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Cytotoxic profiles of *Lithocarpus bancanus* extract and fractions against a lung cancer cell line A549

(Profil sitotoksik ekstrak *Lithocarpus bancanus* dan fraksinya terhadap lini sel kanker paru-paru A549)

Artikel Penelitian

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Abstract: Lithocarpus bancanus *is a medicinal plant used by communities in Riau Province, Indonesia. This plant is a tree an locally known in the province as mempening. In this study, the cytotoxic activity profiles of the methanolic extract, n-hexane fraction, dichloromethane fraction, and ethyl acetate fraction from the leaves of this plant were evaluated against human lung cancer cells (A549 cell line) using the MTT assay. IC_{50} values were calculated from dose–response curves analyzed using the 4-parameter logistic (4PL) model. The results showed IC_{50} values of 455.1, 361.9, 132.9, and 304.7 µg/mL for the methanolic extract, n-hexane fraction, dichloromethane fraction, and ethyl acetate fraction, respectively, with the dichloromethane fraction exhibiting relatively higher cytotoxic activity (IC_{50} = 132.9 µg/mL). Cisplatin was used as the positive control and showed an IC_{50} of 103.9 µg/mL. Meanwhile, the methanolic extract as well as the n-hexane and ethyl acetate fractions showed low cytotoxic activity, with IC_{50} values greater than 300 µg/mL. Therefore, the dichloromethane fraction can serve as a basis for further isolation to identify bioactive anticancer compounds.*

Keywords: *L. bancanus*, cytotoxic, cell line A549, IC_{50}

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Abstrak: *Lithocarpus bancanus* merupakan tumbuhan obat yang digunakan oleh masyarakat Provinsi Riau, Indonesia. Tumbuhan ini berupa pohon yang di provinsi ini dikenal dengan nama mempening. Dalam penelitian ini telah dikaji profil aktivitas sitotoksik ekstrak metanol, fraksi *n*-heksana, fraksi diklorometana, dan fraksi etil asetat dari daun tumbuhan ini terhadap sel kanker paru –paru (lini sel A549) dengan menggunakan metode MTT assay. Metode perhitungan IC_{50} menggunakan kurva dosis-respon yang dianalisis dengan model 4PL. Hasil penelitian menunjukkan bahwa aktivitas sitotoksik ekstrak metanol, fraksi n-heksana, fraksi diklorometana, dan fraksi etil asetat dengan aktivitas IC_{50} berturut-turut 455,1, 361,9, 132,9 dan 304,7 µL dengan fraksi diklorometana memiliki aktivitas sitotoksik yang relatif tinggi dengan nilai IC_{50} sebesar 132,9 µL. Senyawa obat untuk kontrol positif yang digunakan adalah cisplatin dengan IC_{50} 103,9 µL. Sementara itu ekstrak metanol, fraksi *n*-heksana dan etil asetat memiliki aktivitas sitotoksik yang rendah dengan nilai IC_{50} besar dari 300 µL. Dengan demikian dapat disimpulkan bahwa fraksi diklorometana dapat dijadikan dasar untuk pencarian senyawa bioaktif antikanker melalui proses isolasi lebih lanjut.

Kata kunci: *L. bancanus*, sitotoksik, lini sel A549, IC_{50}

Introduction

Research for bioactive compounds as anti-cancer agents in general can be started from screening plant extracts and their fractions. This strategy is based on the principle that most anticancer agents are derived from secondary plant metabolites of varying polarities, so gradual fractionation helps to concentrate several compounds that have the potential to have biological activity against cancer cells. In the context of research on *Lithocarpus bancanus,* methanol extract and its fractions have been used as the starting point for screening, with a separation sequence that follows the solvent's polarity gradation to increase the chances of identification of bioactive compounds [1]. The resulting fractions are *n*-hexane, dichloromethane, and ethyl acetate, which represent a wide polarity range and practically allow the filtering of anticancer activity of various classes of phenolic compounds, terpenoids, steroids, and flavonoids commonly found in plants [1, 2, 3].

Lithocarpus bancanus is a medicinal plant used by the people of the Riau area known as mempening and belongs to the Fagaceae family. The leaves of this plant are known to contain steroid, flavonoid, phenolic, and saponin compounds, as well as the extracts and fractions that have toxic activity against the larvae of *Artemia salina* Leach, where the *n*-hexane fraction has high activity with an LC_{50} value of 3.15 ppm [4].

Other results show that leaf extracts and fractions of *L. bancanus* have antioxidant activity against DPPH radicals, where methanol extract and ethyl acetate fraction have very high antioxidant activity with IC_{50} values of 39.469 ± 0.273 and 40.063 ± 1.604 ppm, respectively [5].

Research on the anticancer activity of several plants in Fagaceae family reports that the crude leaf extract of *L. polystachyus,* which contains flavonoids such as phloridzin, phloretin, and quercetin, has anticancer activity against breast cancer cells (MF-7) [6]. However, anticancer activity in *L. bancanus* has not been reported. Therefore, the purpose of this study is to determine the cytotoxic activity of extracts and fractions of *L. bancanus* against the lung cancer (cell line A549). Extracts and fractions that have cytotoxic activity can be used as the basis for searching for anti-cancer active compounds through the process of isolating pure compounds.

Materials and Methods

Materials

Mempening leaves were obtained from Kelayang Village, Indragiri Hulu Regency. The lung cancer cell line (A549) is a collection of cultures from the Department of Pharmacology and Clinical Pharmacy, Universitas Padjadjaran, Bandung.

Method

Extraction

Dried samples of *L. bancanus* leaves that have been mashed are macerated using methanol solvent pa. (Merck), then the macerate is concentrated with *a rotary evaporator* at a temperature of 40°C to form a thick methanol extract. Furthermore, the methanol extract is diluted with *n*-hexane pa. (Merck), dichloromethane pa. (Merck) and ethyl acetate pa. (Merck) sequentially until *n*-hexane, dichloromethane and ethyl acetate fractions were obtained. The solvents contained in these fractions were evaporated until a thick fraction is obtained.

Cytotoxic activity test against lung cancer cells (A549)

The lung cancer cell line was grown in Dulbecco's Modified Eagle's (DMEM) medium containing 10% fetal bovine serum and 1% *penicillin-streptomycin* solution (Gibco, Paisley, UK). The culture is maintained at 37°C in an atmosphere of 5% CO_2 in a *96-well tissue culture plate*. Cells with *a confluency* of 70-80% were rejuvenated with a new serum-free medium, further incubated for 4 hours, and then given extracts with concentrations of 1000, 500, 250, 125, 62.5 and 31.25 ppm. After 24 hours of incubation, the reagent Cell Counting Kit-8 (Dojindo, Rockville-MD, USA) was added, and the mixture was incubated for 2 hours. Cell suspension absorbance was measured using the

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Tecan Infinite spectrophotometer (Tecan, Grodig, Austria) at 450 nm with Cisplatin with (200, 100, 50, 25, 12.5 and 6.25 ppm) as a positive control tested by the same method as the extract [7].

Statistical Test

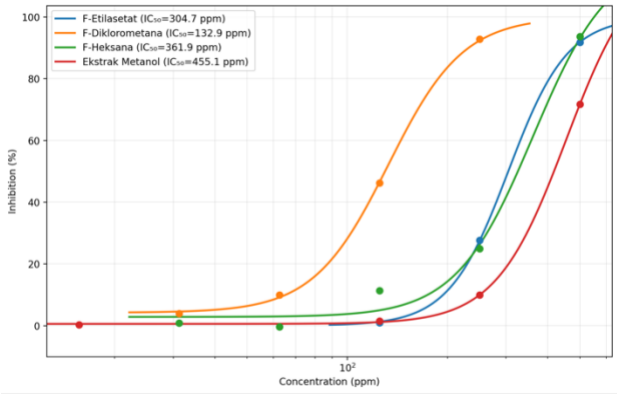
The absorbance data obtained is converted into a percentage of live cells. The percentage of deaths is calculated using the following formula:

$$\%\ \textbf{Cell Death} = \frac{\sum \text{Control Viable Cells} - \sum \text{Treated Viable Cells}}{\sum \text{Control Viable Cells}} \times 100\%$$

The final analysis was carried out by calculating the IC_{50} value with a dose-response curve an analyzing it using the 4PL method.

Results and Discussion

Figure 1 is a graph of the calculation of the IC_{50} value with the dose-response curve analyzed by the 4PL method.



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No.	Extract and fractions	IC_{50} (µL)
1	*n*-hexane fraction	361.9
2	Dichloromethane fraction	132.9
3	Ethyl acetate fraction	304.7
4	Methanol extract	455.1
5	Cisplatin	103.9

Figure 2 shows the comparison of IC_{50} in the form of a bar chart. Extracts or fractions in different solvents exhibit different inhibitory patterns to their cytotoxic activity due to their different polarities [5]. The low cytotoxic activity of methanol extract is due to various compounds with different polarities, while the n-hexane and ethyl acetate fractions are due to the content of secondary metabolites contained in these extracts and fractions that do not support this activity. From this diagram, it can be seen that the difference between IC_{50} cisplatin and the dichloromethane fraction is not very significant.

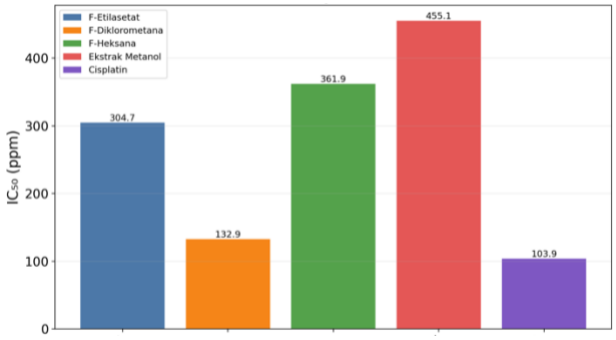

Figure 2. IC_{50} (µL) diagram of *L. bancanus* leaf extract and fractions against lung cancer (cell line A549).

The dichloromethane fraction has a fairly high cytotoxic activity; this is influenced by the content of secondary metabolites contained in the fraction. In general, dichloromethane solvent is able to extract compounds that are semi-polar, especially the terpenoid group, where this group of compounds is widely reported to have anticancer activities [8]. Based on phytochemical tests in previous studies, extracts fractionated with chloroform are known to contain terpenoid compounds [4].

Conclusion

The dichloromethane fraction of the leaves of *L. bancanus* has strong cytotoxicity when compared to methanol extracts, *n*-hexane fractions and ethyl acetate. Thus, this dichloromethane fraction can be used as a basis in the search for anticancer bioactive compounds through the process of isolating pure compounds.

Conflict of Interest

There are no conflicts of interest to be considered in this study.

Acknowledgments

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