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Effects of *Borassus flabellifer* L. fruit seed coat on HSC-3 cell line

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Abstract

Background: The seed coat of *Borassus flabellifer* L. fruit contains antioxidants that can neutralize the toxic effects of free radicals and can fight DNA damage due to exposure to oxidative stress. The extract of *B. flabellifer* is proven to have cytotoxic effects and can inhibit the growth of several types of cancer cells such as HeLa cell lines.

Objective: To determine the cytotoxicity effect of extract of *B. flabellifer* L. fruit seed coat against HSC-3 cell line. **Methods:** Laboratory experimental research was conducted using HSC-3 cell lines treated with *B. flabellifer* L. fruit seed coat extract at concentrations of 93.75 µg/mL, 187.5 µg/mL, 375 µg/mL, 750 µg/mL, and 1,500 µg/mL, negative control DMEM-FBS 20%, and positive control anti cancer drugs Doxorubicin 3 µM. HSC-3 cell line cytotoxicity assay was performed using CCK-8 reagent. Micro plate reader at 450 nm wavelength was used to determine the viability of HSC-3 cell lines by measuring the optical density of formazan.

Results: *B. flabellifer* fruit L. seed coat extract was able to reduce viability and was cytotoxic to HSC-3 cell lines at concentrations of 93.75 µg/mL, 187.5 µg/mL, 375 µg/mL, 750 µg/mL, and 1,500 µg/mL, with an IC_{50} value of 141.9 µg/mL.

Conclusion: *B. flabellifer* L. fruit seed coat extract can reduce the ability of HSC-3 cell line cancer cells to survive by inhibiting cell proliferation due to its cytotoxicity effect.

Keywords: *Borassus flabellifer* L; Fruit seed coat; Cancer cell; HSC-3 cell line; Cytotoxicity; CCK-8.

1. Introduction

Oral cancer including lip cancer, tongue, other parts of the oral cavity and oropharynx is the 15th most common cancer worldwide. The number of patients with lip and oral cancer is estimated to be around 377,713 for new cases with a mortality rate of 177,757 in 2020.[1,2] In Indonesia, the prevalence of oral cancer ranges from 3-4% of all malignancies that occur with various causes with mortality rates due to oral cancer ranging from 2-3%. Exploring new strategies for early diagnosis and treatment of oral cancer is the key to improving cure rates in cancer patients.[3] There are so many methods to treat cancer such as tumor surgery, radiotherapy, immunotherapy, chemotherapy, cancer vaccination, photodynamic therapy, stem cell transformation or their combinations which are often accompanied by severe side effects. These side effects include limited bioavailability, being toxic to body cells and being nonspecific to target cell.[4]

These day, medicinal plants are beneficial to humans to provide better health. Plants and their bioactive compounds have been used in medicinal practices since ancient times.[5] Several species of medicinal plants such as Dutch Eggplant (*Cyphomandra betacea* Sendtn.), Palasa (*Butea monosperma*), Papaya (*Carica papaya* L.), and their phytochemicals have been shown to inhibit cancer growth and development.[6,7,8] The content of primary and secondary metabolites in medicinal plants, such as: alkaloids, flavonoids, lignans, saponins, terpenes, vitamins, and minerals play an important

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role in inhibiting cancer cell activating proteins.[5] In Indonesia, *Borassus flabellifer* L. is widely found which has a variety of functions, such as: antibiotics, antioxidants, anti-inflammatory, antidiuretic, etc.[9] Previous research states, *B.flabellifer* L. fruit seed coat extract contains antioxidants such as flavonoids, tannins, and phenols.[10]

Antioxidants are compounds that can neutralize the toxic effects of free radicals and can fight DNA damage due to exposure to oxidative stress.[11] The extract of *B. flabellifer* L. fruit seed coat is proven to have cytotoxic effects and can inhibit the growth of several types of cancer cells such as HeLa cell line.[12] Currently, there is no research on the cytotoxicity effect of *B.flabellifer* L. fruit seed coat on HSC-3 cell line so it is necessary to conduct research on the effect of *B.flabellifer* L. fruit seed coat extract in inhibiting cancer cells or as an anticancer in HSC-3 cell line with CCK-8 method. The aim of this study was to determine the cytotoxicity effect of *B.flabellifer* L. fruit seed coat on Tongue squamous cell carcinoma HSC-3 cell line. The hypothesis of this study was from that the *B.flabellifer* L. fruit seed coat extract would exerts cytotoxicity effect of HSC-3 cell line.

2. Materials and Methods

The fruit used in this study was the plant species *Borassus flabellifer* L., sourced from Sekolah Ilmu dan Teknologi Institut Teknologi Bandung Hayati (SITH ITB). This study was conducted in the Biomedical Department and the Integrated Laboratory of YARSI University, from September to November 2023. The sample used was HSC-3 cell line from the Bio Bank of the Integrated Laboratory of YARSI University. *B.flabellifer* L. fruit seed coat extract was obtained by maceration technique through diluting 62.2 g of the *B. flabellifer* L. dried seed coat in 1,500 mL of 70% ethanol for 3 days, filtrated, and then evaporated in a rotary vacuum evaporator to obtain a thick extract. The extract was diluted using serial dilution to obtain five concentrations : (93.75 µg/mL, 187.5 µg/mL, 375 µg/mL, 750 µg/mL, 1,500 µg/mL). These concentrations were used for treatment *B.flabellifer* L. fruit seed coat extract groups of this study based on the result of the previous study. [12]

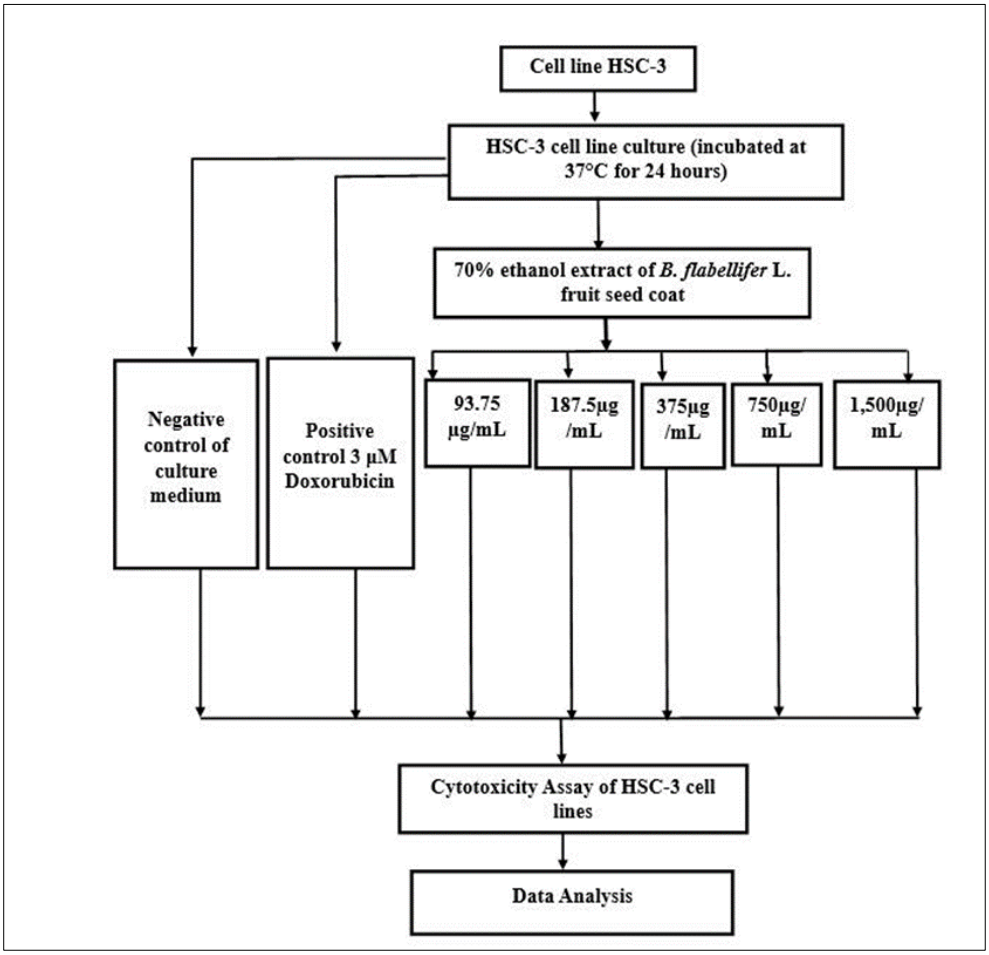


Figure 1 Study protocol and various concentrations of *B. flabellifer* L. seed coat tested.

A subculture of HSC-3 cell line in nitrogen liquid was warmed in a water bath at 37°C and then diluted in the culture medium up to 10 times, centrifuged to obtain the cell plate, and re-suspended with culture media. A T-flask was used for the cell culture, with incubation from 24h up to 7 days. The cells were then collected for further analysis. The total sample used for each well was 5,800 cells that were cultured for group samples consisting of five treatment extract groups, a positive control, and a negative control. Let it in an incubator (37°C) for 24h and then put Cell Counting Kit-8 (CCK-8) into the well. After 1h, the viability test was measured using a micro plate reader with ƛ = 450nm. The measurement was done four times.

The cytotoxicity test in this study used the Cell Counting Kit-8 (CCK-8) test (Sigma Aldrich, Germany). In contrast with the MTT assay that determined the cytotoxic cells, the CCK-8 test counts the viability cells based on the color changes of the HSC-3 cells. This CCK-8 test used in this study determines viability cells using 96 wells, which consisted of 15,000 cells with volume 200 µL. CCK-8 reagent was added to each well and the 96-well plate was placed in an incubator (37°C) for 24h. After 1h, the viability of the HSC-3 cells was measured using a micro plate reader with ƛ = 450nm. The scheme of the research protocol is shown in Figure 1.

The cell viability measurement was performed four times. Cell viability was determined based on color changes of HSC-3 cell line treated with the CCK-8 reagent. Cell viability was determined using the following formula as followed :[13]

$$\% \text{ Viability of cells} = \frac{\text{absorbance of treated group}}{\text{absorbance of negative control}} \text{ X } 100\%$$

Perform % inhibition calculation using the formula as followed:[12]

$$\% \text{ Inhibition} = 100\% - \% \text{ viability of cells}$$

2.1. Statistical analysis

This study used *Saphiro Wilk* test to know the normality of data within groups. The data of this study were not normally distributed (p<0,05); therefore, non parametric statistical was done to find out the difference between groups.

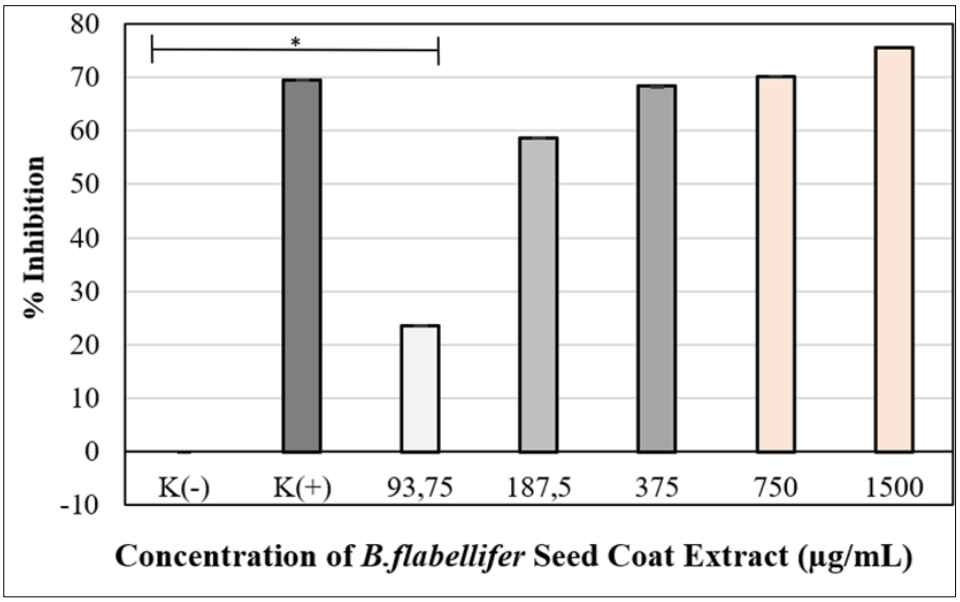
3. Results

The phytochemical content of *Borassus flabellifer* L. fruit seed coat extract is shown on Table 1. The results of the % inhibition *B.flabellifer* L. fruit seed coat extract towards HSC-3 cell line are shown in Figure 2. *Saphiro-Wilk* test showed that the data were not distributed normally (p<0,05). *Kruskal-wallis* test and *Mann Whitney* post hoc test showed that there were significant differences (p<0.05) between concentrations of 93.75 µg/mL, 187.5 µg/mL, 375 µg/mL, 750 µg/mL, and 1,500 µg with negative control and between concentrations of 93.75 µg/mL against concentrations of 187.5 µg/mL, 375 µg/mL, 750 µg/mL, and 1,500 µg/mL. The extract with a concentration of 187.5 µg/mL showed a significant difference (p<0,05) with the extract concentration group of 1,500 µg/mL. The positive control group also gave a significant difference (p<0,05) with the extract group at a concentration of 93.75 µg/mL.

Table 1 Phytochemical Content of Borassus flabellifer L. Seed Coat Extract

No.	Secondary metabolit	Method of test/ reagent	Result
1.	Phenolics	$FeCl_3$	Positive
2.	Flavonoid	HCl concentrated + Mg H_2SO_4 2N NaOH 10%	Positive Positive Positive
3.	Steroid	Lieberman-Burchard	Negative
4.	Terpenoid	Lieberman-Burchard	Positive
5.	Saponin	HCl + H_2O	Negative
6.	Tanin	$FeCl_3$ 1%	Positive
7.	Alkaloid	Hager Wagner Dragendroff	Positive Positive Positive

This study obtained the value IC_{50} 141.9 µg/mL. Among all the extract concentrations tested, the 1,500 µg/mL concentration had the strongest cell viability suppressing effect compared to the other extract concentrations, which means it gave the greatest cytotoxicity effect.



*indicates significant difference (p<0.05)

Figure 2 % HSC-3 cell line viability inhibition test result of *B.flabellifer* L. fruit seed coat extract various concentration, positive control, and negative control.

4. Discussion

Borassus .flabellifer L. fruit seed coat extract is effective in reducing the viability cancer cell (HSC-3 cell line) because it has a cytotoxicity effect, especially at a concentration of 1,500 µg/mL. The cytotoxicity of *B.flabellifer* L. fruit seed coat extract against cancer cell lines as shown in this study is attributed to its phytochemical content (i.e., phenolics, flavonoids, terpenoids, tannins, and alkaloids). The result of this study is in accordance with previous studies that *B.flabellifer* L. fruit seed coat extract has anticancer effects on HeLa cell line due to its phytochemical properties (flavonoids, tannins, alkaloids, and phenolics). Flavonoids can function as antioxidants both directly and indirectly. Directly by providing hydrogen ions so as to neutralize the toxic effects of free radicals. Indirectly by increasing the expression of endogenous antioxidant genes in various ways. One of them is the activation of nuclear factor erythroid 2 related factor 2 (Nrf2), causing an increase in the expression of genes that play a role in the synthesis of the superoxide dismutase (SOD) gene.[14]

Tannins can inhibit Vascular Endothelial Growth Factor/ Vascular Endothelial Growth Factor Receptor (VEGF/VEGFR), thus inhibiting the main angiogenesis signaling pathway in cancer. It also inhibits sex-determining region Y-box 2 (SOX-2) gene expression and inhibits the Epidermal Growth Factor/ Epidermal Growth Factor Receptor (EGF/EGFR) signaling pathway. [15] Alkaloids can cause apoptosis of breast, lung, and colorectal cancer cells in the G2/M phase of the cell cycle. In addition, Alkaloids can also activate Mitogen Activated Protein Kinase (MAPK) p38 which affects dose-dependent cytotoxicity in breast, ovarian, and lung cancer.[16] Terpenoid compounds can inhibit early initiation, apoptosis in cancer cells, suppress cancer growth, inhibit angiogenesis and metastasis of cancer cells.[17]

5. Conclusions

Borassus flabellifer L. fruit seed coat extract showed cytotoxicity effect against HSC-3 cell line starting from the concentration of 93.75 µg/mL with the optimum concentration of 1,500 µg/mL.

The cytotoxicity of *Borassus flabellifer* L. fruit seed coat extract against cancer cells is due to its phytochemicals content such as flavonoids, tannins, alkaloids, terpenoids.

Compliance with ethical standards

Disclosure of Conflict of Interest

The author has no conflict of interest in this research.

References

[1] Saraswat N, Everett B, Pillay R. Knowledge, attitudes and practices of general medical practitioners in developed countries regarding oral cancer: an integrative review. Oxford Journals : Family Practice. 2020; 37(5)

[2] Peres MA, Macpherson LMD, Weyant RJ et al. Oral diseases: a global public health challenge. Lancet 2019; 394

[3] Bray F, Ferlay J, Soerjomataram I et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clinic 2018; 68: 394–424

[4] Day. TA, Davis. BK, Gillespie. MB, Joe. JK, Kibbey. M, Martin-Harris. B, Neville. B, Reed. SG, Richardson. MS, Rosenzweig. S, Sharma. AK, Smith. MM, Stewart. S, Stuart. RK. Oral cancer treatment. Curr Treat Options Oncol. 2003 Feb;4(1):27-41. doi: 10.1007/s11864-003-0029-4. PMID: 12525277.

[5] Iqbal. J, Abbasi. BA, Mahmood. T, Kanwal. S, Ali. B, Shah. SA, Khalil. AT. Plant-derived anticancer agents: A green anticancer approach. Asian Pacific Journal Of Tropical Biomedicine 2017;7;2:1130.

[6] Saqila S, Sudiono J. Efek Ekstrak Biji Buah Terung Belanda (*Cyphomandra betacea* sendt.) terhadap Sel Kanker Lidah (HSC-3). Scientific Dental Journal. 2022: 141

[7] Karia P, Patel K V., Rathod SSP. Breast cancer amelioration by *Butea monosperma* in-vitro and in-vivo. J Ethnopharmacol. 2018;217:54–62.

[8] Nisa FZ, Astuti M, Murdiati A, Haryana SM. Anti-proliferation and apoptosis induction of aqueous leaf extract of *Carica papaya* L. On human breast cancer. Pakistan Journal of Biological Science. 2017;20(1) :36-41

[9] Lina SMM, Mahbub KMM, Ashab I, Al-Faruk M, Atanu SH, Alam MJ, et al. Antioxidant and Cytotoxicity Potential of Alcohol and Petroleum Ether Extract of *Borassus Flabellifer* Linn. 2013; 4(5): 1853.

[10] Susanto GR, Sudiono J. Antioxidant Content of Palm Fruit (*Borassus flabellifer* L.) Seed Coat. Biomedical Journal of Scientific & Technical Research. 2021;34(3):26699.

[11] Dai J, Mumper RJ. 2010. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. J mol 15,7313. Doi : 10.3390/molecules 15107313.

[12] Duddukuri RG, Sastry NY, Babu A, Chaitanya KK, Rao KK, et al. Preliminary Studies on In Vitro Anti-Tumor Activity of Tender Seed Coat Extract of *Borassus flabellifer* L. on Hela Cell Line. International Journal of Current Research. 2011; 3(7): 76

[13] Fitria N, Bustami DA, Komariah K, Kusuma I. The antioxidant activity of lemongrass leaves extract against fibroblasts oxidative stress. BDS. 2022; 25(4) : 3.

[14] Syaiffudin. Uji aktivitas antioksidan bayam merah (*Alternanthera Amoena voss*.) segar dan rebus dengan metode DPPH [disertasi]. Universitas Islam Negeri Walisongo Semarang; 2015.

[15] A Youness R, Kamel R, A Elkasabgy N, Shao P, A Farag M. Recent Advances in Tannic Acid (Gallotannin) Anticancer Activities and Drug Delivery Systems for Efficacy Improvement; A Comprehensive Review. Molecules. 2021 Mar 9;26(5):1486. doi: 10.3390/molecules26051486. PMID: 33803294; PMCID: PMC7967207.

[16] Habli Z, Toumieh G, Fatfat M, Rahal ON, Gali-Muhtasib H. Emerging Cytotoxic Alkaloids in the Battle against Cancer: Overview of Molecular Mechanisms. Molecules. 2017 Feb 8;22(2):250. doi: 10.3390/molecules22020250. PMID: 28208712; PMCID: PMC6155614.

[17] Akhtar MS, Ansari IA. Natural Bio-active Compounds. Springer Nature Singapore; 2019 [cited 2024 January 5]. Available from : https://doi.org/10.1007/978-981-13-7205-6_3