract was 10.08%. Phytochemical screening showed that young leaves contained phenols, tannins, and saponins. Old leaves contained alkaloids, phenols, tannins, and saponins. The antioxidant activity test results are shown in Table 1. The young leaf extract had an IC~50~ of 14.46 ppm and an AAI of 2.76. The old leaf extract had an IC~50~ of 17.82 ppm and an AAI of 2.24. Based on the AAI value, both extracts are categorized as having very strong antioxidant activity. The results of the normality and homogeneity tests showed that the data were normally distributed and homogeneous. The Independent Sample T-Test results showed a significance value of 0.257 (p

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> 0.05), indicating no significant difference in antioxidant activity between young and old leaf extracts.

Table 1. Antioxidant Activity Test Results of Young and Old Cajuput Leaf Extracts

Leaf Type	IC50 (ppm)	AAI Value
Young	14.46	2.76
Old	17.82	2.24

DISCUSSION

The higher yield of the young leaf extract indicates that more soluble compounds were extracted from young leaves compared to old leaves. Phytochemical screening revealed that old leaves contained alkaloids, which were not found in young leaves. This is likely due to the greater ability of older leaves to synthesize certain secondary metabolites as they age. The antioxidant activity, measured by IC~50~ and AAI, was slightly higher in young leaves. This is supported by the presence of phenolic compounds, tannins, and saponins, which are known to act as antioxidants by donating hydrogen atoms to stabilize free radicals like DPPH. The higher activity in young leaves may be because they are still actively producing secondary metabolites as part of their defense mechanism during the early growth stage. However, statistical analysis confirmed that the difference in antioxidant activity between young and old leaves was not significant. This means that while young leaves may have a slight edge, both young and old cajuput leaves are excellent and statistically equivalent sources of potent natural antioxidants. The choice of leaf maturity, therefore, does not strongly impact its effectiveness as an antioxidant source, allowing for flexibility in the utilization of this plant resource.

CONCLUSION(S)

In conclusion, both young and old cajuput leaf extracts possess very strong antioxidant activity. Although the young leaf extract showed a slightly higher AAI value, there is no significant difference in the antioxidant activity between the 70% ethanol extracts of young and old cajuput leaves (Melaleuca cajuputi). Therefore, both types of leaves can be utilized effectively as natural antioxidant sources.

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Conflict of Interest

The author(s) declare that they have no conflict of interest.

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