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Anti-Proliferative Effect Of Duku (*Lansium Domesticum Corr*) Extracts on Human Colorectal Adenocarcinoma Cell Lines.

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ABSTRACT

In the treatment or prevention of cancer, fruits and plants have a high content of natural antioxidants and the potential to protect humans against cancer. The *duku* fruit or *Lansium Domesticum Corr*, is rich in bioactive compounds and nutrients which important to health benefits. The purpose of this study is to screen possible anticancer activity of various extracts and fractions of *duku* fruit extracts that inhibit the proliferation of human colorectal adenocarcinoma (HT29). The *duku* fruit extract process involved three different solvents (methanol, ethanol and ethyl acetate). The human colorectal adenocarcinoma cell line was treated with concentration methanol, ethanol and ethyl acetate extracts (0-60µg/ml) from the *duku* fruit. The anti-proliferative activities of human colon adenocarcinoma cell line treated with *duku* fruit extracts were determined using MTT Assays (570nm). This study found that only the methanol extract had inhibited the proliferation activity of cancer cell lines. The degree of inhibited proliferation is defined as a concentration that reduced the number of cells to 50% compared to the untreated sample (IC_{50}). Methanol extract inhibited the proliferative HT-29 cell lines at concentration of 6.8 µg/ml. Methanol extract from the *duku* fruit was found to be more successful in inhibiting HT29 cell proliferation compared to the Ethanol and Ethyl Acetate extracts.
Keyword: *Lansium Domesticum Corr*, HT-29 Cell, Antiproliferative, MTT Assays, Methanol

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INTRODUCTION

Cancer is recognised as the uncontrolled proliferation and differentiation of cells that eventually invade organs and tissues. It is known to be a major public health burden in both developed and developing countries. According to the American Cancer Society [22], colorectal cancer is a term used for 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 where they start. Adenocarcinoma is the most common type of colorectal cancer. According to the National Cancer Institute, Adenocarcinoma is a cancer that begins in glandular (secretory) cells. Glandular cells are found in the tissue that lines certain internal organs and makes as well as releases substances in the body, such as mucus, digestive juices, and other fluids.

Colon cancer is a serious health problem in most developed countries and is one of the leading causes of cancer mortality throughout the world [1, 2]. According to the National Cancer Society of Malaysia, colon cancer is the second most common cancer affecting about 2,900 Malaysians each year, mostly those above the age of 50 although it could afflict anyone at any age. It is the second most common cancer in Malaysian men across all age groups. Current therapy includes a combination of radiation, surgery and chemotherapy. The rate of relapse after surgical resection of the tumour is very high [3]. The main chemotherapy available is temolozolamide, which is quite expensive and has a high level of toxicity. Thus, 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, 5].

In the treatment or prevention of cancer, fruits and plants as natural products, have a high content of natural antioxidants and the potential to protect humans against cancer. Fruits and vegetables are the main source of antioxidant vitamins, making these foods essential to human health [6, 7]. Esmaeilbeig *et al.* [8] believe that safe, nontoxic origin of herbs and plants have valuable sources for novel anti-cancer drugs. Epidemiological studies, experiments on laboratory animals and investigations on humans have shown that consumption of a diet rich in vegetables and fruits is associated with a low risk of contracting some diseases, including cardiovascular diseases and cancer [9].

Lansium domesticum Corr. is a genus of small trees from the family Meliaceae. The fruit is known locally as *langsat, duku , duku –langsat*, or *dokong*. The tree grows to a height of 40–50 ft with long leaves that are pinnate, dark green, and glossy on the surface [10, 11]. The raw *duku* and *langsat* fruits are green in colour and have a very sour-gummy taste. As the fruit matures, the skin will turn yellowish and the fruit's flesh will become sweet. Most of the fruit, especially the flesh, is eaten fresh. The nutritional composition of 100 grams of *duku* and *langsat* is reported to contain 70-74 calories, 1.0-1.5 g protein, 0.2-0.5 g fat, 13-15 g carbohydrates, 0.7-1.0 g minerals, 18-20 mg calcium, 9-11 g phosphorus and 0.9-1.5 mg of iron [12].

Duku is a famous fruit in South East Asia, with Malaysia, Thailand, Philippines and Indonesia being the biggest producers of *L. Domesticum* [13]. Traditionally, the bark of the *duku* and *langsat* trees are often used to treat venomous insect bites, dysentery and eradicate cancer cells [14]. The seeds are used as febrifuge, vermifuge, antipyretic and anthelmintic . Recently, researchers have focused on the potential of *duku* as having antimalarial [10], anticancerous [15] and antibacterial properties. According to Manosroi *et al.* [15], the extract of young duku fruit by using hot chloroform (YFCH) demonstrated apoptosis in KB and HT-29 cell lines. In the present study, the *in-vitro* anticancer activities in human colorectal adenocarcinoma cell lines (HT-29), including the anti-proliferation properties of extracts from various parts of *Lansium Domesticum Corr*, will be investigated in order to evaluate its potential as a cancer treatment. MTT Assays were used to determine the anti-proliferative properties of *Lansium Domesticum Corr*.

MATERIALS AND METHODS

Duku Extraction

The method introduced by [23] was used in the extraction process. The *Duku* fruits (*Lansium Domesticum Corr*) were obtained from the local market in Kuala Terengganu, Terengganu, Malaysia. The fruit extracts were obtained by using different solvents of increasing polarity. The solvents used were Ethanol,

Methanol and Ethyl Acetate. The fruits were carefully washed under running tap water, dried with a soft cloth and the skin peeled with the fresh flesh then macerated.

Then, 10g of the macerated fruit sample was soaked into the solvents (Ethanol, Methanol and Ethyl Acetate) with a ratio 1:10 for 24-hours. Finally, all the extracts for each solvent were filtered using Whatman ® No. 41 filter paper (pore size 20-25 µm) and were then concentrated under reduced pressure at a temperature of 40^{o}C and stored at a temperature of -20^{o}C until it is used for further analysis.

To screen the extracts of the *Duku* fruit (*Lansium Domesticum Corr*), 100mg of the sample was dissolved in 1mL of DMSO to obtain a 100 mg/mL stock solution of extracts. All extracts were kept at a temperature of 4^{o}C throughout the experiment. Stock solutions were further diluted in a RPIM1640 (Sigma, MO, USA) media without serum to obtain a final concentration of 100 µg/mL.

Anti-proliferative Activity of Duku Extracts

Cell Culture

The human colorectal adenocarcinoma cell line (HT-29) was obtained from the cell bank in the Department of Biotechnology, Universiti Sultan Zainal Abidin. The cancer cells were grown and maintained at a temperature of 37^{0}C in a humidified CO_2 incubator with 5% CO_2 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.

Measurement of cell proliferation (MTT Assay)

According to Mosmann [16], the anti-proliferative activity of extracts from the *L. Domesticum Corr* sample were obtained using ethanol, methanol and ethyl acetate solvents to determine the anti-proliferation rate of HT-29 cell lines using the micro-titration colometric method of tetrazolium salt reduction. The tetrazolium salt 3-[4, 5-dimethylthiazol-2-yl]-2-5-diphenyltetrazolium bromide (MTT) was used to determine cell viability in assays that involved cell proliferation and cytotoxicity.

The viability of cells was determined with the trypan blue reagent. Exponentially growing cells were harvested, enumerated using the haemocytometer and diluted with a particular medium. A cell culture with a concentration of 2.0 x 10^5 cells/mL was prepared and plated (100 µL/well) onto 96-well plates (NUNCTM, Denmark). Prior to the addition of cells into the plates, the stock solution was diluted with media, transferred into the wells and sequentially added with cells in the media to achieve the required starting concentration of 100 µg/mL in 1% DMSO. The 100 µg/mL extract in each well was serially diluted to achieve the concentration range of 60-0 µg/mL. The proliferative activity was determined using the MTT assay (3- [4, 5 - dimethylthiazol - 2-yl]- 2,5-diphenyl tetrazolium bromide) [17].

Thereafter, various concentrations of the *L. Domesticum Corr* extract samples were plated out in triplicates. Each plate included untreated cell controls and a blank cell-free control. After 72 hours of incubation, the MTT (20 µg/ml) was added to each well and re-incubated for a further 4 hours. Then, the media was removed and DMSO was added into each well to solubilize the formazan crystals and incubated for another 15 minutes. Finally, the absorbance was read at wavelengths of 570nm using a flouorometer 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, using the following formula:

$$\% \text{ Cell Viability} = \frac{[\text{A490 of treated cells}]}{[\text{490 of control cells}]} \text{ x } 100\%$$

The anti-proliferative dose (IC_{50}) was determined by nonlinear regression analysis of the corresponding dose response curve and expressed as microgram's ± SD.

Statistical Analysis

Data were expressed as mean ± SD of three independent experiments. The SPSS 20.0 software was used to perform statistical analysis. Differences were analysed using one-way analysis of variance (ANOVA) or two-way ANOVE while a $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

In fact, plant extracts and isolated compounds are formulated in a variety of dietary supplements as antioxidants and providing protective effects against ROS, of which an excess is associated with ageing, cataracts, cardiovascular and neurodegenerative diseases as well as certain types of cancer [9]. The effect of methanol, ethanol and ethyl acetate extracts of *L. Domesticum Corr* as having an anti-proliferative effect on the human colon adenocarcinoma cell line (HT-29) was determined by using MTT Assays.

The MTT was reduced from an insoluble purple formazan by mitochondrial dehydrogenase activity in viable tumour cells into an insoluble coloured formazan product, which could be measured spectrophotometrically after dissolution [17]. Cell proliferative activity was measured by comparing the purple colour formations. Dead cells, on the other hand, do not form the purple formazan due to their lack of the enzyme [18]. The exact cellular mechanism of MTT reduction into formazan is not well understood, but it likely involves reaction with NADH or similar reducing molecules that transfer electrons to MTT [19].

The anti-proliferative activity of *Lansium Domesticum Corr* methanol extracts on the growth of the HT-29 Cell line in-vitro is shown in Table 1. According to the National Cancer Institute standards, criteria crude extracts possessing an IC_{50} value of less than 20 µg/mL are considered active against tested cancer cells [17, 20, 21]. In this study, methanol, ethanol and ethyl acetate extracts from *Lansium Domesticum Corr* were evaluated for their anti-proliferative effects on HT-29 cell lines.

Table 1: Inhibition of the proliferative effect HT-29 Cell Line by the Methanol, Ethanol and Ethyl Acetate Extracts of *Duku* (*Lansium Domesticum Corr*)

Extract	HT-29 IC_{50} Value (µg/mL)
Methanol	6.8 ± 0.11
Ethanol	nil
Ethyl Acetate	nil

Out of the three extracts from *Lansium Domesticum Corr*, only Methanol was found to inhibit the proliferation of cells at $IC_{50.}$ The degree of proliferative inhibit is defined as a concentration that reduces the number of cells to 50% as compared to the untreated (IC_{50}). The IC_{50} value was determined quantitatively after staining the cells with crystal violet and using the MTT assay. The Methanol extract from *Lansium Domesticum Corr* inhibited the proliferation of HT-29 cells at a concentration 6.8 µg/ml. The extract was also found to be sensitive towards the inhibition of HT29 cell proliferation compared to Ethanol and Ethyl Acetate extracts.

Ethanol and Ethyl Acetate extracts were not very sensitive to the inhibition of HT-29 cell proliferation probably due to several factors. *Lansium Domesticum Corr* might contain insufficient value of anti-cancer properties that could inhibit the proliferation of HT-29 cell or contain a high glucose level in the extract, which enhances the proliferation activity of cancer cells. It has been known since the 1920s [23] that tumour cells have a much higher rate of glucose consumption through a glycolysis pathway that does not send pyruvate to the Krebs cycle (i.e. the oxidative phosphorylation pathway) but rather converts pyruvate to lactate: the so called Warburg effect [23-24].

CONCLUSION

In summary, this study aimed to screen the potential effects of *duku* (*Lansium domesticum corr*) extraction towards anti-proliferative on human colorectal adenocarcinoma cell line (HT-29) with various

concentration. *Lansium Domesticum Corr* is rich with important nutrient for health benefits. *Lansium Domesticum* had potential as antimalarial, antimicrobial, anticancer and antibacterial. Study done by Mitsuoko [14] claimed that *Lansium Domesticum* relatively high in dietary fiber which gives great benefits for the digestive system in preventing cancer of the colon and act to cleanse the body from cancer-causing free radicals. Based on previous study done by Manosoroi [15] reported that *Lansium Domesticum* extracted with chloroform demonstrate apoptosis against KB and HT-29 cell line 13.84 ± 4.21 and 8.68 ± 1.85, respectively.

This study generates new information on anticancer of *Lansium Domesticum* fruit toward anti-proliferative HT-29 cell. The anti-proliferative of HT-29 cell was determined using MTT Assays. Result were read after 72 Hours of incubation *Lansium Domesticum* was extracted by mixing with three solvent which are Methanol, Ethanol and Ethyl acetate separately. Compared among all the three sample only *Lansium Domesticum* extracted with Methanol shows inhibition of HT-29 Cell with an IC_{50} 6.8 ± 0.11 µg/mL. It shows *Lansium Domesticum* extracted with Ethanol and Ethyl Acetate have negative reaction toward anti-proliferative activity in HT-29 cell lines. The results from this study have demonstrated that the anticancer activity of the Methanol extract from the *Lansium Domesticum* might be further developed for the treatment of colorectal cancers. In the future, *Lansium Domesticum* can be used for deep investigation toward mechanism reaction between Methanol extract of *Lansium Domesticum* with HT-29 cells line.

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