ORIGINAL ARTICLE

Extraction of Local *Artocarpus Heterophyllus* (Jackfruit) Seeds and Its Effects on The Migration of Oral Squamous Cell Carcinoma (OSCC)

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is an aggressive malignancy with poor prognosis and limited therapeutic success. *Artocarpus heterophyllus* (jackfruit) is a tropical fruit rich in phytochemicals such as flavonoids, saponins, tannins, and lectins like jacalin, which have demonstrated antioxidant and anticancer properties in previous studies. Extracts from jackfruit seeds have been reported to inhibit cancer cell proliferation and metastasis through the suppression of cell migration and induction of apoptosis. This study investigates the anti-migratory effects of aqueous *Artocarpus heterophyllus* seed extract and purified jacalin on ORL-48 OSCC cell culture. **Methods**: Jackfruit seeds were first prepared and subjected to aqueous extraction to obtain the crude extract. ORL-48 cells were cultured in DMEM F-12 supplemented with FBS and antibiotics under standard incubator conditions. The cells were then either treated with the jackfruit seed crude extract, purified jacalin, cisplatin (a chemotherapy drug), or left untreated as a control. Cell migration was assessed using a scratch assay, in which a uniform wound was created on the cell monolayer. Wound closure was monitored over time to indicate cell migration. The extent of cell migration was then quantified using ImageJ software. **Results**: The ORL-48 cells that were treated with crude extract demonstrated the lowest wound closure (6.37%), followed by jacalin (13.45%), cisplatin (20.80%), and control (25.98%). Although not statistically significant ($p>0.05$), the ORL-48 cells treated with crude extract showed minimal cell migration, indicated by the least wound closure. These findings suggest the crude extract had the strongest OSCC cell inhibitory potential amongst the three treatments. **Conclusion**: The aqueous extract of jackfruit seeds exhibited potential anti-migratory properties against OSCC cells, warranting further investigation in preclinical studies as a potential anti-migratory agent for oral cancer.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is one of the most aggressive malignancies affecting the head and neck region, accounting for a significant proportion of cancer-related morbidity and mortality worldwide (Zhou et al., 2025). Despite advances in surgical, radiotherapeutic, and chemotherapeutic interventions, the prognosis of OSCC remains poor due to its high recurrence rate, rapid metastasis, and resistance to conventional treatments. These limitations highlight the urgent need for alternative therapeutic strategies that are more effective and less toxic (Xue et al., 2025).

Natural products, particularly plant-derived bioactive compounds, have attracted considerable attention as potential anticancer agents due to their diverse pharmacological properties and relatively low side effects. *Artocarpus heterophyllus* (jackfruit), a tropical fruit widely cultivated in Malaysia, contains seeds rich in bioactive compounds, including flavonoids, saponins, and lectins such as jacalin (Fabil et al., 2024). Previous studies have demonstrated that jacalin exhibits anticancer activities in breast and colon cancer cell lines, primarily through modulation of cell proliferation, apoptosis, and migration (Geraldino et al., 2017).

However, research on the anti-migratory effects of jackfruit seed extracts and jacalin specifically on OSCC cells remains scarce. Understanding these effects is crucial, as cancer cell migration is a key step in metastasis, which significantly contributes to poor clinical outcomes. Therefore, this study aims to investigate the potential anti-migratory properties of aqueous jackfruit seed extract and purified jacalin using the ORL-48 OSCC cell line. The findings of this study may provide insights into the

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therapeutic potential of jackfruit-derived compounds and support the development of novel complementary approaches for OSCC management.

MATERIALS AND METHODS

Materials

Fresh jackfruit (variety J33, Tekam Yellow) was sourced locally. The seeds were separated, washed, air-dried, and dehydrated before being ground into fine powder. Aqueous extraction was performed by soaking 10 g of powdered seeds in 100 mL distilled water for 24 hours, followed by filtration and evaporation using a rotary evaporator. The crude extract was concentrated, filtered, and stored at 4°C for use.

The OSCC cell line (ORL-48) was obtained from the Cancer Research Malaysia (CRM) and cultured in DMEM-F12 supplemented with 10% fetal bovine serum (FBS) and antibiotics.

Methods

Aqueous Extraction of Jackfruit Seeds

Jackfruit (Artocarpus heterophyllus) seeds were obtained from a local supplier in Kuantan, Pahang (Figure 1). The seeds were peeled, cleaned, and sliced into approximately 2 mm chips with the spermoderm intact. They were air-dried for three days, further dried using a food dehydrator, and then ground with a dry blender for 10 minutes to obtain a fine powder (<0.5 mm). The powder was stored in plastic pouches at room temperature until used (Mohd Ali. et al., 2015).

For extraction, 10 g of the powdered seeds were dissolved in 100 mL of distilled water (1:10 w/v) and soaked at room temperature for 24 hours. The mixture was filtered using Whatman filter paper, and the filtrate was concentrated with a rotary evaporator. The crude extract obtained was stored in an airtight container at 4 °C, then passed through a 0.45 µm syringe filter to yield a clear supernatant, which was kept at 4 °C until further analysis (Sharifah et al., 2019).

Cell Culture

ORL-48 oral squamous cell carcinoma (OSCC) cells, derived from gingival epithelium, exhibited polygonal epithelial morphology and formed cohesive monolayers. The cells were cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F12 (DMEM F-12; Gibco, USA) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin–streptomycin). Cultures were maintained at 37 °C in a humidified incubator with 5% CO_2 under aseptic conditions.

Frozen stocks were rapidly thawed at 37 °C, washed to remove cryoprotectant, and seeded into T25 flasks with fresh complete medium. Subculturing was performed by rinsing with phosphate-buffered saline (PBS), detaching cells with trypsin, and reseeding into new flasks. For long-term storage, cells were suspended in freezing medium (complete medium with 10% DMSO) at 1.0–1.5 × 10^6 cells/mL, aliquoted into cryovials, frozen gradually at –80 °C and transferred to liquid nitrogen for preservation.

Scratch Assay

The scratch assay was performed to assess the anti-migratory effects of crude *Artocarpus heterophyllus* seed extract and purified jacalin on ORL-48 oral squamous cell carcinoma (OSCC) cells. On Day 1, cells harvested at 80–90% confluency were seeded at 3.0 × 10^5 viable cells/well in 12-well plates with sterile glass coverslips and incubated overnight in DMEM-F12 containing 1% fetal bovine serum (FBS) to form uniform monolayers.

On Day 2, a straight wound was created using a sterile 200 µL pipette tip. Detached cells were removed by PBS washing, and the medium was replaced with serum-reduced DMEM-F12 (1% FBS). Cells were then treated with crude extract (100 mg/mL), jacalin (100 µg/mL), cisplatin (8 µg/mL), or medium alone. Treatments were administered at 1 mL per well and incubated for 24 hours at 37 °C with 5% CO_2.

On Day 3, images of the wound area were captured at 0 and 24 hours using an EVOS inverted microscope. Migration was quantified as the percentage of wound closure using ImageJ software. Each treatment was performed in triplicate, and the assay was conducted in duplicate for reproducibility.

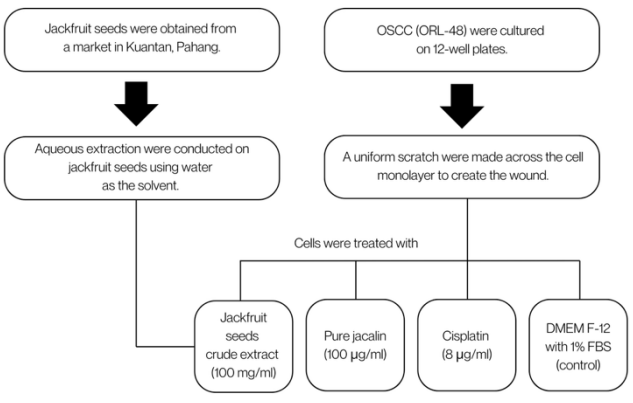

Figure 1: Research flow chart

RESULTS

The final yield of Jackfruit seed extraction was 27.5 g, corresponding to 25% of the initial seed weight, which is relatively high for aqueous extractions. The extract was concentrated to 916.7 mg/mL, providing a suitable stock solution for *in vitro* assays. Scratch assay analysis revealed that the untreated control showed the highest wound closure (25.98%), indicating normal migration. Cisplatin treatment reduced wound closure to 20.80%. Purified jacalin further decreased migration to 13.45%, while the crude extract produced the lowest closure at 6.37%, showing the strongest inhibitory trend (Table 1). Although differences were not statistically significant ($p>0.05$), the data demonstrated clear trends of reduced migration under treatment conditions.

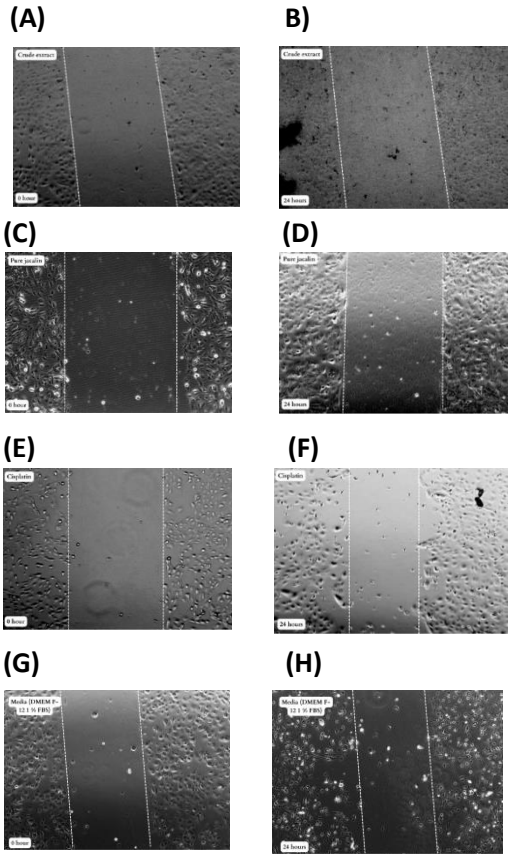

Figure 2: Wound healing assay assessing ORL-48 OSCC cell migration in response to different treatments over 24 hours. Phase-contrast microscopy images show cells treated with:

(A-B) *Artocarpus heterophyllus* aqueous extract
(C-D) Purified Jacalin
(E-F) Cisplatin
(G-H) DMEM F-12 supplemented with 1% FBS (*untreated control*)

Table 1: Summary of wound closure percentages in ORL-48 OSCC cells following different treatments. For the untreated control, cells received DMEM F-1 supplemented with 1% FBS without any exposure to cisplatin, jacalin or jackfruit crude extract.

Treatment	N	Mean (%)	Standard deviation	*Min.	Max.
Media (DMEM F-12 1% FBS)	5	25.98	22.26	2.42	57.41
Cisplatin (8 µg/ml)	6	20.80	15.43	5.36	49.60
Pure Jacalin (100 µg/ml)	6	13.45	4.41	7.14	19.19
Crude extract of jackfruit seeds (100 mg/ml)	4	6.37	2.91	2.18	8.93

**Min. = Minimum , Max.= maximum*

DISCUSSION

In this study of the ORL-48 oral squamous cell carcinoma (OSCC) line, the aqueous seed extract of *Artocarpus heterophyllus* (jackfruit) demonstrated an inhibitory trend on cell migration, while the purified lectin, Jacalin showed a weaker effect under the same conditions. The observation that the crude extract produced a stronger anti-migratory effect than the isolated jacalin suggests that multiple phytochemicals in the seed extract may act synergistically to inhibit migration more effectively than a single purified compound (Cotoraci et al., 2021).

This finding is supported by prior reviews identifying jackfruit seed bioactive compounds (lectins, flavonoids, saponins, tannins) as collectively contributing to anticancer properties (Ranasinghe et al., 2019). The superior effect of the crude extract may be explained by several possibilities. First, besides jacalin, the seed extract likely contains flavonoids, tannins and saponins that could each target different aspects of cell migration. For example, flavonoids have been shown to interfere with signalling pathways (PI3K/Akt, MAPK, NF-κB) that regulate migration and invasion (Mazuráková et al., 2024). Secondly, saponins and tannins may inhibit matrix metalloproteinases (MMPs) or reduce cell adhesion or interaction with extracellular matrix which is key steps in cell migration (Kang et al., 2008). Although our study did not assess these mechanisms directly, the phytochemical

composition of jackfruit seeds supports their plausible involvement.

Nevertheless, it is crucial to emphasise that the results did not reach statistical significance, which limits the strength of the conclusion. The possible reasons for this include the use of only a single concentration of extract or lectin, which prevents assessment of dose–response and might have missed an optimal inhibitory concentration (Cotoraci et al., 2021). Besides that, a relatively small number of replicates or lower effect size, reducing statistical power.

Other than that, the exclusive use of one cell line (ORL-48) and one migration assay format (scratch assay), which may not capture the full biological variability of OSCC. Moreover, short incubation time which is 24 hours could be one of the limitations in this study. The migration assay was conducted over a 24-hour period, which may have been too short to fully capture the anti-migratory effects of the tested compounds, especially for plant-based treatments that often act more gradually than synthetic drugs. Some bioactive compounds may require longer exposure times to interfere with migration-related signaling pathways, such as epithelial–mesenchymal transition (EMT), cytoskeletal rearrangement, or matrix metalloproteinase (MMP) expression. Without these, the observed effect remains phenotypic rather than mechanistically explained.

Based on these limitations, future work should prioritise the following, conducting dose-response experiments across multiple concentrations to determine the effective inhibitory range and calculate IC_{50}/ED_{50} values for migration suppression. Next, including time-course analysis to see if the inhibition is stable or transient over time. Incorporating mechanistic studies, for instance, assessing expression of MMP-2, MMP-9, integrins, E-cadherin/vimentin, focal adhesion kinase, or performing signalling pathway analyses (e.g., Akt, ERK phosphorylation) to uncover the underlying molecular mechanisms of migration inhibition. Moreover, testing additional OSCC cell lines (and perhaps normal oral epithelial controls) to examine whether the effect is generalisable across OSCC heterogeneity.

CONCLUSION

The aqueous extract of jackfruit seeds demonstrated the strongest anti-migratory effect on OSCC cells compared to jacalin and cisplatin. These results highlight the potential of jackfruit seeds as a source of bioactive compounds with anticancer properties. Further studies are recommended to isolate active compounds, optimize dosages, and evaluate *in viv*o efficacy for oral cancer therapy.

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