

COMPARATIVE STUDY OF ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF *ABRUS PRECATORIUS* EXTRACTS FROM THE BIOFARMAKA HERBAL GARDEN AND THE MATERIA MEDICA GARDEN BATU

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Abstract

Breast cancer is the leading cause of cancer incidence and death in women. Doxorubicin plays an important role in breast cancer therapy, although its application is restricted by toxic and adverse effects. *Abrus precatorius* (APC) has bioactive compounds that show antioxidant and anticancer potential, which can be affected by different growing environments. This study evaluated the antioxidant and cytotoxic activities of 70% ethanol leaf extracts of APC from the Biofarmaka Garden (Bogor, West Java) and the Materia Medica Garden (Batu, East Java). Qualitative screening was used to identify secondary metabolites. Antioxidant activity was evaluated using the DPPH assay with vitamin C as the positive control, while cytotoxic activity was assessed against MCF-7 cells using the MTT assay with doxorubicin as the positive control. IC₅₀ values were calculated and analyzed using independent t-test and Mann-Whitney test. Both extracts contained saponins, flavonoids, tannins, alkaloids, and steroids. Antioxidant activity was moderate with IC₅₀ values of 175.70 ± 0.69 µg/mL (Bogor) and 173.07 ± 0.07 µg/mL (Batu) (p = 0.021). Cytotoxic activity was weak with IC₅₀ values of 264.51 ± 8.11 µg/mL (Bogor) and 257.35 ± 33.92 µg/mL (Batu) (p = 0.513). Both extracts showed similar phytochemical profiles, with moderate antioxidant and weak cytotoxic activities against MCF-7 cells.

Keywords: *Abrus precatorius*, antioxidant, cytotoxic, MCF-7, IC₅₀

Introduction

Breast cancer is the type of cancer with the highest incidence and mortality among women worldwide. According to GLOBOCAN 2022 data, approximately 2.3 million women were diagnosed with breast cancer, with more than 670,000 deaths annually, making it the leading cause of cancer-related mortality in women.¹ In Indonesia, breast cancer has the highest incidence among all cancers (16.2% of total cancer cases) and is the third leading cause of cancer-related death (9.3%).² One of the main therapeutic approaches for breast cancer is chemotherapy using a combination of Cyclophosphamide, Adriamycin, and

Fluorouracil (CAF). Doxorubicin (Adriamycin) exerts its anticancer effects by intercalating DNA, inhibiting topoisomerase II, and generating reactive oxygen species (ROS), which lead to mitochondrial damage and trigger apoptosis.³⁻⁴ However, despite its effectiveness, doxorubicin is associated with significant toxic effects, including cardiotoxicity, hepatotoxicity, and bone marrow suppression, which limit its clinical use.⁵

These toxic effects and limitations have driven the development of safer alternative therapies, including the utilization of herbal medicines. Medicinal plants contain bioactive compounds such as flavonoids, saponins, and alkaloids that have antioxidant and anticancer effects. These compounds can reduce reactive oxygen species (ROS) and trigger apoptosis.⁶ According to the World Health Organization (WHO), more than 88% of the world's population has used herbal medicines as part of traditional healthcare.⁷ One plant that shows promising potential is *Abrus precatorius* (APC), known as rosary pea. This plant contains saponins, flavonoids, and tannins with antioxidant, anti-inflammatory, and cytotoxic activities against several cancer cell types, including MCF-7 cells.⁸ Saponins are known to promote apoptosis and DNA fragmentation, while flavonoids help neutralize free radicals and inhibit cancer cell growth.⁹

The biological activity of herbal plants can vary depending on environmental factors such as altitude, soil pH, and sunlight exposure.¹⁰ The result, the same plants (species) may produce different levels of secondary metabolites and show different biological effects. In Indonesia, we can find *Abrus precatorius* (APC) in the Biofarmaka Garden in Bogor (West Java) and the Materia Medica Garden in Batu (East Java), two regions with different environmental characteristics. Until now, there are no studies comparing the antioxidant and cytotoxic activities of *Abrus precatorius* extracts from these two locations on MCF-7 breast cancer cells. Therefore, this study aims to compare the antioxidant and cytotoxic activities of 70% ethanol extracts of *Abrus precatorius* leaves from both locations to explore its potential as an adjuvant therapy for breast cancer.

Materials and Methods

We used an experimental design to evaluate antioxidant and cytotoxic activities. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was used to test the antioxidant activity. The cytotoxic activity was evaluated on MCF-7 breast cancer cells, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Absorbance was measured using a spectrophotometer to determine the inhibitory concentration 50% (IC₅₀) values. The study population consisted of MCF-7 breast cancer cells and the samples were 70% ethanol extracts of *Abrus precatorius* leaves collected from the Biofarmaka Garden in Bogor (West Java) and the Materia Medica Garden in Batu (East Java).

Data were collected through qualitative phytochemical screening, antioxidant testing, and cytotoxic testing. Phytochemical screening was performed to identify secondary

metabolites related to antioxidant and cytotoxic activities, including saponins, flavonoids, tannins, alkaloids, triterpenoids, and steroids. Antioxidant activity was evaluated using the DPPH assay by mixing extract solutions at different concentrations with DPPH solution and we used vitamin C as a positive control. The mixtures were incubated in the dark for 30 minutes, and then we looked for the absorbance that measured at 517 nm. Cytotoxic activity was evaluated on MCF-7 cells using the MTT assay, with doxorubicin as a positive control. After incubation, the MTT reagent was added and absorbance was measured at 590 nm.

Antioxidant and cytotoxic activities were reported as IC_{50} values, representing the concentration required to reduce 50% of DPPH radical activity or 50% of MCF-7 cell viability. IC_{50} values were calculated using logarithmic equations based on linear absorbance curves generated in Microsoft Excel. All experiments were performed in triplicate for each sample in both the DPPH and MTT assays to ensure data reliability. Antioxidant activity was classified based on IC_{50} values as follows: very strong ($< 50 \mu\text{g/mL}$), strong ($50\text{--}100 \mu\text{g/mL}$), moderate ($101\text{--}250 \mu\text{g/mL}$), weak ($250\text{--}500 \mu\text{g/mL}$), and inactive ($>500 \mu\text{g/mL}$).¹¹ Cytotoxic activity was categorized according to the National Cancer Institute (NCI) criteria, in which IC_{50} values $\leq 20 \mu\text{g/mL}$ were considered very active, $21\text{--}200 \mu\text{g/mL}$ as moderate, $201\text{--}500 \mu\text{g/mL}$ as weak, and $> 500 \mu\text{g/mL}$ as inactive.¹²

Statistical analysis was performed using IBM SPSS Statistics version 29.0. Data normality was assessed using the Shapiro–Wilk test. Normally distributed data ($p > 0.05$) were presented as mean \pm standard deviation and analyzed using an independent *t*-test, while non-normally distributed data ($p < 0.05$) were presented as median ($Q_1\text{--}Q_3$) and analyzed using the Mann–Whitney test. A *p*-value < 0.05 was considered statistically significant.

This study did not require ethical approval from the Faculty of Medicine, Universitas Indonesia Ethics Committee as it did not involve human or animal subjects. Sample batch numbers were APC-Bogor (November 2024) and APC-Batu (December 2024).

Results

Extraction of *Abrus precatorius* (APC)

In this study, the extraction of *Abrus precatorius* (APC) simplicia obtained from the Biofarmaka Garden in Bogor (200 g) and the Materia Medica Garden in Batu (500 g) was carried out using the cold maceration method with 70% ethanol as the solvent. Solvent removal was performed using an evaporation method. However, during the evaporation process of the *Abrus precatorius* extract from the Biofarmaka Garden in Bogor, a technical issue occurred in the form of bumping (the formation of large splashing bubbles), resulting in an unstable evaporation process and a longer evaporation time than usual. The final products were two viscous extracts with a thick consistency, dark greenish-black in color, and a strong characteristic aroma of saga leaves.

Phytochemical Screening of APC Extracts

In this study, phytochemical screening was used to identify secondary metabolites present in both APC extracts, including saponins, flavonoids, tannins, alkaloids, steroids, and triterpenoids. The results of phytochemical screening are shown in Table 1.

Table 1. Phytochemical Screening Results of APC Extracts

| Phytochemical Test | APC B | APC M |
|--------------------|-------|-------|
| Saponin | + | + |
| Flavonoids | + | + |
| Tannins | + | + |
| Alkaloids | + | + |
| Steroids | + | + |
| Triterpenoids | - | - |

Notes: APC B: *Abrus precatorius* extract from Biofarmaka Garden (Bogor); APC M: *Abrus precatorius* extract from Materia Medica Garden (Batu); +: compound present; -: compound absent.

Antioxidant Effects of APC Extracts

The antioxidant activity of APC extracts was measured using IC_{50} values. Vitamin C was used as a positive control, and its inhibition percentage was calculated for comparison. The results of the DPPH assay are shown in Table 2.

Table 2. IC_{50} Values of Antioxidant Activity of APC Extracts and Vitamin C

| | Treatment with APC extract | | |
|---------------------------------|---------------------------------|-----------------------------------|-----------------------------------|
| | Vitamin C ($\mu\text{g/mL}$) | APC B ($\mu\text{g/mL}$) | APC M ($\mu\text{g/mL}$) |
| IC_{50} | 3,32 | 176,19 | 173,14 |
| | 3,32 | 176,00 | 172,99 |
| | 3,32 | 174,91 | 173,07 |
| Mean \pm SD | 3,32\pm0,00 | 175,70\pm0,69 | 173,07\pm0,07 |

Notes: APC M: *Abrus precatorius* extract from Biofarmaka Garden (Bogor); APC M: *Abrus precatorius* extract from Materia Medica Garden (Batu); $\mu\text{g/mL}$: micrograms per milliliter.

The results showed that the IC_{50} value of the APC extract from the Biofarmaka Garden, Bogor was $175.70 \pm 0.69 \mu\text{g/mL}$ and the APC extract from the Materia Medica Garden, Batu had an IC_{50} value of $173.07 \pm 0.07 \mu\text{g/mL}$. Vitamin C, as a positive control can exhibited an IC_{50} value of $3.32 \mu\text{g/mL}$. An independent *t*-test was performed to compare the IC_{50} values of the two APC extracts, and the results showed a significant difference between the

Bogor and Batu samples ($p = 0.021$). The mean difference was $2.64 \mu\text{g/mL}$ (95% CI: 0.95–4.33).

Cytotoxic Effects of APC Extracts

Cytotoxic activity of APC extracts from Bogor and Batu was evaluated using the MTT assay based on IC_{50} values. Doxorubicin served as a positive control, and the results are shown in Table 3.

Table 3. IC_{50} Values of Cytotoxic Activity of APC Extracts and Doxorubicin

| Variable | Treatment with APC extract | | |
|---------------------------------|-----------------------------------|-------------------------------------|--------------------------------------|
| | Doxorubicin ($\mu\text{g/mL}$) | APC B ($\mu\text{g/mL}$) | APC M ($\mu\text{g/mL}$) |
| IC_{50} | 25,57 | 268,99 | 228,21 |
| | 47,03 | 255,14 | 249,26 |
| | 31,43 | 269,39 | 294,58 |
| Mean \pm SD | 34,68\pm11,09 | 264,51 \pm 8,11 | 257,35 \pm 33,92 |

Notes: APC M: *Abrus precatorius* extract from Biofarmaka Garden (Bogor); APC B: *Abrus precatorius* extract from Materia Medica Garden (Batu); $\mu\text{g/mL}$: micrograms per milliliter.

The results showed that the IC_{50} value of the APC extract from the Biofarmaka Garden, Bogor was $264.51 \pm 8.11 \mu\text{g/mL}$ and the APC extract from the Materia Medica Garden, Batu exhibited an IC_{50} value of $257.35 \pm 33.92 \mu\text{g/mL}$. Doxorubicin that used as a positive control, showed a much lower IC_{50} value ($34.68 \pm 11.09 \mu\text{g/mL}$). The IC_{50} values of the two APC extracts were compared using the Mann–Whitney test. There is no significant difference in cytotoxic activity between the Bogor and Batu extracts ($p = 0.513$). The median IC_{50} value was 268.99 (255.14–269.39) for the Bogor extract and 249.26 (228.21–294.58) for the Batu extract.

Discussion

The simplicia of *Abrus precatorius* (APC) from both locations was extracted using cold maceration with 70% ethanol as the solvent. Ethanol was chosen because semi-polar properties allow it to extract both polar and non-polar compounds effectively. According to Gul et al. (2013) study, ethanol and ethyl acetate extracts of *Abrus precatorius* demonstrated stronger antioxidant and antiproliferative activities against several cancer cell lines compared to aqueous and hexane extracts. These findings support the use of ethanol as a solvent for APC extraction, as it can effectively extract bioactive compounds with antioxidant and cytotoxic potential.¹³ Solvent evaporation was performed using a rotary evaporator. However, the APC ethanol extract from the Biofarmaka Garden in Bogor repeatedly experienced bumping during evaporation, which

slowed down the process and required careful monitoring to avoid sample loss. This bumping is likely related to the high saponin content, since saponins have surfactant properties that lower surface tension and promote foam formation in aqueous or alcoholic solutions.¹⁴

Qualitative phytochemical screening showed both of APC extracts contained saponins, flavonoids, tannins, alkaloids, and steroids. These findings are consistent with previous studies. Dewi BS et al. (2024) reported that APC extracts contain tannins, saponins, alkaloids, and flavonoids,¹⁵ while Rambe R et al. (2021) demonstrated the presence of flavonoids, saponins, and alkaloids in APC extracts.¹⁶ These results are in line with a recent ethnopharmacological review reporting that more than 160 compounds have been identified from *Abrus precatorius* L., including flavonoids, phenolics, terpenoids, steroids, alkaloids, and polysaccharides, which contribute to various pharmacological activities such as antitumor, antimicrobial, anti-inflammatory, antioxidant, immunomodulatory, and antidiabetic effects.¹⁷ Thus, the presence of secondary metabolites in this study supports existing literature and confirms the consistency of the secondary metabolite profile of *Abrus precatorius* leaves with their potential biological activities.

In this study, antioxidant activity was evaluated using the DPPH assay, while cytotoxic activity was assessed using the MTT assay. In the DPPH assay, vitamin C was used as a positive control because it is known to possess very strong antioxidant activity and is commonly employed in DPPH testing. The results showed an IC₅₀ value of vitamin C of 3.32 µg/mL, which is categorized as very strong antioxidant activity. This finding is consistent with previous studies reporting vitamin C IC₅₀ values ranging from 2.26 to 12.36 µg/mL.¹⁸⁻²¹

For cytotoxic activity testing, doxorubicin was used as a positive control because it is a commonly used chemotherapeutic agent for breast cancer and is known for its high cytotoxic potency. The results showed an IC₅₀ value of doxorubicin of 34.68 ± 11.09 µg/mL, which is categorized as very strong cytotoxic activity. Previous studies have reported doxorubicin IC₅₀ values ranging from 0.37 to 5.4 µg/mL. Although there is a difference between the IC₅₀ values obtained in this study and those reported previously, doxorubicin remains classified as a highly active cytotoxic agent.²²⁻²⁴

In this study, APC extracts from the Materia Medica Garden in Batu exhibited slightly better antioxidant activity, with an IC₅₀ value of 173.07 ± 0.07 µg/mL (moderate activity category), compared to APC extracts from the Biofarmaka Garden in Bogor, which had an IC₅₀ value of 175.70 ± 0.69 µg/mL (moderate activity category). From the theory, differences in altitude between the two locations may lead to differences in secondary metabolite content. Batu is located at an altitude of approximately ±875 m above sea level, with relatively cooler temperatures and different light intensity compared to Bogor (±200–300 m above sea level). Environmental factors such as altitude, humidity, light intensity, and harvesting time are known to influence the biosynthesis of flavonoids and phenolic compounds that function as antioxidants.

Several studies on other plants, such as *Sinopodophyllum hexandrum* from eight different locations and *Scrophularia striata* from three locations with different altitudes, have demonstrated significant differences in antioxidant activity due to geographical factors²⁵.²⁶ However, the results of the present study showed nearly similar antioxidant activities. This may be attributed to several factors, including species uniformity, differences in extraction yield, and limitations of the DPPH method, which measures only a single antioxidant mechanism and may not detect minor differences in secondary metabolite composition.

This study also found differences in IC_{50} values for cytotoxic activity, although these differences were not statistically significant. APC extracts from the Biofarmaka Garden in Bogor had an IC_{50} value of $264.51 \pm 8.11 \mu\text{g/mL}$, while APC extracts from the Materia Medica Garden in Batu showed an IC_{50} value of $257.35 \pm 33.92 \mu\text{g/mL}$. Both values fall within the moderate cytotoxic activity category. These results indicate that despite differences in altitude and environmental conditions between Bogor (± 200 – 300 m above sea level, warmer temperature, high humidity) and Batu (± 875 m above sea level, cooler temperature, different light intensity), the cytotoxic activity of APC extracts remained relatively similar. This may be influenced by factors such as uniformity of plant varieties, consistent extraction methods, and the possibility that the main bioactive metabolites responsible for cytotoxic activity are relatively stable across environmental variations at both locations.

Several studies have also shown that variations in the cytotoxic activity of *Abrus precatorius* are more strongly influenced by the plant organ used and the extraction technique applied.²⁶⁻²⁸ For example, APC seeds are known to contain abrin and abrine, which exhibit higher toxicity and therefore tend to show stronger cytotoxic activity compared to leaves.²⁷ Other studies have reported that ethanol extracts of APC seeds have an IC_{50} value of $60.89 \mu\text{g/mL}$ (moderate activity).²⁸ Therefore, the relatively small differences in cytotoxic IC_{50} values observed in this study are acceptable, considering that research on APC leaf extracts remains limited. These findings provide further insight into the cytotoxic potential of *Abrus precatorius* leaf extracts.

Overall, this study shows that the antioxidant and cytotoxic activities of *Abrus precatorius* leaves remain relatively consistent despite being grown in different locations (Bogor and Batu). This suggests that geographical factors such as altitude and climate do not have a major effect on the levels of key bioactive compounds in APC leaves. The results also emphasize the importance of using the same plant species and consistent extraction methods when evaluating biological activity. From a practical perspective, the findings indicate that APC leaves grown in Bogor have antioxidant and cytotoxic activities comparable to those grown in Batu. This provides an advantage for research and phytopharmaceutical development, as saga leaves can be sourced locally in Bogor without the need to obtain samples from more distant areas such as Batu, East Java, especially for communities in the Greater Jakarta area.

Conclusions

The phytochemical screening showed that both APC extracts contained the same secondary metabolites, including saponins, flavonoids, tannins, alkaloids, and steroids. APC extracts from the Materia Medica Garden, Batu and the Biofarmaka Garden, Bogor showed similar antioxidant activity, which was classified as moderate. The IC₅₀ values were 173.07 ± 0.07 µg/mL for APC M and 175.70 ± 0.69 µg/mL for APC B. Similarly, APC extracts from both locations exhibited comparable cytotoxic activity, which was classified as weak. The IC₅₀ values were 257.35 ± 33.92 µg/mL for APC M and 264.51 ± 8.11 µg/mL for APC B.

Competing Interests

The authors declare there are no conflicts of interest related to this study. The research was conducted independently without influence from external parties and only for academic purposes.

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