

Cytotoxicity Effect and Morphological Study of Different Duku(*Lansium domesticum* corr.) Extract towards Human Colorectal Adenocarcinoma Cells Line (HT-29)

Rohin Mohd Adzim Khalili, Jumli Mimie Noratiqah, Ridzwan Norhaslinda, Abd Hadi Norhayati, Baig Atif Amin, Arshad Roslan, A. Latif Ahmad Zubaidi

ABSTRACT

Context: *Lansium domesticum* corr. is a member of the family Meliaceae, and known locally as duku and has been used traditionally in the prevention and treatment of various illness. **Aim:** To study the cytotoxic effect and morphological changes of human colorectal adenocarcinoma cells (HT-29) treated with different duku (*Lansium domesticum* corr.) extracts. **Methods:** The *L. domesticum* corr. fruit extracts were processed involving three different solvents; methanol, ethanol and ethyl acetate. HT-29 cell lines were treated with different concentrations of *L. domesticum* corr. (0-100 µg/ml) extracts for a total of 24, 48 and 72 hours. Cytotoxicity of cells line was determined by using MTT assay as per IC₅₀ values. **Results:** Methanol extract of *L. domesticum* corr. showed IC₅₀ value at 6.79 ± 0.00 µg/ml and 50.0 ± 0.00 µg/ml respective, while ethyl acetate extract of *L. domesticum* corr. reached IC₅₀ value at 86.00 ± 0.08 µg/ml, and 96.0 ± 0.12 µg/ml. There was no IC₅₀ value of ethanol extract from *L. domesticum* corr. Only methanol extract showed toxicity towards HT-29 cells line. **Conclusion:** To the best of our knowledge, this is the first repeat the exploring the effect of duku (*L. domesticum* corr.) extract on HT-29 cells line. **Key words:** Anti-proliferative effect, colorectal cancer, MTT assay, polarity extracts, IC₅₀ value.

Rohin Mohd Adzim Khalili,
Jumli Mimie Noratiqah,
Ridzwan Norhaslinda, Abd
Hadi Norhayati, Baig Atif
Amin, Arshad Roslan, A.
Latif Ahmad Zubaidi

Faculty of Health Sciences, Universiti
Sultan Zainal Abidin, Gong Badak
Campus, Hafsa Block, 21300 Kuala
Nerus, Terengganu Darul Iman,
MALAYSIA.

Correspondence

Dr. Mohd Adzim Khalili Rohin, Faculty of
Health Sciences, Universiti Sultan Zainal
Abidin, Gong Badak Campus, Hafsa Block,
21300, Kuala Nerus, Terengganu Darul
Iman, MALAYSIA.

Phone numbers: +609 6688522;
Facsimile numbers: +609 6275771
E-mail: mohdadzim@unisza.edu.my

History

- Submission Date: 28-05-2017;
- Review completed: 08-06-2017;
- Accepted Date: 31-08-2017

DOI : 10.5530/pj.2017.6.119

Article Available online

<http://www.phcogj.com/v9/6>

Copyright

© 2017 Phcog.Net. This is an open-
access article distributed under the terms
of the Creative Commons Attribution 4.0

INTRODUCTION

According to the American Cancer Society,¹ colorectal cancer is a term specific for a cancer that starts in the colon or the rectum. These cancers can also be referred to separately as colon cancer or rectal cancer, depending on the individual side. The most common type of colorectal cancer includes adenocarcinoma, with their origin in glandular cells.¹ Colon cancer is a serious health problem in most developed countries and it is one of the contributing causes of cancer mortality globally.² It affects mostly those above the age of 50 years' and, it is the second most cancer cause among Malaysian men across all age groups. Current therapy includes a combination of radiation, surgery and chemotherapy, but rate of relapse is very high.³ Therefore, the scarcity of therapeutic options and the need to improve the patient's quality of life requires the exploration of an alternative or complementary form of therapy.⁴

In the treatment of cancer, antioxidants from various fruit extracts had been known to have beneficial effects against free radicals by reducing the cancer mortality.⁵ Fruits and veggies are the primary source of antioxidants' vitamins, making these foods essential to human health.⁶ Esmaeil beiget *al.*⁷ believes that safe, nontoxic

origin of herbs and plants have valuable sources for novel anti-cancer drugs. According to Bauman,⁸ in fruits, there are important nutrient besides other vitamins, minerals, flavonoids and phytochemicals, which have been reported as a good contributing factor to the health. In addition, epidemiological studies, including experiments performed on animal models and investigations on humans have proven that consumption of a diet rich in vegetables and fruits is associated with a low risk of contracting some diseases, including cardiovascular diseases and cancers.⁹

The genus, *L. domesticum* corr. belongs to the family Meliaceae, and known locally as duku. Duku is a famous fruit in Southeast Asia, beside Malaysia, Thailand, Philippines and Indonesia being its' biggest producers.¹⁰ The tree grows to a peak of 40–50 feet (ft) with long leaves that are pinnate and dark green.¹¹ The raw dukufruits are greenish in colour and have a very sour-gummy taste. As the fruit matures, the skin will turn yellowish, the fruit's flesh will become sweet and can be eaten freshly. The nutritional composition of 100 grams of duku is reported to contain 70-74 calories, includes of 1.0-

Cite this article : Khalili RMA, Noratiqah JM, Norhaslinda R, Norhayati AH, Atif BA, Roslan A, Zubaidi ALA. Cytotoxicity Effect and Morphological Study of Different Duku(*Lansium domesticum* corr.) Extract towards Human Colorectal Adenocarcinoma Cells Line (HT-29). Pharmacog J. 2017;9(6):757-61.



1.5 g protein, 0.2-0.5 g fat and 13-15 g carbohydrates.¹² Traditionally, the bark trees and seed of the duku used to treat dysentery, eradicate cancer cells¹³ as anti-pyretic and anthelmintic. Lately, researchers have focused on potential of dukuas having anti-malarial,¹⁴ anti-cancerous¹⁵ and anti-bacterial properties. This study focused on the screening the cytotoxic effect of *L. domesticum* corr. extracts towards, studying the morphological changes of human colorectal adenocarcinoma cells lines (HT-29).

MATERIALS AND METHODS

Extraction and Isolation of *L. domesticum* corr

For processing extraction, method described previous was used.¹⁵ The duku fruits (*L. domesticum* corr.) were obtained from Kuala Terengganu, Terengganu, Malaysia. The fruits were skin peeled and carefully washed under running tap water, dried with a soft cloth and macerated. A total of 10 g of the macerated fruit sample was soaked into three different polar solvents namely, ethanol, methanol and ethyl acetate with in a ratio of 1:10; w/v for 24-hours.

Then, all the extracts from all solvent were filtered using Whatmann® No. 41 filter paper (pore size 20-25 µm) and were then concentrated under reduced pressure at 40°C. All the extracts were stored at -20°C until a total of used for further analysis. For screening the extracts of the duku fruit (*L. domesticum* corr.), 100 mg of the sample was dissolved in 1mL of DMSO to obtain 100 mg/mL stock solution of extracts. All extracts were kept at a temperature of 4°C throughout the experiment. Stock solutions were further diluted in a RPMI 1640 (Sigma, MO, USA) media to obtain a final concentration of 100 µg/mL.

Cell Culture and Harvesting

The human colorectal adenocarcinoma cells (HT-29) were purchased and maintained at a temperature of 37°C, in a humidified CO₂ incubator with 5% CO₂ in a RPMI-1640 media supplemented with 10% fetal bovine serum and at 95% relative humidity, while changing the media at least twice a week for harvesting.

MTT Assay and Determination of IC₅₀

According to Mosmann,¹⁶ the anti-proliferative activity of the *L. domesticum* corr. extracts were obtained using ethanol, methanol and ethyl acetate solvents via micro-titration colorimetric method of tetrazolium salt reduction. The viability of cells used was determined by the trypan blue method. Exponentially, cells were harvested, enumerated using the haemocytometer and diluted with a particular medium. A total cell volume with a concentration of 2 x cells/cm² was prepared and plated (100 µl/well) into 96-well plate (SPL Life Sciences, Korea). After a 24-hour recovery period, a serial dilution of *L. domesticum* corr. samples was plated out, in triplicates. Each plate was included with untreated cells as controls and a blank cell-free control.

After 0, 24, 48 and 72 hours of incubation, tetrazolium salt 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (20 µg/mL) was added to each well and plate was re-incubated for a further 4 hours. The media then was removed and DMSO (100 µl) was pipetted into each well to solubilize the formazan crystals and incubated for another 15 minutes. Finally, the absorbance was measured at 570 nm using a flourometer micro-plate reader (Tecan, Infinite M2000) and the percentage of cell viability was calculated with the appropriate controls taken into account. The relative viability of the treated cells as compared to the control cells was expressed as the % cell viability; (% cell viability = [A₅₇₀ of treated cells]/ [A₅₇₀ of control cells] x 100%). The inhibition concentration (IC₅₀) will then determine by non-linear regression analysis of the corresponding dose response curve.

Morphology Studies/Pattern of HT-29

HT-29 cells line were grown in 4 plates and treated with extract residue from the fruit extract obtained using ethanol, methanol and ethyl acetate as a solvent. The morphological study was conducted at a temperature of 37°C for duration of 0, 24, 48 and 72 hours; morphological changes occurring in the cells were observed under an inverted microscope (Nikon, Japan).

Data Analysis

The SPSS 20.0 software was employed for statistical analysis. Data were expressed as mean ± SD of three independent values.

RESULTS

The cytotoxicity effect of *L. domesticum* corr. was measured in triplicate for every extract against untreated cells for an interval of 24 hours, 48 hours and 72 hours incubation, respectively. The effect of methanol, ethanol and ethyl acetate extract on HT-29 cells line is shown in Figure 1 and Table 1. The value of IC₅₀ for anti-proliferative effect of methanol extract *L. domesticum* corr. at 48 hours treatment was found to be; 50.0±0.00 µg/ml, while treatment at 72 hours shown that IC₅₀ value is 6.79±0.00 µg/ml. For ethyl acetate extract, anti-proliferative effect showed IC₅₀ value at 24 hours, treatment with 86.00±0.08 µg/ml and at 72 hours treatment with 96.0±0.12 µg/ml. Meanwhile, ethanol extract had no anti-proliferative effect on HT-29 cells line as none of ethanol extract reached IC₅₀.

Cell morphology study was assessed at 24, 48 and 72 hours after treatment with *L. domesticum* corr. extract [Figure 2]. In the experiment, under treated control conditions (0 hours), cells appeared healthy and grew up to 90% of confluency in methanol, ethanol and ethyl acetate extracts. For methanol extract, HT-29 cells line slowly exhibited the characteristic features of loss normal shape with some cell floating after 48 hours. After 72 hours, around 40% cells line continues to exhibit progressive characteristic features of HT-29 cells. Meanwhile, in ethyl acetate extract after 24 hours, there are few cells that looked smaller in size or shrunk, stated as per N/C ratio (nuclear-cytoplasmic ratio). After 48 hours, 5-10% cell slowly exhibiting the characteristic features of loss in normal shape with few cells floating in the medium and increased to be about 20% after 72 hours treatment but in slow progress.

On the other hand, the other intervals of after 24 hours treatment with methanol extract and after 24, 48, 72 hours treatment with ethanol extract showed that the HT-29 cells exhibited the characteristic features of blabbing and polygonal shape, as compared to control conditions (0 hours). There is no major presence of cell shrinkage, rounding and partial detachment, floating of cell and lobulated appearance to be apoptotic cells.

DISCUSSION

The cytotoxicity activity of *L. domesticum* corr. different extracts on the growth of the HT-29 cells is shown in Table 1. According to the National Cancer Institute standards, crude extracts possessing an IC₅₀ value of less than 20 µg/mL are considered to be active, potential cytotoxic extract against tested cancer cells.¹⁷ Among the three extracts of *L. domesticum* corr., methanol and ethyl acetate extracts were found to inhibit the proliferation of cells line at IC₅₀. The degree of proliferative inhibiting is defined as a concentration that reduces the number of cells to be 50% as compared to the untreated (IC₅₀). However, only methanol extract from *L. domesticum* corr. was found as a potential cytotoxic extract to HT-29 cells line at concentration 6.79±0.00 µg/ml. The extract was also found to be sensitive towards the inhibition of HT-29 cells proliferation compared to ethanol and ethyl acetate extracts.

Table 1: Anti-proliferative effect of HT-29 cells line by methanol, ethanol and ethyl acetate extracts of *L. domesticum corr.*

		IC ₅₀ Value (µg/mL)	
<i>Lansium domesticum corr.</i> Extract			HT-29 Cell lines
Methanol	0 Hour		NA
	24 Hours		NA
	48 Hours		50.00 ± 0.02
	72 Hours		6.79 ± 0.01
Ethanol	0 Hour		NA
	24 Hours		NA
	48 Hours		NA
	72 Hours		NA
Ethyl Acetate	0 Hour		NA
	24 Hours		86.00 ± 0.08
	48 Hours		NA
	72 Hours		96.00 ± 0.12

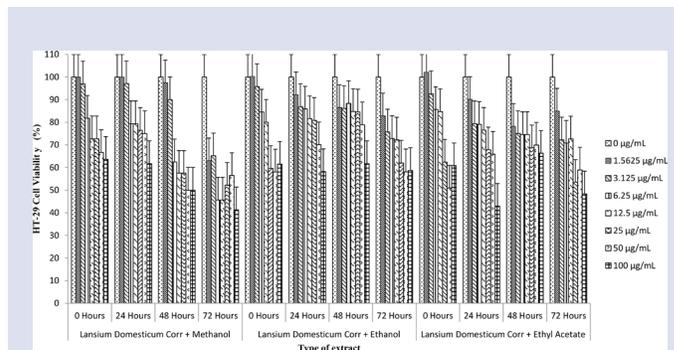


Figure 1: Inhibition of human *colorectal adenocarcinoma* cells line (HT-29) by methanol, ethanol and ethyl acetate extract of *L. domesticum corr.* fruit on time dependent at 0 hours, 24 hours, 48 hours and 72 hours. * Values shown are means of 3 independent experiments. The cell growth of treated groups was standardized with untreated control group as A/A0 (%), where A is the value of A570 generated at a given concentration of extracts by MTT assay, A0 is from untreated control group.

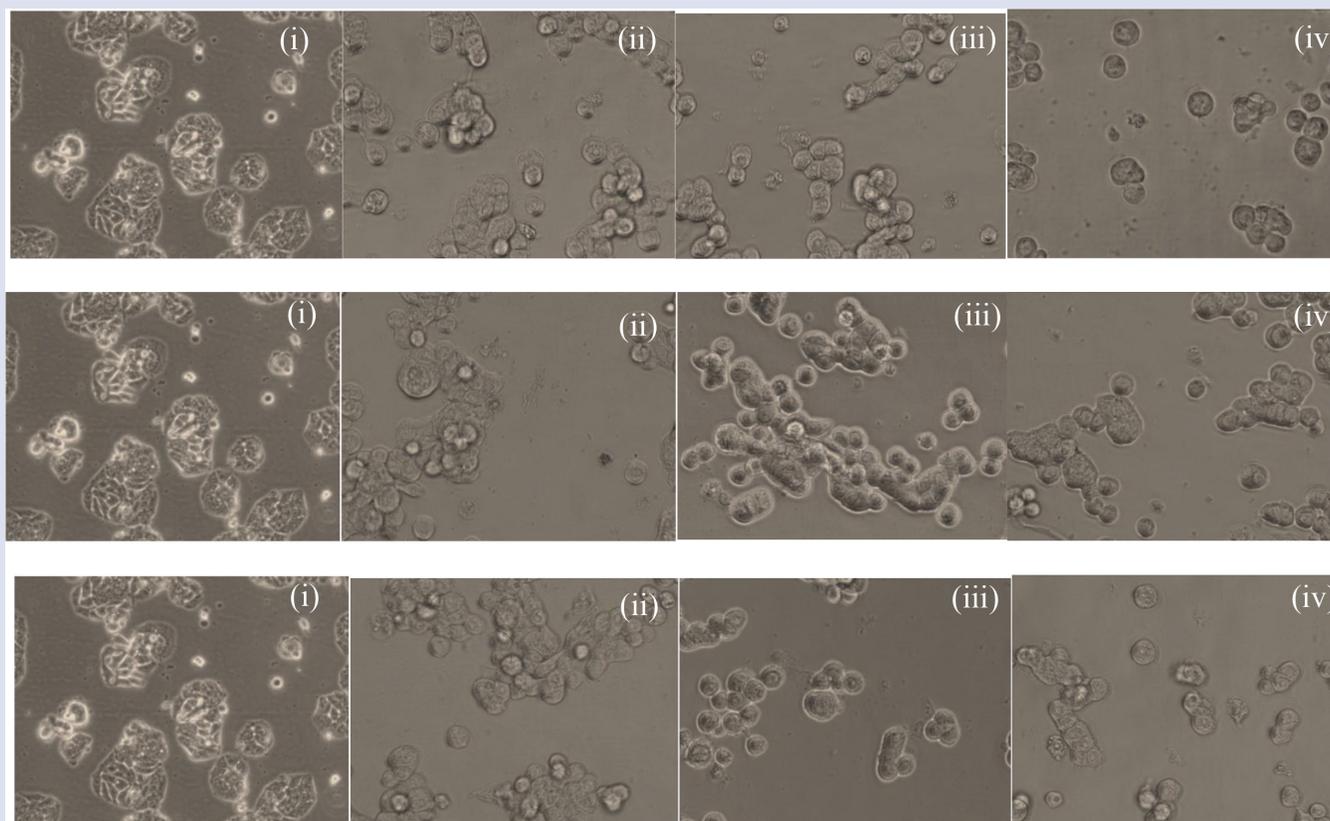


Figure 2: Inhibition of human *colorectal adenocarcinoma* cells line (HT-29) by methanol (A.), ethanol (B.) and ethyl acetate (C.) extract of *L. domesticum corr.* fruits for 3 days. Cell morphology of HT-29 was examined after being treated with (i) IC₅₀ at 0 hours, (ii) IC₅₀ at 24 hours, (iii) IC₅₀ at 48 hours and (iv) IC₅₀ at 72 hours. The photographs were taken at x20 magnification with inverted microscope (Nikon, Japan).

Contrast with a study done by Khalili *et al.*,¹⁸ ethyl acetate extract does not give anti-proliferative effect to HT-29 cells line as none of ethyl acetate extract at any concentrations reached IC_{50} . According to Manosroi *et al.*,¹⁹ the extract of young duku (*L. domesticum* corr.) fruit in hot chloroform demonstrated apoptosis in HT-29 cells lines at 5 mg/mL. In agreement with a previous study, the present study found that *L. domesticum* corr. extract can induce the cytotoxic effect and morphological changes towards HT-29 cells line at $6.79 \pm 0.00 \mu\text{g/ml}$

Klungsupya *et al.*⁵ had stated that *L. domesticum* corr. contains high antioxidant and ascorbic acid, which has a potential effect as an anti-proliferative agent towards cancer cells. However, ethanol and ethyl acetate extracts were not sensitive to the inhibition of HT-29 cells line proliferation probably due to several factors; *L. domesticum* corr. may contain an insufficient value of anti-proliferative properties that could inhibit the proliferation of HT-29 cells line or contain a high glucose level in the extract, which enhances the proliferation activity of cancer cells. It has been known since the 1920s²⁰ that tumour cells have a much higher rate of glucose consumption through a glycolysis pathway by block the pyruvate to be sent to continuous Krebs cycle (i.e. the oxidative phosphorylation pathway) but rather converts pyruvate to lactate: the so-called Warburg effect.^{20,21}

CONCLUSION

This study aimed for screening the cytotoxicity effect of *L. domesticum* corr. extracts towards study the morphological changes of human colorectal adenocarcinoma cell line (HT-29). The study showed that methanol and ethyl acetate extraction of *L. domesticum* corr. have potential in inhibiting the growth of HT-29 cells without any toxic effect. While, ethanol extract showed no IC_{50} value obtained. Morphology of HT-29 cells line changed and continues exhibited characteristic features of cell, but slowly progressing after treatment with methanol extract, but none of concentration showed toxic. Further studies are required to reveal the important chemical constituents and bioactive molecules, responsible for anti-proliferative activity.

ACKNOWLEDGEMENT

The authors would like to thank the Ministry of Higher Education, Malaysia and Universiti Sultan Zainal Abidin (UniSZA) for the financial aid (UNISZA/2015/DKP/23) and the Faculty of Health Sciences for providing the facilities. The authors would also like to acknowledge all staffs from Teaching Laboratory 1, Faculty of Medicine and Faculty of Health Sciences, UniSZA.

CONFLICT OF INTEREST

No conflict of interests exist.

ABBREVIATION USED

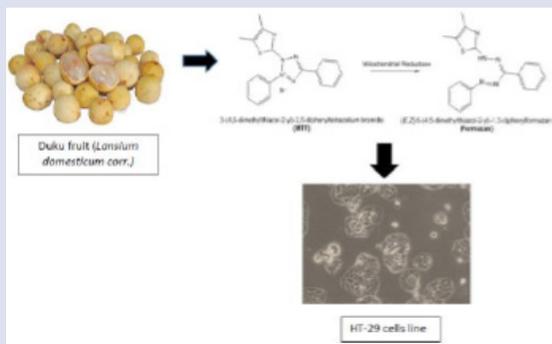
%; percentages; $\mu\text{g/ml}$: microgram per milliliter; $\mu\text{l/well}$: microlitre per well; μm : micrometer; cells/cm_2 : cells per centimetre square; CO_2 : carbon dioxide; DMSO: dimethyl sulfoxide; IC_{50} : half maximum inhibition

concentration; mg/ml : milligram per milliliter; **MTT**: tetrazolium salt 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; **N/C**: nuclear-cytoplasmic; **nm**: nanometer; $^{\circ}\text{C}$: degree Celsius; **w/v**: weight per volume.

REFERENCES

- American Cancer Society. Colorectal cancer. 2015. Available from <http://www.cancer.org/cancer/colonandrectumcancer/detailedguide/colorectal-cancer-what-is-colorectal-cancer>
- Labianca R, Beretta G, Gatta G, *et al.* Colon cancer. *Crit Rev Oncol Hematol.* 2004;51:145-70.
- Scheck AC, Shapiro JR, Coons SW, *et al.* Biological and molecular analysis of a low-grade recurrence of a *glioblastoma multiforme*. *Am J Clin Cancer Res.* 1996;2(1):187-99.
- Buchanan DR, White JD, O'Mara AM, *et al.* Research-design issues in cancer-symptom-management trials using complementary and alternative medicine: lessons from the National Cancer Institute Community Clinical Oncology Program Experience. *J Clin Oncol.* 2005;23(27):6682-9.
- Klungsupya P, Suthepakul N, Laovithayangoon S, *et al.* Investigation on antioxidant, antimutagenic and cytotoxic properties of active fractions of Thai long-kong (*Lansium domesticum* corr.) fruits. *J Ethnobiol Ethnopharmacol.* 2012;1(1):1-9.
- MohdAdzimKhalili R, Norhayati AH, Rokiah MY, *et al.* Determination of radical scavenging activity and Vitamin A, C and E in organically grown Red Pitaya (*Hylocereus* sp.). *Int Food Res J.* 2010;17(2):405-9.
- Esmailbeig M, Kouhpayeh SA, Amirghofran Z. An investigation of the growth inhibitory capacity of several medicinal plants from Iran on tumor cell lines. *Iran J Cancer Prev.* 2015;8(5):e4032.
- Bauman AE. Updating the evidenced the physical activity is good for health: an epidemiological review 2000-2003. *J Sci Med Sport.* 2004;7:6-19.
- Encalada MA, Hoyos KM, Rehecho S, *et al.* Anti-proliferative effect of *Melissa officinalis* human colon cancer cell line. *Plant Foods Hum Nutr.* 2011;66(4):328-34.
- Hanum L, Kasiamdari RS. The phylogenetic relationship among varieties of *L. domesticum* corr Correa based on its rDNA sequences. *Int J Syst Evol Microbiol.* 2013;18(2):123-32.
- Morton JF. Fruits of warm climates. Miami, FL: Winterville, 1987:201.
- Mabberley DJ. *Jupiter Botanicus: Robert Brown of the British Museum.* Braunschweig. J Cramer London. 1985:420.
- Mitsuoka T. Beneficial microbial aspects (probiotics). *Proc. International Dairy Congress.* 1990;2:1226-37.
- Yapp DTT, Yap SY. *Lansium domesticum*: skin and leaf extracts of this fruit tree interrupt the lifecycle of *Plasmodium falciparum*, and are active towards a chloroquine-resistant strain of the parasite (T9) *in-vitro*. *J Ethnopharmacol.* 2003;85(1):145-50.
- Manosroi A, Jantrawut P, Sainakham M, *et al.* Anticancer activities of the extract from Longkong (*Lansium Domesticum*) young fruits. *Pharm Biol.* 2012;50(11):1397-407.
- Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods.* 1983;65(1-2):55-63.
- Geran RI, Greenberg HM, McDonald M, *et al.* Protocols for screening chemical agents and natural products against animal tumours and other biological systems. *Cancer Chemother Rep.* 1972;33:1-17.
- Khalili MAR, Mimie NJ, Norhaslinda R, *et al.* Anti-proliferative effect of Duku (*Lansium domesticum* corr) extracts on human colorectal adenocarcinoma cell lines. *Res J Pharm Biol Chem Sci.* 2016;1078-83.
- Manosroi A, Jantrawut P, Sainakham M, *et al.* Anticancer activities of the extract from Longkong (*Lansium domesticum*) young fruits. *Pharma Biol* 2012; 50: 1-11.
- Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. *J Gen Physiol.* 1927;8:519-30.
- Annibaldi A, Widmann C. Glucose metabolism in cancer cells. *Curr Opin Clin Nutr Metab Care.* 2010;13:466-70.

GRAPHICAL ABSTRACT



HIGHLIGHTS OF PAPER

- Three different polarity solvents were used to extract duku fruits which is methanol, ethanol and ethyl acetate.
- Extraction of duku fruits (*L. domesticum corr.*) shows cytotoxicity effect to HT-29 cells line.
- Methanol and ethyl acetate extraction of *L. domesticum corr.* have potential in inhibiting the growth of HT-29 cells line.
- Morphology of HT-29 cells line changed and continuous exhibited characteristics features of cell.
- To the best of our knowledge, this is the first repeat the exploring the effect of duku extraction on HT-29 cells line.

AUTHOR PROFILE



Dr. Mohd Adzim Khalili Rohin: Got his undergraduate degree and postgraduate degree from Universiti Putra Malaysia (UPM) in Food Study (1999 – 2002) and Community Nutrition (2003 – 2006). He finished PhD in Functional Food and Nutraceuticals (2009 – 2014) from Universiti Sultan Zainal Abidin (UniSZA). Work as Director, Centre for Continuing Education and Senior Lecturer at Universiti Sultan Zainal Abidin (UniSZA). Currently on research functional food towards opioid dependence study.

Cite this article : Khalili RMA, Noratiqah JM, Norhaslinda R, Norhayati AH, Atif BA, Roslan A, Zubaidi ALA. Cytotoxicity Effect and Morphological Study of Different Duku (*Lansium domesticum corr.*) Extract towards Human Colorectal Adenocarcinoma Cells Line (HT-29). *Pharmacog J.* 2017;9(6):757-61.