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Inhibition of HSC-3 cell migration by Borassus flabellifer L. extract

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ABSTRACT: Objectives: to discover the bio active compounds of *B. flabellifer* L. seed coat ethanol extract and examine its potential to inhibit the migration of HSC-3 cells. Methods: A colorimetric assay was used to determine the bio active compounds of the ethanol extract of *B. flabellifer* L. seed coat. This study involved seven groups: five treatment groups with extract concentrations of 93.75, 187.5, 375, 750, and 1500 µg/mL, a negative control (0.1% DMSO in culture medium), and a positive control (doxorubicin). Cell migration was assessed using a wound healing assay, with wound closure percentage as the outcome measure. Results: Colorimetric analysis confirmed the presence of phenolics, flavonoids, terpenoids, tannins, and alkaloids in the *B. flabellifer* L. seed coat ethanol extract. The extract inhibited HSC-3 cell migration, with the greatest effect observed at 750 µg/mL, showing the lowest wound closure. Conclusion: The extract demonstrates anticancer potential in OSCC by suppressing cell migration.

Keywords: Borassus Flabellifer, HSC-3, Migration, Wound Healing

1 INTRODUCTION

As the most common oral malignancy in the world, oral squamous cell carcinoma (OSCC) accounts for 90% of cases and is the sixth most common malignancy overall (Xu *et al.* 2023). Even with the introduction of new treatment options in the last ten years, like radiation, chemotherapy, and surgery, the overall survival rate is still low (Bai *et al.* 2022). Lymph node metastasis and recurrence are common in OSCC, which has a less than 50% 5-year survival rate. Eradicating lesions with surgery and chemotherapy is very challenging due to the high incidence of metastasis (Chang *et al.* 2023). Therefore, the development of low toxicity and efficient novel drugs for the treatment of OSCC is urgently required. To achieve this goal, significant focus has been placed on exploring natural substances found in plants, marine creatures, and microorganisms.

The *Borassus flabellifer* L. *(B. flabellifer* L.), a summer fruit in India, shows promise as a therapeutic herb due to its rich bioactive compounds. Prior research has shown that *B. flabellifer* L. has anti-tumor effects on human cancer cell lines like Hela and Vero cell lines (Banu *et al.* 2022, Duddukuri *et al.* 2011), and has also demonstrated a notable decrease in body weight gain, tumor volume, and tumor weight in Swiss albino mice with Ehrlich ascites carcinoma and Dalton ascites lymphoma (Babu and Jeenachandran 2019). Prior research has shown the potential of *B. flabellifer* L. extracts in fighting cancer, but limited research has been conducted on the inhibitory effects of *B. flabellifer* L. seed coat ethanol extract specifically on HSC-3 cell migration. This study aims to address this research gap by examining the inhibitory potential of *B. flabellifer* L seed coat ethanol extract on HSC-3 cell migration. By addressing this aim, this study helps to develop new natural therapeutic agents for the management of oral squamous cell carcinoma.

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2 METHOD

2.1 *Preparation Of B.Flabellifer L. seed coat ethanol extract*

The fruit of *B. flabellifer* L. is rinsed with tap water before the outer skin is peeled off. Next, the seed coat of *B. flabellifer* L. is allowed to dry for 4-5 days. Extraction is done through maceration by mixing 62.2 grams of dried seed coat of *B. flabellifer* L. with 1500 milliliters of 70% ethanol for three days, filtering, and then evaporating in a rotary vacuum evaporator to yield a thick extract. The sample was thinned out through a series of dilutions to yield five different concentrations. (93.75, 187,5, 375, 750, 1500 μg/mL).

2.2 *Phytochemical test*

Qualitative phytochemical analyses were conducted at Yarsi University's Integrated Research Laboratory to determine the active constituents present in *B. flabellifer*. L ethanolic extract of the seed coat.

2.3 *Cell culture*

The human oral squamous cell carcinoma (OSCC) cell line (HSC-3) was obtained from the Integrated laboratory at Yarsi University in Jakarta, Indonesia. Cells were cultured in a complete DMEM medium containing 10% FBS, 1% Penicillin-Streptomycin, and 1% Amphotericin B.

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2.4 *Wound healing assay*

The effects of *B. flabellifer* L. seed coat ethanol extract on HSC-3 migration were examined using a wound healing assay. The stable cells were seeded onto 24-well cell culture plates containing DMEM supplemented with 10% FBS and 1% penicillin-streptomycin, and grown to near 100% confluence. After scratching with a 10 μL pipette tip, the cells were rinsed with phosphate-buffered saline (PBS) and incubated with different concentrations (93.75, 187.5, 375, 750, 1500 μg/mL) of *B. flabellifer* L. seed coat ethanol extract. Images of cells migrating at the corresponding wound sites were taken at 0, 2, 4, 6 and 22 h using a Nikon inverted light microscope with a 40x objective lens. The T-Scratch software was used to identify the wound area. Cell migration inhibition was determined by assessing the percentage of wound closure. The equation for determining wound closure is :

$$\text{Percentage wound closure } = \frac{[(At = 0h - At = xh)]}{At = 0h} x100$$

At=0h is the area of the wound measured immediately after scratching.

At=xh is the area of the wound measured at four time intervals (2, 4, 6, and 22 hours after scratching) (Babu and Jeenachandran 2019).

2.5 *Statistical analysis*

The normality was evaluated using the Shapiro-Wilk test. One-way analysis of variance (ANOVA) with post hoc test was used to analyze the variations between experimental groups. A p-value of less than 0.05 was deemed to be statistically significant.

3 RESULT

3.1 *Phytochemical test*

Qualitative phytochemical assay shows *B. flabellifer* L. seed coat ethanol extract contains phenolic, flavonoid, terpenoid, tannin, and alkaloid (Table 1).

Table 1. Phytochemical test results.

Secondary Metabolites	Methods	Results
Phenolic	$FeCl_3$ 5% Reagent	+
Flavonoid	a. Concentrated HCl + Mg reagent	+
	b. H_2SO_4 2N reagent	+
	c. NaOH 10% reagent	+
Steroid	Lieberman-Burchard reagent	-
Terpenoid	Lieberman-Burchard reagent	+
Saponin	HCl + H_2O reagent	-
Tannin	$FeCl_3$ 1% Reagent	+
Alkaloid	a. Hager reagent	+
	b. Wagner reagent	+
	c. Dragendorff reagent	+

3.2 *Wound healing assay*

In order to examine the impact of *B. flabellifer* L. seed coat ethanol extract on HSC-3 cell lines' ability to migrate, we initially took pictures and recorded the scratches at various intervals following the intervention of HSC-3 cells with various extract groups (Table 2 and Figure 1). The cells moved toward the scratch area over time, as illustrated in Figure 2. In comparison to the control group, the ethanol extract from *B. flabellifer* L. seed coat can decrease the migratory ability of HSC-3 cells. The wound healing assay results indicate that a greater percentage indicates the wound is in the process of closing. The control group without treatment demonstrated a 48.29% reduction in wound size after six hours, and complete closure of the wound after 22 hours. After 22 hours, the least amount of wound closure was observed when using a concentration of 750 μg/mL of *B. flabellifer* L. It means *B. flabellifer* L. seed coat ethanol extract has the ability to inhibit the migration of HSC-3 cells.

Table 2. Wound closure results.

	Wound Closure (%)			
Concentration of *B.flabellifer* L (μg/mL)	0-2h	0-4h	0-6h	0-22h
93.75 μg/mL	12.68	21.11	35.32	99.57
187.5 μg/mL	15.91	16.71	20.26	28.38
375 μg/mL	5.01	8.02	10.63	15.67
750 μg/mL	0.50	1.25	3.70	7.18
1500 μg/mL	9.23	11.48	14.41	19.26
Doxo	18.14	21.50	32.34	38.74
Control	9.13	17.31	48.29	100

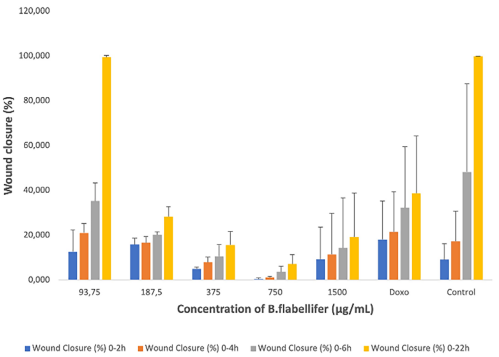

Figure 1. Wound closure diagram.

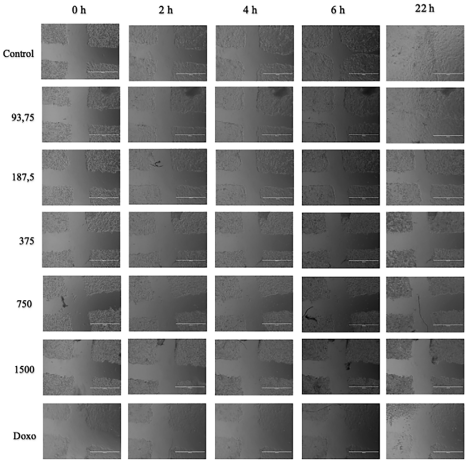

Figure 2. Images of wound healing assay results were taken within 0,2,4,6, and 22 hours.

4 DISCUSSION

Oral squamous cell carcinoma (OSCC) is a prevalent type of oral cancer with a grim outlook, frequent recurrence, and metastasis. The health benefits of natural products have sparked significant interest in disease prevention at recent times. *B. flabellifer* L. shows impressive anti-cancer characteristics (Banu *et al.* 2022). The current study examined the effectiveness of *B. flabellifer* L. seed coat ethanol extract in inhibiting HSC-3 cell migration.

The process of cancer cell metastasis or migration involves several factors, such as cancer cell growth, angiogenesis, invasion, and interaction with micro environmental conditions. The spatiotemporal synchronization of actin cytoskeleton remodeling and cell-ECM adhesion is essential for cell migration (Casalou *et al.* 2019, Zhou *et al.* 2024). MMPs are linked to poor prognosis and tumor progression, including invasion, angiogenesis, and metastasis. They are also over expressed in several human cancer types. Since their amplification in tumor cells is linked to metastasis and advanced tumor stage and because it plays a crucial role in tumor cell migration, invasion, and angiogenesis by releasing ECM-associated growth factors, MMP-2 and MMP-9 are the most studied MMPs in cancer (Dalirsani *et al.* 2019).

Borassus flabellifer L. seed coat ethanol extract with a concentration of 750 µg/mL had the greatest cell migration inhibitory effect compared to other groups (Figure 1). The extract

inhibited HSC-3 cell migration, with the 750 μg/mL concentration showing the lowest wound closure after 22 hours of observation (Table 2 and Figure 2). This is in line with a prior study which proved that 50% inhibition of *B. flabellifer* L. against HeLa cancer cells was found at this concentration (Duddukuri *et al.* 2011). The results of the phytochemical analysis indicated that *B. flabellifer* L. seed coat ethanol extract contains several bioactive compounds, such as : phenolic, flavonoid, terpenoid, tannin, and alkaloid (Table 1). This result relates to previous studies that revealed pomegranate extract, which contains terpenoids, could inhibit HSC-3 cell migration and MMP-2/-9 activation through ERK activation (Peng *et al.* 2020).

5 CONCLUSION

Medicinal plants have provided crucial treatments for cancer through the discovery of natural products. This research showed that terpenoids found in the seed coat ethanol extract of *B. flabellifer* L. have the ability to prevent the movement or migration of HSC-3 cells. The most effective concentration to prevent HSC-3 cell migration was 750μg/mL.

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