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Anticancer Potential of Water Fraction of Cashew Leaf (*Anacardium occidentale* L.) on A-375 Melanoma Cell Line In Vitro

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ABSTRACT: Melanoma is an aggressive form of skin cancer with a high potential for metastasis and resistance to conventional therapies, highlighting the need for safer and more effective treatment alternatives. Natural products, particularly plant-derived compounds, have gained attention as promising sources of anticancer agents. This study aimed to evaluate the anticancer potential of the aqueous fraction of cashew leaves (Anacardium occidentale L.) against melanoma in vitro. Anticancer activity was assessed through cytotoxicity and cell migration assays. Cytotoxic effects were determined using the MTT assay on A-375 melanoma cells, while selectivity was evaluated using normal HaCaT keratinocyte cells. Antimetastatic activity was examined using a scratch wound healing assay to determine the ability of the fraction to inhibit cancer cell migration. The results demonstrated that the aqueous fraction exhibited cytotoxic activity against melanoma cells, with an IC_{50} value of 184.29 ± 3.16 µg/mL, and showed good selectivity toward normal cells (SI = 6.68). Furthermore, the fraction significantly inhibited melanoma cell migration after 24 hours of treatment, indicating potential antimetastatic activity. This effect may be attributed to the presence of flavonoid and phenolic compounds, which are known to induce apoptosis and reduce oxidative stress. In conclusion, the aqueous fraction of A. occidentale leaves shows promising potential as a natural anticancer agent and may serve as a candidate for adjunctive therapy in melanoma treatment.

KEYWORDS: Anticancer; MTT cytotoxicity; cashew leaves; cell migration.

1. INTRODUCTION

Cashew (*Anacardium occidentale* L.) is a tropical plant widely distributed across Southeast Asia, including Indonesia, and is well recognized for both its economic and medicinal value. While its seeds are extensively utilized in the food and industrial sectors, various parts of the plant—including the leaves, bark, fruit, and seeds—have long been employed in traditional medicine to manage a wide range of conditions, such as inflammation, infections, diabetes, and skin disorders (Chan et al., 2023). These therapeutic properties are largely attributed to the rich content of bioactive compounds, particularly flavonoids and phenolic constituents, which exhibit diverse pharmacological activities, including antioxidant, anti-inflammatory, antibacterial, and anticancer effects (Sassi et al., 2022).

Melanoma is the most aggressive form of skin cancer, characterized by rapid proliferation, high metastatic potential, and notable resistance to conventional treatments such as chemotherapy and immunotherapy (Carr et al., 2020). According to Global Cancer Statistics (GLOBOCAN, 2020), the global incidence of melanoma continues to rise, with mortality rates remaining substantial, particularly in advanced metastatic stages. These challenges highlight the urgent need for novel, effective, and safer therapeutic agents, particularly those derived from natural sources.

Previous studies have demonstrated that extracts of *A. occidentale* leaves possess significant anticancer activity against various cancer cell lines, including breast, colon, and lung cancers (Barros et al., 2020). The presence of flavonoids and phenolic compounds has been reported to contribute to the inhibition of cancer cell proliferation and the induction of apoptosis (Salehi et al., 2019). Despite these promising findings, studies specifically investigating the antiproliferative and antimetastatic effects of the aqueous fraction of cashew leaves against A-375 melanoma cells remain limited.

Therefore, this study aims to evaluate the anticancer potential of the aqueous fraction of *A. occidentale* leaves against A-375 melanoma cells in vitro. Cytotoxic activity was assessed using the MTT assay, while antimetastatic potential was evaluated through a scratch wound healing assay to examine the inhibition of cancer cell migration. The findings of this study are expected to provide a scientific basis for the development of cashew leaf-derived compounds as effective and selective natural anticancer agents, as well as to support their potential application as adjunctive therapy in melanoma treatment.

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2. EXPERIMENTAL SECTION

2.1. Materials

Anacardium leaves were obtained from Kendari, Southeast Sulawesi. A total of 400 g of cashew leaves (*A. Occidentale*) were extracted using the maceration method with 4 L of methanol for 3 × 24 hours. The maceration product was concentrated using a rotary evaporator, yielding 101g of thick extract. The extract yield was 25% (Ribeiro et al., 2021). The thick extract was then fractionated using the liquid-liquid fractionation method. The extract was dissolved in methanol at a ratio of 1:10 (w/v), then fractionated sequentially using n-hexane and ethyl acetate. The remaining aqueous layer was designated as the aqueous fraction. All fractions obtained were reconcentrated using a rotary evaporator (Pham *et al*., 2023).

2.2. Cell culture

A-375 melanoma cells were obtained from the American Type Culture Collection (ATCC, USA). A-375 cell cultures were cultured using DMEM medium (Gibco™, USA) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution (Invitrogen™, USA). The incubation process was carried out at 37°C and 5% CO_2 until the cell density reached 70% confluence.

2.3. Cytotoxicity test by MTT assay

A-375 melanoma cells were cultured in a 96-well plate until 70% confluent. The culture medium was discarded, and the cell surface was washed using a PBS solution with a pH of 7.4 (Invitrogen, USA). The cells were incubated for 24 hours with various treatments. Each well was added 100 µL of MTT solution in PBS at a concentration of 0.5 mg/mL (Merck, Germany), then incubated again for 4 hours at 37°C. After incubation, the MTT solution was discarded, and the formazan crystals formed were dissolved using 100 µL of DMSO (Merck, Germany). The absorbance value of each well was measured using a microplate reader (Spectrostar Omega, BMG Labtech, Germany) at a wavelength of 570 nm. The IC_{50} value was calculated based on the curve relating the percentage of cell viability and the concentration of the extract (Chan *et al*., 2023; Purnama *et al*., 2024). The percentage of cell viability (CV) was calculated using the following equation (Purnama *et al*., 2024) :

$$Cell\ Viablity\ \% = \frac{OD\ sampel}{OD\ kontrol}\ x\ 100$$

The OD (optical density) values of the samples and controls were obtained from wells containing the treated samples and wells containing only untreated cells, respectively. A sample was considered to meet the criteria if the cell viability percentage (CV) exceeded 70%.

The dose-response curve was created based on the concentration of the test sample against the CV value to determine the IC_{50} value. The selectivity index (SI) was then calculated by comparing the IC_{50} values in normal cells and cancer cells (Khuanekkaphan et al., 2024).

$$SI = \frac{IC_{50}\ of\ A.occidentale\ leaf\ aqueous\ fraction\ on\ HaCat\ cells}{IC_{50}\ of\ A.occidentale\ leaf\ aqueous\ fraction\ on\ A-375\ cells}\ x\ 100$$

2.4. Anti-migration test by the scratch method

A-375 melanoma cells were cultured in a 24-well microplate, then incubated for 24 hours at 37°C until 70% confluent. The cells were washed with PBS, medium containing 0.5% FBS was added, and they were incubated again for 24 hours. The bottom surface of the wells was scratched using a sterile yellow tip. The old medium was discarded, the cells were rinsed again with PBS, and then added with cashew leaf (*A. occidentale*) water fraction at concentrations of 25, 50, and 100 µg/mL. The cells were observed at 0 and 24 hours after treatment. The scratch area was documented at each observation time with the same microscope magnification, then analyzed using ImageJ software to determine the scratch area (Wahyuni et al., 2021; Bobadilla et al., 2019).

2.5. Data analysis

Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A p-value of <0.05 was considered statistically significant. All analyses were conducted using SPSS version 22 (IBM Corp., Armonk, NY, USA).

3. RESULTS AND DISCUSSION

Cytotoxicity testing was conducted to evaluate the ability of cashew leaf (*A.occidentale*) water fraction to inhibit the growth of A-375 melanoma cells in vitro. This test aims to determine the antiproliferative potential of the fraction by measuring the IC_{50} value, which represents the concentration of the fraction that inhibits 50% cell viability (Boo *et al.*,

2018). The results of the cytotoxicity test showed that the water fraction of cashew leaves (*A. occidentale*) had inhibitory activity against the growth of A-375 melanoma cells with an IC_{50} value of 184.29 ± 3.61 µg/mL, which means it has moderate cytotoxic ability against A-375 melanoma cells. The administration of water fractions of cashew leaves (*A. occidentale*) showed a gradual increase in toxic effects as the treatment concentration increased (**Figure 1**).

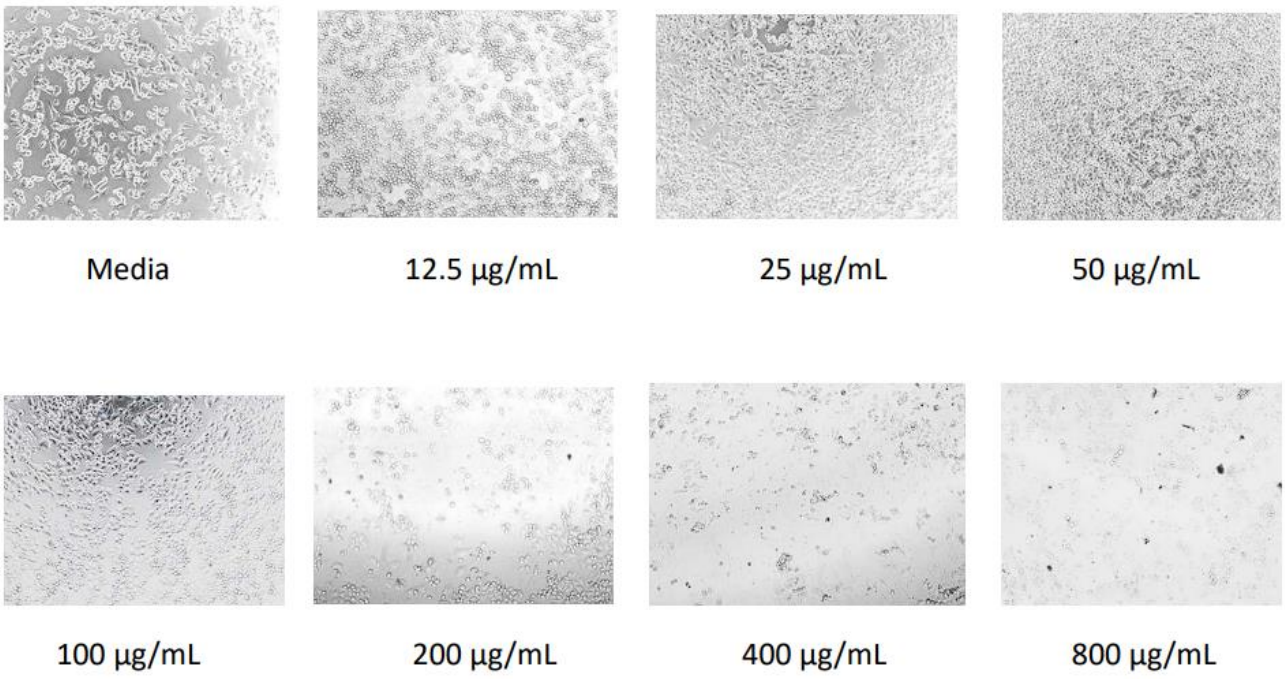


Figure 1. Morphological appearance of A-375 melanoma cells in cytotoxicity assay

This effect was evident from changes in cell morphology and a significant decrease in the number of living cells (Lee et al., 2020). The control group, which contained only the medium, showed that the A-375 melanoma cells grew normally, spread evenly, and adhered strongly to the surface of the well, with a viability rate of 100%. At a concentration of 12.5 µg/mL, the water fraction of cashew leaves showed cell viability of around 95%, with no significant morphological changes compared to the control. When the concentration increased to 50 µg/mL, cell viability decreased to around 60–70%, indicating the onset of cytotoxic effects. At a concentration of 100 µg/mL, cell viability dropped to 40–50%, the number of adherent cells decreased significantly, and cell morphology changed to become rounded, pale, and showed decreased metabolic activity. An increase in concentration to 200 µg/mL reduced cell viability to around 20–30%, while at 400 µg/mL the number of living cells decreased further, with viability only 5–10%. At the highest concentration of 800 µg/mL, cell viability dropped dramatically to below 5%, indicating that almost all cells died.

Testing of the viability of normal HaCaT cells showed that the water fraction of cashew leaves (*A. occidentale)* did not cause any significant toxic effects at various treatment concentrations. The IC_{50} value for HaCaT cells was recorded at 1,230.33 ± 61.83 µg/mL, resulting in a selectivity index (SI) of 6.68. This value indicates that the water fraction has a much higher toxicity to A-375 melanoma cancer cells than to normal cells. Based on general criteria, an SI value > 2 indicates that a compound is selective against cancer cells, so the water fraction of cashew leaves can be categorized as having good selectivity (Sarkhail et al., 2024;Valderrama et al., 2016). The use of HaCaT cells in this study aims to assess the relative safety of the extract to normal cells, where the comparison of IC_{50} values between the two cell types shows that the water fraction is more toxic to cancer cells but remains safe for normal cells (De la Cruz-Concepción *et al*., 2021).

Cell migration is a critical biological process in cancer development, particularly during the metastasis stage. Migration enables cancer cells to move from the primary tumor to surrounding tissues, penetrate the basement membrane, and enter the bloodstream or lymphatic system, thereby leading to the spread of cancer to other organs. Inhibition of wound closure in the scratch area reflects reduced cell migration capacity, which indirectly indicates the antimetastatic potential (Byambaragchaa et al., 2014).

The water fraction also exhibited inhibitory activity against A-375 melanoma cell migration within 24 hours. The migration rate at 25 µg/mL was 37.60%, and at 50 µg/mL, it decreased to 31.93% as shown in **Figure 2**. This did not result in a statistically significant reduction in migration (p=0.750). At a concentration of 100 µg/mL, wound closure decreased sharply to 8.74%, and this decrease was found to be significant compared to the 50 µg/mL concentration (p=0.052) as well as the 25 µg/mL concentration (p=0.022). These findings indicate that the inhibitory effect on migration

of the aqueous fraction becomes significant at a concentration of 100 µg/mL, while the increase from 25 to 50 µg/mL did not yield a significant difference.

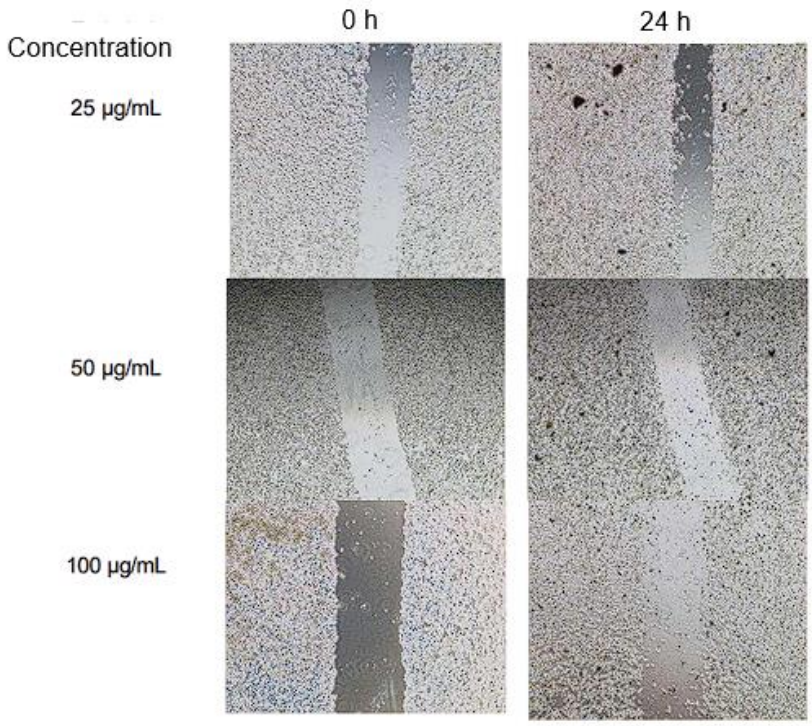


Figure 2. Microscopic migration activity of the water fraction of cashew leaves on melanoma A-375 Cell Line

Overall, these findings suggest that the aqueous fraction of cashew leaves has potential as an antimetastatic agent, particularly at higher concentrations. The ability to inhibit the migration of A-375 melanoma cells suggests that the active compounds in the aqueous fraction likely play a role in disrupting the mechanisms of cancer cell movement. Therefore, further research is needed to identify the active compounds involved and to understand the molecular mechanisms underlying this activity, thereby supporting the development of the aqueous fraction of cashew leaves as a candidate for effective and selective anticancer therapy.

4. CONCLUSION

The aqueous fraction of cashew leaves (*A. occidentale* L.) demonstrates promising potential as a natural anticancer agent against A-375 melanoma cells. This fraction exhibits selective cytotoxic activity toward cancer cells while maintaining relative safety toward normal cells. Moreover, it effectively inhibits cancer cell migration, indicating its potential as a natural antimetastatic agent for the treatment of melanoma.

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Ethical Approval: Not applicable

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