Effect of Lectin from Artocarpus Heterophyllus Seed on Cancer Cell Lines

Zuraidah M. A. and Mimi Sakinah A. M.
University of Malaysia Pahang/FFKSA, Gambang, Malaysia
Email: mimi@ump.edu.my; idamohdali@yahoo.com
Wan Azizi W. S.
International Islamic University Malaysia, Kuantan, Malaysia
Email: drwanazizi@iium.edu.my

Abstract—The objective of this paper to present a potential of jacalin (protein extract from A. heterophyllus seed) against human breast cancer (MCF7) and non-small lung carcinoma (H1299). The extraction and purification were carried out by phosphate buffer precipitation and reverse miceller, respectively. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and trypan blue exclusion methods were performed to study the cytotoxic activity. Jacalin and crude protein was compared in cytotoxic activity against MCF7 and H1299 cancer cells and the result showed jacalin more effective than crude protein, 64.73 and 75.54 percent for MCF7 and 67.71 and 68.39 percent for H1299, respectively. Jacalin also found very active against MCF7 compared to H1299 cancer cells and expected IC_{50} for both cancer cells would be reached if concentration of jacalin will extended in cytotoxic activity analysis.

Index Terms—artocarpus heterophyllus, jacalin, crude protein, cytotoxicity, cancer cell, MCF7, H1299

I. INTRODUCTION

Jacalin is a plant lectin from Artocarpus heterophyllus seed, is a tetrameric two-chain lectin with molecule structure weight is 66kDa. It is combining a heavy α chain and light β chain with of 133 and 20 amino acid residues respectively [1]-[3]. Jacalin is galactose binding lectin type with symbol AIL and ligand motif (Sia)Galβ1-3GalNAcα1-Ser/Thr [4]. The recovery of more than 50% of the jacalin from crude protein seed extracts which it is single major protein and the resultant product is very expensive because of the extensive purification procedures [5]. However, the abundance of sources material (jackfruit seed) for the production of jacalin has made it an attractive cost-effective lectin, especially in Malaysia. Recent advancement in medicine studies, have been carried out on jacalin revealing its importance as a lectin of diverse applications ranging from the isolation of human IgA and AIDS research [2]. Jacalin also useful tool for studying serum, which is it can be used for the purification of glycoprotein containing O-linked oligosaccharides and as histochemical detection of the Thomsen-Friedenreich antigen tumours.

In the production of jacalin, crude protein is recovered from A. heterophyllus seed by phosphate buffer saline (PBS) precipitation and followed by purification of jacalin. The crude protein contains total protein, thus the purification process is necessary in order to purify the crude seed and recover the purified lectin or jacalin. There are many pharmacological research studies focusing intensively on jacalin now days, however this paper to investigate the antiproliferation effect on MCF7 and H1299 in comparative of crude protein and jacalin extracted from A. heterophyllus seed.

II. MATERIALS AND METHODS

A. Cell Culture

MCF7 and H1299 cancer cells were kindly provided by Dr. Masa-aki Ikeda; Department of Molecular and Craniofacial Embryology, Tokyo Medical and Dental University; were maintained in DMEM medium (with high glucose and glutamine) supplemented with 10% heat inactivated fetal bovine serum (FBS) and 1% penicillin streptomycin, at 37° C in a humidified atmosphere containing 5% CO_{2}. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) medium containing 10% FBS, 200 U/mL penicillin G, and 200 lg/mL streptomycin and incubated at 37°C with 5 % CO_{2} [6].

B. Extraction and Purification of A. heterophyllus seed

Artocarpus heterophyllus or jackfruit species of Mada Mastura was selected to use their seeds for this study. The A. heterophyllus were bought direct from the jackfruit farm in Temerloh. A. heterophyllus seeds were collected, cleaned and sliced with around 2mm thickness and sun dried for 7 days without remove the thin brown spermoderm covers the fleshy white cotyledons. Dried chip seeds were put in a mixer-grinder cum blender which having 550 watts, 17000 rpm rotating speed electrical motor. The seeds were grinded and crushed well and uniformly for 10 minutes with highest precaution to avoid any contamination and made them as
particle-sized powder (<0.5mm). The powdered materials were packed in plastic pouches and stored in normal room temperature until use. The details procedures for jackfruit seed powder extraction have been previously reported in the literature [7].

Briefly, a seed powder was dissolved in phosphate buffer saline (PBS, 0.1M, pH7.4). The mixture was soaked, shook, centrifuged and clear supernatant was passed through the filter paper and collected as crude protein sample. The supernatant or crude protein of A. heterophyllus seed (5ml, protein 8mg/ml) were applied onto reverse micelle method to purification. The reverse micelle system was constituted by the anionic surfactant, sodium di(2-ethylhexyl) sulfosuccinate (AOT) in isooctane [8]. Forward extraction was carried out by mixing with ratio 1:1 of organic phase (AOT in isooctane) with aqueous phase salt (NaCl) and 5 min stirred, wherein lectin extracted into reverse micelles. Extraction back-extraction assays were performed by phase contact (1:1), stirred (5min) and separated by centrifugation (3000Xg, 10min) [8][9] “unpublished” [10].

C. Determination of Protein Concentration

The determination of protein concentration during the extraction and purification process was made according to the Lowry method as modified for both aqueous and organic phases by UV spectrophotometer Varian-Cary 50 [5]. Bovine serum albumin (BSA) with concentration 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0mg/ml was used to develop standard calibration curve to calculate concentration of the protein. Concentration of standard and sample should be diluted with distilled water to ensure that they are effective in inhibit proliferation of MCF7 cancer cell. The concentration range. Fig. 1 showed dropping of viability cancer cell for both crude protein and jacalin, matched with jacalin standard. The pattern for the inhibition of cell viability was very similar for cell lines, crude protein and jacalin. However, jacalin gave a better result compared to crude protein, which are 64.73 and 75.54 percent viability cell respectively at maximum concentration 10µL and 72 hours exposures. A 10.81 percent of different between crude protein and jacalin potential on inhibit of viability cancer cells was proved that purification of crude protein is necessary to more effective in inhibit proliferation of MCF7 cancer cell.

D. Cell Viability and Antiproliferation Assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to evaluate the antiproliferative activities of the protein against the cancer cell lines. The assay depends on the cleavage of the tetrazolium salt into formazan blue by the mitochondrial enzyme succinate dehydrogenase. The conversion takes place only in living cells and the amount of formazan produced is proportional to the number of viable cells present. Thus, the MTT assay is potentially useful for assaying both cell viability and antiproliferative activities of materials. For this purpose the cancer cells were seeded in complete medium in a 96-well plate at a density of 1x10^4 cells/ml. After reaching confluent, the cells were incubated with different concentrations of the sample (900-3µg/ml) for 72 hours. The medium was then discarded and the adherent cells were washed twice with PBS, then 20 µl of MTT stock solution (5 mg/ml in PBS) were added to each well and the plates were further incubated for 4 hours at 37 °C. 100 µl of DMSO were added to each well to solubilize the formazan crystals produced by viable cells. After complete dissolving of formazan blue, the absorbance was measured at 570 and 630 nm, as reference wavelength, using TECAN infinite M200 microplate reader. The percentage of cell viability was calculated according to the equation described as below [11]:

\[
\% \text{ of cell viability} = (\text{OD of treated cells} / \text{OD of control cells}) \times 100
\]

The concentrations required for inhibition of 50% of cell viability (IC50) were calculated.

III. RESULTS AND DISCUSSIONS

The MTT assay is a common practice to study the action of natural products on cell viability, proliferation and cytotoxicity. This assay is based on reduction of tetrizolium salt to a purple insoluble formazan by metabolically active cells [12]. The absorbance of the solubilized formazan is taken as a measure of the number of living cells.

A. Effect of Lectin from A.heterophyllus Seed to MCF7 Cancer Cell Lines

As shown in Fig. 1, the effect of the crude protein and jacalin from A.heterophyllus seed on viability MCF7 cancer cell control by standard of jacalin in dose dependent manner (from 0-10µL), with little change in effect the concentration range. Fig. 1 showed dropping of viability cancer cell for both crude protein and jacalin, matched with jacalin standard. The pattern for the inhibition of cell viability was very similar for cell lines, crude protein and jacalin. However, jacalin gave a better result compared to crude protein, which are 64.73 and 75.54 percent viability cell respectively at maximum concentration 10µL and 72 hours exposures. A 10.81 percent of different between crude protein and jacalin potential on inhibit of viability cancer cells was proved that purification of crude protein is necessary to more effective in inhibit proliferation of MCF7 cancer cell.

![Figure 1](image-url)
the biological effect to MCF 7 cancer cell is similar to jacalin standard. Similar to previous study mentioned jacalin have been used to study tissue binding properties in benign and malignant lesions of the breast and the thyroid cancer [2]. Equation from graph in Fig. 1, \( Y = -4.3004x + 106.56 \), was used to calculate expected IC\(_{50}\) of viability cell for jacalin will be achieved at concentration 13.15µL.

### B. Effect of Lectin from A.heterophyllus seed to H1299 Cancer Cell Lines

Fig. 2 presented the comparison of affecting cell proliferation by crude protein and jacalin from A.heterophyllus seed against viability of H1299 cancer cell and standard of jacalin as control. The proliferation cells were studied after 72 hours exposure in concentration range 0-10µL (0, 0.3125, 0.625, 1.25, 2.5, 5 and 10µL). The graph showed reducing trend line of cell viability; it gave same trend for crude protein, jacalin and also standard of jacalin. However, at the maximum concentration (10µL), standard of jacalin most affecting against H1299, it gave the lowest of percent of cell viability, 56.40 followed by jacalin and crude protein, 67.72 and 68.39 respectively. From the graph in Fig. 2, it can be seen that the rate proliferation of cell at concentration 5 to 10µL showed trend slowing down with rate -0.3497 % for crude protein and compared to jacalin was -2.7727 %. Additionally, jacalin have a sharp decreasing trend of the proliferation from concentration 0.125 to 10µL, if extended the concentration it expected would be reach IC\(_{50}\) at concentration 16.39µL with the same rate decreasing. Besides, extended concentration for crude protein it not worth and it would reach a plateau condition with the very low rate decreasing previously.

![Graph](image)

**Figure 2.** The effect of the crude protein and jacalin of A.heterophyllus seed on viability non-small lung carcinoma cell (H1299) controlled by jacalin standard. Cells (1x10^4/well) were incubated for 24 hrs with different concentrations (0-10µL/ml) of A.heterophyllus seed ethanolic extract (0-100µg/ml of cell growth media). Cell viability was evaluated as the ability of cells to reduce MTT to blue formazan crystals.

### C. Jacalin Effect to Viability of MCF7 and H1299 Cancer Cell Lines

The jacalin was found to significantly decrease the cell viability of MCF7 and H1299 with 24 hours exposure.

![Graph](image)

**Figure 3.** Effect of jacalin from A.heterophyllus seed on the viability of MCF7 and H1299 cancer cells at different concentration (0-10µL). Result presented as mean ± standard error of mean from triplicate data.

### IV. CONCLUSIONS

The results of the present study have demonstrated that a crude protein and jacalin of A.heterophyllus seed prevented growth of proliferating cells for both type cancer cell MCF7 and H1299. Nevertheless, the both graph (Fig. 1 and Fig. 2) indicated further extended concentration of jacalin from A.heterophyllus seed would reach IC\(_{50}\) for against viability cell of MCF7 and H1299. Therefore, crude protein gave slowing down trend decreasing of viability cell for both, means it not worth to prolong the concentration and recommended to purify the crude protein prior the treatment. Through detailed *in vitro* study studies, the results propose that protein derivatives from A.heterophyllus seed or called jacalin have potential to inhibit the growth of cancer cells.

These finding was parallel suggestion by [2], jacalin have potential as a therapeutic agent for cancer. Jacalin has been used as a histochemical reagent to study tissue binding properties in benign and malignant lesions of the breast and the thyroid cancer cells. Results of the present study, however, for the first time, recognized that jacalin causes a growth inhibition of MCF7 and H1299 cancer cell lines, which is accompanied by significant apoptotic death. Upon determination of the effect of jacalin to MCF7 and H1299, study was further identified jacalin (purified protein) was more effective compared to crude protein in treatment against viability cell. The reason of
this, crude protein are mixtures with complicated and unknown in their details compared to purified protein with selective and filtered compound as suggest by [13]. Lectins are involved in cell recognition and aggregation [14] but all biological functions of lectins in these organisms have not yet been determined. The mechanism of the antiproliferative activity of jacalin is still unknown, and it could reveal some new and interesting facts about the role of lectins as treatment agent in prohibit viability cancer cells. Whereas, report by [15] found that native jacalin from A. Intergrifolia seed was to be a cytotoxic inhibitor of proliferation of other cancer cell, which is epidermoid carcinoma (A431), and it was proved that jacalin can be one of the agent in cancer cell treatment nowadays and future.

Acknowledgment

This research has been carried out with the practically hands-on by Putri Nur Hidayah Al-Zikri, Faculty of Pharmacy, IIUM (Bandar Indera Mahkota, Kuantan), materials and analysis equipments support by Assoc. Prof. Dr. Muhammad Taher, Faculty of Pharmacy, IIUM (Bandar Indera Mahkota, Kuantan), and financially support by Universiti Malaysia Pahang (UMP) and MyPhD scholarship by Ministry of High Education.

References


Zuraidah Mohd Ali was born in Terengganu, Malaysia, on 11 September 1981. Zuraidah Mohd Ali received first degree from the University Technology Malaysia (UTM), Malaysia in 2003, and Bachelor Degree in Chemical (Gas) Engineering. Started doing Master in Chemical Engineering from University Malaysia Pahang (UMP), Malaysia and graduated in 2010. Currently Zuraidah Mohd Ali is doing PhD study also in UMP, Malaysia since 2011 major in Bioprocess Engineering.

In 2004, after graduate her degree, she working as engineer at oil and gas company in Pahang, Malaysia. On year 2006, she joined the Faculty of Chemical Engineering and Natural Resources, UMP as fulltime master student and working with company under UMP as an engineer immediate after finished her Master. She decided to continue study for PhD in end of year 2011 and until now. She also does a part time job as Research Assistant and Lecturer in UMP. She was presented in ICCBPE, SOMCHE on November 2014 about time effect in extraction of lectin from jackfruit seed. During her master study she was presented in The 3rd Regional Conference on Natural Resources in The Tropics (NRTrop3) at UNIMAS, Malaysia in 2009 and member of Board of Engineer Malaysia (BEM) since 2012.

Mimi Sakinah Abdul Mumain was born in Malaysia on 11 July 1977. Mimi Sakinah Abdul Mumain finished Diploma and Degree in Chemical Engineering from University Technology Malaysia (UTM), Malaysia. Completed Master study in Environmental from University Putra Malaysia (UPM), Malaysia and graduated PhD in Bioprocess Engineering from University Malaysia Pahang (UMP), Malaysia.

In 2003, she joined the Faculty of Chemical Engineering and Natural Resources, University Malaysia Pahang, formerly known as Collage of Engineering and Technology Malaysia as Vocational Training Officer and in September 2005 became a Lecturer. She was appointed as Deputy Dean of R&D and Postgraduate Study in 2005 and as Deputy Dean of Academic & Student Affair 2 years later. She was promoted as a Senior Lecturer in 2008 and currently she was awarded as Associate Professor since 2010. Her current research interests include bioprocess separation technology, enzymatic membrane reactor, enzymatic reaction and specialty chemicals. Asst. Prof. Dr. Mimi Sakinah has received 7 international awards and 48 national awards, has published 27 journal with 50.79 total impact factor, wrote 2 books, attended 64 proceeding and seminar and 10 patent filled. Other than that, Asst. Prof. Dr. Mimi Sakinah joined member of Board of Engineering Malaysia (BEM), Institution of Engineers Malaysia (IEM) and European Desalination Society (EDS).
Wan Mohd Azizi Bin Wan Sulaiman was born in Kelantan, Malaysia on 1970. Wan Mohd Azizi Wan Sulaiman are Medical Doctorate, Master in Herbal Medicine and PhD in Pharmacology. Currently, he is academician and researcher at Kulliyah Pharmacy, International Islamic University Malaysia. His research works mainly in natural product development for diabetes mellitus, wound care and cancer.

He also successfully introduced complementary therapy Maggot Therapy for Diabetic Foot ulcer patients in Malaysia. He has secured various grants from public and private funds including MOSTI, MOHE, Biotechcorp, MARDI and various private companies. His current consultancy works include the Head of Research for Herbal Development Office (HDO) BAUNKEM project under the Ministry Agriculture for NKEA High Impact Economy project and Head of Commercialization unit for Integrated Care for research Animal and Use (ICRACU) under IIUM Kuantan promoting the use animal testing under the OECD and GLP accreditation.