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Antiproliferative Activity of *Borassus flabellifer* L. Seed Coat Extracts on Oral Carcinoma Cells

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ABSTRACT

Borassus flabellifer L. is a plant in Arecaceae family, widely distributed and cultivated in tropical Asian countries. Different parts of *Borassus flabellifer* L., including the fruit, seeds, leaves, and roots, are used in traditional medicine due to their rich bioactive compound content. The present study aims to investigate the in vitro anti-proliferative activity of EBSC on HSC-3 cells. The extract were prepared using an ethanolic solvent, and its phytochemical composition were analyzed to identify potential bioactive compounds. The antiproliferative effects of the extract were evaluated using the Cell Counting Kit-8 (CCK-8) assay. The phytochemical analysis revealed the presence of phenolic, flavonoid, terpenoid, tannin, and alkaloid in EBSC. In CCK-8 assay, EBSC exhibited dose-dependent antiproliferative effects on HSC-3 cells. Significant inhibition of cell proliferation was observed at 750 µg/mL, comparable to doxorubicin (3 µM, $p < 0.001$), following 24, 72, and 120 hours of treatment. EBSC shows promising potential as a natural therapeutic candidate for OSCC treatment and warrants further in vivo studies.

Keywords: Borassus flabellifer L.; Seed Coat Extract; Antiproliferative; OSCC; Natural Anticancer Agent; CCK-8 Assay

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1. Introduction

Oral squamous cell carcinoma (OSCC) represents a significant global health issue [1], In 2020, lip and oral cavity cancer was estimated to be the 16th most common type of cancer globally in terms of both incidence and mortality, accounting for a substantial proportion of oral cancers [2]. Despite advancements in treatment modalities, OSCC continues to have high morbidity and mortality rates [3], underscoring the need for novel therapeutic agents. Traditional medicine, with its rich repository of bioactive compounds, has emerged as a promising avenue for discovering new cancer therapies [4],[5].

Borassus flabellifer L. a member of the Arecaceae family, is widely distributed and cultivated in tropical Asian countries [6]. Known commonly as the palmyra palm, it has been used extensively in traditional medicine. Various parts of the plant, including the fruit, seeds, leaves, and roots, are valued for their medicinal properties, attributed to the presence of diverse bioactive compounds. The potential therapeutic effects of plant

extracts, particularly their anti-proliferative properties, are often linked to the presence of phytochemicals such as phenolics, flavonoids, terpenoids, tannins, and alkaloids. These compounds are known to exhibit antioxidant, anti-inflammatory, and anticancer activities [7],[8],[9],[10].

Previous studies have highlighted the medicinal potential of *B.flabellifer* L. extracts in various contexts. Research has demonstrated that *B.flabellifer* L. male flower extracts possess antimicrobial, antioxidant, and anti-inflammatory properties [6]. Another study reported that the aqueous extract from the endosperm of germinated B. flabellifer L. seeds exhibited strong antiproliferative activity against MOLT-4 blood cancer cell lines [10]. However, studies focusing specifically on the antiproliferative effects of Borassus flabellifer L. seed coat extract on oral cancer cell lines remain limited. Therefore in the present study, we investigated the effect of *B.flabellifer.* L seed coat ethanol extract (EBSC) on the proliferation of human oral squamous carcinoma-3 (HSC-3) cells. Understanding the mechanisms by which this extract influences cancer cell viability and proliferation could provide valuable insights into its potential therapeutic applications.

2. Metode

This study is an in vitro laboratory experimental research investigating the effects of EBSC on HSC-3 cell lines. The research includes the preparation of EBSC at various concentrations and the assessment of its effects on HSC-3 cell proliferation. This study has been approved by Universitas Trisakti of the Dentistry Ethics Commission under ethical clearance number 173/KEP-UY/EA.10/VII/2023.

Sample Collection

B.flabellifer L. were collected from our local area Bogor, Indonesia. Seed coat was taken out from the *B.flabellifer* L. fruit and was cut into small pieces. These small pieces of *B.flabellifer* L. seed coat were dried for 4-5 days. The dried seed coat samples were stored at room temperature in order to be used for this study [8].

Plant Extraction

The extraction process was conducted using the maceration technique, where 62.2 g of dried *B. flabellifer* L. seed coat was immersed in 1,500 mL of 70% ethanol for three days. After the maceration period, the mixture was filtered and then concentrated using a rotary vacuum evaporator to produce a thick extract. This extract was then subjected to serial dilution to achieve five different concentrations: 93.75, 187.5, 375, 750, and 1500 µg/mL [7].

Phytochemical Test

Phytochemical tests were carried out at the Integrated Research Laboratory of Yarsi University to identify the active compounds contained in EBSC. Phytochemical tests were carried out qualitatively [6].

Cell Culture

The human oral squamous cell carcinoma (OSCC) cell line (HSC-3) was acquired from Integrated laboratory of Yarsi University (Jakarta, Indonesia). Cells were maintained in complete Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS), 1% Penicillin-Streptomycin, and 1% Amphotericin B [5].

Cell Proliferation Assay

A cell counting kit-8 (CCK-8; Sigma-Aldrich) was used to characterize the antiproliferative activity of EBSC on HSC-3 cells. HSC-3 cells ($5x10^3$ cells) were seeded

in triplicates in a 96-well plate. Following incubation with various concentrations (93.75; 187.5; 375; 750; 1500 µg/mL) of EBSC for 24, 72, and 120 h. 10 mL of CCK-8 solution was added to each well and incubated for 60 min. Cell counts were quantified using a microplate reader at an absorbance of 450 nm. Doxorubicin 3 µM was used as the positive control, while DMEM served as the negative control [5].

Statistical Analysis

The Shapiro-Wilk test was used to assess normality. Differences between the experimental groups were analyzed using a one-way analysis of variance (ANOVA) followed by a Post-Hoc test. A p-value of less than 0.05 ($p<0.05$) was considered statistically significant [11].

3. Result and Discussion

Phytochemical Test

The qualitative phytochemical analysis revealed that the ethanol extract of *B.flabellifer* L. seed coat contains phenolics, flavonoids, terpenoids, tannins, and alkaloids (**Table 1**).

Table 1. Phytochemical Screening Results of EBSC

Secondary Metabolites	Detected
Phenolic	+
Flavonoid	+
Steroid	-
Terpenoid	+
Saponin	-
Tannin	+
Alkaloid	+

Note: (+) indicates presence; (–) indicates absence of the compound

Phytochemical screening of the ethanol extract of *B. flabellifer* L. seed coat (EBSC) revealed the presence of phenolics, flavonoids, terpenoids, tannins, and alkaloids, which are widely recognized for their anticancer and cytotoxic properties. Flavonoids and phenolics can act as antioxidants and modulators of cell signaling pathways, while alkaloids have been reported to induce apoptosis and interfere with DNA replication in cancer cells [7],[11],[13]. Terpenoids and tannins may also contribute to cytotoxicity by disrupting mitochondrial function and enhancing oxidative stress [8], [10]. The absence of steroids and saponins indicates that the observed antiproliferative effects are likely driven by the dominant phenolic and alkaloid constituents, in line with previous studies on plant-based anticancer agents [12],[14].

Cell Proliferation Assay

The possible effects of different concentrations of *B.flabellifer* L. seed coat extract (93.75; 187.5; 375; 750; 1500 µg/mL) on the proliferation of OSCC cell line HSC-3 were investigated further using CCK-8 assay. *B.flabellifer* L. seed coat extract treatment significantly suppressed the proliferation of HSC-3 cells in concentration-dependent manner. The best results are at a concentration of 750 µg/mL (**Figure 1** & **2**).

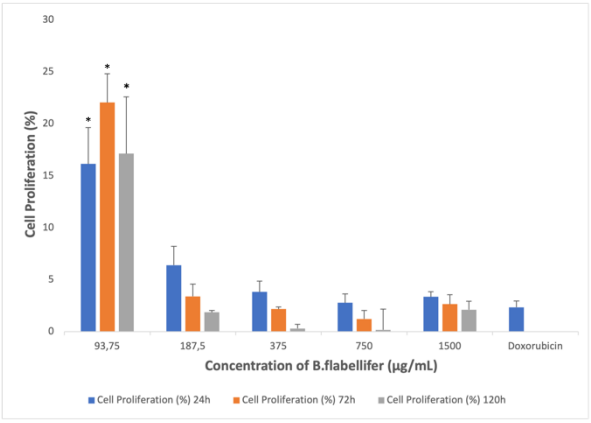

Figure 1. Proliferation of HSC-3 cells measured using a CCK-8 assay. CCK-8 assays were performed at 24, 72 and 120 h. Data are presented as the mean ± standard error of the mean of 3 repeats.* P<0.05 vs positive control.

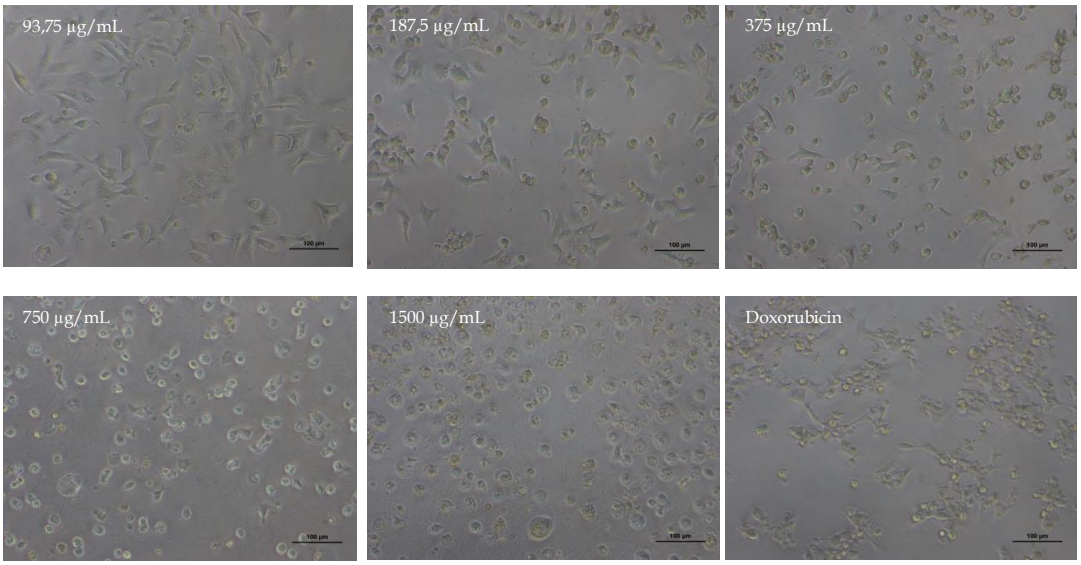

Figure 2. Representative image of the CCK-8 assay after 24 h of treatment. HSC-3 cells were treated with different concentration of EBSC, with 750 µg/mL shows the most significant anti proliferative-effect. EBSC : a ethanol extract of *B.flabellifer* L. seed coat

At present, the pathogenesis of oral squamous cell carcinoma (OSCC) is considered a complex multifactorial process that includes genetic predisposition, environmental exposures, and lifestyle factors such as tobacco and alcohol consumption [12]. Among various cell types involved in OSCC progression, oral keratinocytes and carcinoma-associated fibroblasts play pivotal roles in promoting tumor growth, invasion, and metastasis through dysregulated proliferation, resistance to apoptosis, and increased inflammatory signaling [13]. Despite advancements in surgical techniques, radiotherapy, and chemotherapy, the prognosis of OSCC remains unsatisfactory due to high rates of recurrence and metastasis [14]. Thus, there is a pressing need for alternative and adjunctive therapeutic agents derived from natural sources. *Borassus flabellifer* L., commonly known as palmyra palm, has been reported to possess multiple

pharmacological activities, including antioxidant, anti-inflammatory, and anticancer properties [10][8]. Based on these properties, we investigated the antiproliferative effects of *B. flabellifer* L. seed coat extract on human OSCC HSC-3 cells.

Previous studies showed that *B.flabellifer* L. not only exert significant cytotoxicity towards several cancer cell lines [8], but also showed significant reduction in the percent increase of body weight, tumor volume and tumor weight of Ehrlich ascites carcinoma and Dalton ascites lymphoma in Swiss albino [7]. In addition, Abirami et al demonstrated that hydrogel containing *B.flabellifer* L. male flower extract can exert significant inhibition towards blood cancer (MOLT-4) cell lines proliferation [10].

In the present study, CCK-8 assays results showed that EBSC significantly decreased the proliferation of HSC-3 cell lines in a dose-dependent manner, with effects comparable to those induced by the clinical drug Doxorubicin. The optimal inhibitory effect on cell proliferation was observed at a concentration of 750 µg/mL of EBSC. At this concentration, the inhibition of HSC-3 cell proliferation was not significantly different from that of the positive control. Interestingly, at a higher concentration of 1500 µg/mL, the inhibitory effect was reduced compared to the 750 µg/mL concentration (**Figure 1**). This may suggest that 750 µg/mL represents the maximal effective dose of the extract, and that higher concentrations may exceed a cytotoxicity threshold or trigger off-target effects that compromise efficacy. A similar trend was reported by Duddukuri et al., who discovered that 50% inhibition of *B.flabellifer.* L against HeLa cancer cells occurred at this concentration [8].

Species of the genus *Borassus* have been known for their richness in phenolic compounds. Our study also showed that EBSCs are rich in flavonoids compounds such as phenolics, flavonoids, terpenoids, tannins, and alkaloids (**Table 1**). Those phenolic compounds are non-harmful to human health and have many health benefits; therefore, there is an increasing use of plants with high phenolic content in cancer therapy. The antiproliferative activity of the plant was correlated with the content of polyphenolic compounds. Flavonoid compounds, saponins, quinones, steroids, terpenoids, and tannins are believed to have antiproliferative activity by direct and indirect mechanism. Flavonoids are the most important natural phenolic compounds and are thought to be responsible for the antioxidant and the antiproliferative activities of natural products [15].

Flavonoid compounds can inhibit the cell cycle, either in G1/S or G2/M, by inhibiting cyclin-dependent kinases (CDKs), which are key regulators of cell cycle development [11]. Steroids and terpenoids can inhibit the primary mechanisms in cell proliferation and trigger apoptosis and autophagy of cancer cells [16][17]. Kim et al, found that geraniol, a monoterpene with structural and functional similarities, can effectively induce apoptosis and autophagy in tumor cells. Their study also showed that inhibition of AMPK promotes cell proliferation and enhances malignant behavior. These findings suggest that activating AMPK may enhance the anticancer effects of AKT pathway inhibition. Furthermore, geraniol was shown to inhibit the AKT signaling pathway, activate the AMPK pathway, and suppress the mTOR signaling pathway [18]. Tannin compounds inhibit cancer proliferation indirectly by inducing apoptosis in Caspase 3 and Caspase 9-dependent pathways, down-regulating TGF beta, and causing mitochondrial membrane potential in KB cells. Tannins' pro-apoptotic action in KB cells is most likely due to ROS production and cell cycle arrest mechanisms [19].

Despite these promising findings, this study has several limitations. The molecular mechanisms underlying the antiproliferative effects of EBSC were not examined in detail, and the study was confined to in vitro testing using a single OSCC

cell line without in vivo validation. Therefore, further studies involving mechanistic exploration, animal models, and isolation of specific active compounds are necessary to better elucidate the therapeutic potential of *B. flabellifer* L. seed coat extract as a candidate for OSCC treatment.

4. Conclusion

Natural compounds derived from medicinal plants have been pivotal in cancer treatment. This study demonstrated that the ethanol extract of *B.flabellifer* L. seed coat contains a flavonoid capable of inhibiting the proliferation of HSC-3 cell lines. The optimal inhibition of HSC-3 cell proliferation was observed at a concentration of 750 µg/mL. These results suggest that *B. flabellifer* L. seed coat extract holds potential as a therapeutic agent for the treatment of oral squamous cell carcinoma. The authors may suggest further functional and mechanistic studies to elucidate the molecular pathways involved in its anticancer activity, as well as in vivo studies to validate its efficacy and safety. It is therefore recommended that future research focuses on the isolation and characterization of the active constituents, alongside preclinical evaluations, to support the development of plant-derived adjunctive therapies for oral cancer.

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Conflicts of Interest:

The authors declare no conflicts of interest.

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