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Anti-Proliferative Activity of Fragrant Rice MRQ 74 (*Mas Wangi*) Water Extract Against Colon Cancer Cell Lines (Ht-29)

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Abstract

Fragrant rice MRQ 74 (*Mas wangi*) is one of the rice by the Malaysia Research and Development Institute (MARDI), which is responsible for developing varieties of high-quality rice that is nutritious, suitable for diabetics due to its low glycemic index and low starch content as well as resistant to pest and disease. Colorectal cancer (CRC), also known as colon cancer, is the second most common cancer in the world. This cancer involves many factors, including family history, physical inactivity, high-fat and low-fibre diets, inflammatory bowel disease (IBD), diabetes and other conditions associated with the large intestine. This study aimed to examine the antiproliferative activity of polished rice extract and unpolished rice extract against colon cancer cell line HT-29 using MTT assay. Morphology observation and fluorescence double staining of treated cells were determined using a light inverted microscope and acridine orange/propidium iodide staining (AO/PI). The results showed that unpolished rice extract has better IC50 values (0.46 ± 0.02) mg/mL compared to polished rice extract (0.50 ± 0.06) mg/mL after 72 hours of treatment and showed statistically significant results. Morphological changes at 24, 48, and 72 hours after treatment, the antiproliferative effect was observed, which resulted in a reduction of cell size, cell density, the presence of fragmentation cells and apoptotic bodies. Therefore, unpolished rice extracts have promising natural plants in future as an anticancer properties in the nutraceutical and food industry. These results can provide new information for further in vitro or in vivo biological studies.

Keyword: Fragrant rice MRQ 74, colon cancer, HT-29, MTT assay, anti-proliferative, IC50, morphological changes

Introduction

Around the world, cancer is a terrible disease that causes extreme repressibility and mortality. Due to the need for early illness detection and the fact that the majority of patients are only discovered in the latter stages, administration becomes challenging (Akhtar *et al.*, 2019). Colon cancer is the third most common diagnosis and has become the second deadliest malignancy for both genders (Vettil *et al.*, 2017). According to the Centre for Disease Control and Prevention (2020), colon cancer is a disease which occurs in the colon or rectum. Cells in the colon or rectum grow abnormally, called polyps.

Intake of foods that contain phytochemicals, such as fruits, vegetables, and whole grains, has been linked to a reduced risk of colon cancer (Thanuja *et al.*, 2022). Finding plant food sources and their oxidative stress-targeting bioactive components is, therefore, a crucial component of colon cancer prevention and treatment strategies. According to Malabadi *et al.* (2022), around 50% of people on earth, including a sizable portion of Indians, eat rice as a staple food. This is especially true of cereal grain varieties. Coloured rice varieties are prized in comparison to polished white rice because of their health benefits.

Rice (*Oryza sativa* sp), is an edible starchy food, and the grass plant that comes from the Poaceae family. For more than half of the world, rice is the staple food and boasts the second-highest grain production after corn in 2008 in the human population (Fukugawa *et al.*, 2019). *Oryza sativa* contains two significant variants, which are short japonica rice and long indica rice. Short-grained is a sticky type of rice, usually grown in dry fields that are mainly submerged in East Asia; meanwhile, long-grained rice is a sinica variety and a non-sticky type of rice mainly in lowland rice and grown mostly submerged in Southeast Asia (Wang *et al.*, 2014). Rice comes in a range of colours, types, and origins, such as black, brown, purple, fragrant, or aromatic rice (Saikhrisna *et al.*, 2018).

Fragrance in rice is a crucial component in determining market pricing. It is linked to both local and national identity, making it one of the most essential grain quality attributes in rice. Fragrant rice accounts for an estimated 15-18% of rice trade and is the highest priced in the world market. Countries like the United States, Vietnam and other rice producers and traders are interested in entering this premium segment (Paul *et al.*, 2021). The Malaysian Agricultural Research and Development Institute (MARDI) has introduced four types of fragrant rice varieties: MRQ 50, MRQ 74, MRQ 76 and MARDI Wangi 88.

Fragrant rice MRQ 74 (Mas Wangi) is the second most preferred fragrant rice variety (27%), along with MARDI Wangi 88 (21%), with MRQ 76 (30%) being the most preferred (Ariff *et al.*, 2019). Fragrant rice MRQ 74 was more

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likely the same as the Basmati type of rice and characterized as a healthy food, especially for diabetic patients. Lengthy, fine grain shape, flaky, non-greasy, high starch content, slightly softer gel consistency, and moderate alkali diffusion value are some of the notable features of fragrance rice. Until now, rice has become the most preferred by consumers that are locally produced (Rahman *et al*., 2022).

Furthermore, new research suggests that edible rice bran varieties may have an anticancer impact, including breast, lung, liver, and colorectal cancer (Sohail *et al*., 2017). The fourth most significant cause of death is colorectal cancer, and it usually begins in the cells of the colon and rectum. The capacity of rice bran-derived bioactive compounds to induce apoptosis, limit cell proliferation, and change the development of the cell cycle in malignant cells has been shown to mediate their anticancer effects (Rohin *et al*., 2020). The bioactive components in rice bran protect against tissue damage by scavenging free radicals and suppressing chronic inflammatory responses. The phytochemicals in rice bran have also been shown to trigger antitumor immune responses and influence the microenvironment of colon tumours to support better colon cancer prevention (Saad *et al*., 2022). However, there still needs to be more detailed research regarding the cytotoxic activity of fragrant rice MRQ 74 water extraction in Malaysia. Therefore, cell morphology observation is one of the methods that have been used to identify cytotoxic activity in plants.

Methods

Plant collection and preparation

Fragrant rice MRQ 74 was collected from MARDI, Malaysia. Rice grain samples were carefully separated from any physical residue before being placed in sealed containers.

Extraction Procedure for Rice Samples

The rice grain samples were extracted based on the method introduced by Acquistucci (2017). Rice grain samples were prepared right before analysis by grinding rice grains into a fine flour using an electronic blender (Warring 2-Speed Blender, 240 VAC-Standard Motor) at maximum speed for about 30 seconds, ensuring that the grains are correctly ground. Then, the soaking process (1:10 w/v) was continued with 50 g of refined flour rice grains by using an aqueous solution (deionized water) at 25℃ for 24 hours. The solvent extract was filtered through a vacuum filtration kit nylon membrane filter (0.22 µm) and was further concentrated using a rotary evaporator under reduced pressure. The concentrated extract was placed in a 40℃ oven to allow for complete solvent evaporation. The extract was stored in a -20℃ freezer until the following analysis.

Extraction Yield Procedure of Rice Extracts

The extraction yield was calculated according to the method of Dey and Rathod (2013) using the formula and expressed as a percentage (%):

$$\text{Extraction method} = W2/W1 \times 100\%$$

W1= original weight of sample, W2= weight of dried extract

Preparation of Rice Extracts

The methods used previously by Rohin *et al*. (2017) with certain modifications were implemented for this study. To make a stock solution of 1 mg/mL, 10 mg of rice grains extract was dissolved in 1 mL of DMSO reagent. The extract solutions were stored at 4℃ until they were used. To generate a working solution of 1 mg/mL, the stock solution for each extract was further diluted in finished RPMI-1640 and McCoy's 5A medium with 10% fetal bovine serum and 1% penicillin-streptomycin.

Cells maintain and harvest.

A colon cancer cell line (HT-29) was used in this study. Cells were collected from UniSZA's Faculty of Bioresources and Food Industry in passage 3 (P3). The cell line was grown in RPMI-1640 media supplemented with 10% bovine serum and 1% penicillin-streptomycin at 37°C in an incubator humidified with 5% Carbon dioxide (CO2) and with a relative humidity of 95%. The cell media was replenished twice weekly to restore the nutrients essential for cell growth.

Determination inhibition concentration of 50% (IC50) by fragrant rice extracts

A colourimetric micro-titration method known as the MTT test, or tetrazolium salt reduction assay, was used to determine the inhibitory concentration (IC50) of rice extracts (Kuete *et al*., 2017). A method used previously by Rohin *et al*. (2017), the cells were harvested from the media, counted with a hemocytometer, and diluted further in RPMI and

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McCoy's 5A medium (added with 10 % fetal bovine serum and 1 % penicillin-streptomycin). By using 96-well culture plates (SPL Life Sciences, Korea), a total of 100 µL of cell suspension was seeded in triplicates at an optimized density of 1 x 105 cells/cm² for each cell. After 24 hours, each well was received triplicate serial dilutions of rice grains and cooked rice extracts (1.00–0.016 mg/mL) (Rohin *et al*., 2018) as well as 5-fluorouracil (5FU) (a drug) (1.00–0.016 µg/mL). Blank cells (blank) and untreated cells were seeded in each 96-well plate (positive control). After a 48-hour incubation period, 20 µL (5µg/mL) of MTT assay was put into each well of a 96-well plate and left for another 4 hours. The medium was removed, and 100 µL of DMSO reagent was added to each cell. By using a microplate reader, the absorbance at 570 nm was measured with a reference of 630 nm (TECAN, INFINITE M200, Switzerland). The nonlinear regression of the response curves within the same region was used to calculate the IC50 dose concentration. The relative cell viability of the treated cells was described as % of cell viability and will be calculated based on the following formula:

Cell viability (%) = (A570 of treated cells) / (A570 of control cells) x 100%

Cells Morphology Observation

The effects of rice extract on cellular morphological changes were investigated using the Merlin et al. approach (2010). The inhibitor concentration (IC50) value was measured using the MTT assay in this approach to estimate the effective dose concentration of the extract. A light inverted microscope (Nikon, Japan) with a magnification of 10x was used to observe morphological changes at 37°C for 24, 48, and 72 hours.

Fluorescence Microscopy of Apoptosis using AO/PI Doubles Staining

The method according to Hajiaghaalipour *et al.* (2017), was used in this study. Acridine orange (AO)/propidium iodide (PI) staining was used to determine the apoptotic effects of rice extracts on the cancer cell line, which was subsequently observed under a fluorescence microscope. A total of 1 x105 cells/well cell density was seeded into 96-well plates and treated for 72 hours with each extract's IC50 dose concentrations. Then, both untreated and treated cells were incubated with AO and PI staining at a concentration of 10 g/mL, and apoptosis or cell viability was seen at 10x magnification by using an Olympus-BX51 fluorescence microscope (Olympus, Japan) fitted with a Nikon camera (Nikon, Japan).

Data Analysis

All tests for evaluating percentage yield (%) of fragrant rice MRQ 74 water extract sample and IC50 values of HT-29 cell lines at 72 hours treatment were conducted in triplicates. The data were explored using descriptive and inferential statistical analysis by using the Microsoft Excel Spreadsheet 2019 and Scientific Package for Social Sciences version 26.0 software (IBM Corp. US) with $p<0.05$. Descriptive statistics was applied to measure the extraction yield and an IC50 value as means and standard deviation (SD). Based on the variables that were analyzed, this analysis was related to the Independent T-test in percentages of extraction yield. Other than that, a One-way ANOVA test was used to compare an IC50 value of HT-29 cell lines was tested.

Results and Discussions

Percentage Extraction Yield

Table 1 shows the percentage yield (%) of the fragrant MRQ 74 rice water extract sample. The weight of crude extract (g) ± SD in unpolished fragrant rice MRQ 74 extract is (1.12 ± 0.02) higher than polished fragrant rice MRQ 74 extract (0.43 ± 0.03). The percentage of extraction yield in unpolished fragrant rice MRQ 74 extract (2.24%) also had been observed to be higher than polished fragrant rice MRQ 74 extract (0.86%). Overall, the percentage yield was significant between the different types of samples and the percentage of extraction yield ($p<0.05$) with a p-value of 0.00.

Table 1. Percentage yield (%) of fragrant rice MRQ 74 water extract sample

Sample	Weight of crude extract (g) ± SD	Extraction yield (%)	df	F-statistics	p-value
MRQ 74 aged (polished)	0.43 ± 0.03	0.86 ± 0.05	4	2.66	0.00*
MRQ 74 aged (unpolished)	1.12 ± 0.02	2.24 ± 0.03			

Data represent the mean ± SD of three independent experiments.

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Independent T test, *p<0.05. Extract yield present in percentages (100%).

In this study, the sample extraction is determined for categories, which are fragrant rice MRQ 74, polished rice and unpolished rice. Based on the result, unpolished rice has a higher extraction yield compared to polished rice. This result is similar to Quagliariello *et al.* (2016). The yield of bioactive compounds was increased during the extraction of brown rice with the help of pulsed electrical fields (PEF) and promoted antioxidant activity. Water extraction of rice extract showed lower extraction yield in polished rice extract (0.86%) and unpolished rice extract (2.24%) compared to the study from Sohail *et al.* (2017), the extraction of dried rice bran using ethanol as solvent (9.94%). In vitro antioxidant activity of brown rice extracts varied depending on the extraction method, extract bioactive compound content, brown rice cultivar or variation, and bioactive compound fraction, bound or free (Gao *et al*., 2018; Gong *et al.*, 2017). The extraction yield is based on three types of coloured rice: brown (38.6%), black (33.7%) and red rice (28.5%) (Ziegler *et al*., 2017). It has been shown that brown rice has the highest extraction yield compared to black rice and red rice.

Determination Inhibition Concentration Of 50% (IC50) By Fragrant Rice Extracts

Table 2 shows that unpolished fragrant rice MRQ 74 has a better antiproliferative effect on colon cancer cell lines, which showed the IC50 values (0.46 ± 0.02) mg/mL. This result is similar to the study from Tan *et al*. (2015), which showed that water brewer extracts with IC50 values of 38.33 ± 6.51 µg/mL justified that water brewer extract meets two essential criteria, which are tumour specificity and minimal toxicity against normal cells and become a promising antiproliferative agent for HT-29 colon cancer (p<0.05).

Table 2. IC50 values of HT-29 cell lines after treatment with fragrant rice MRQ 74 water extract at 72 hours.

Sample	IC50 values ± SD (mg/mL)	df	F-statistics	p-value
	72 hours			
MRQ 74 age (polish)	0.50 ± 0.06	7	2.12	0.10
MRQ 74 age (unpolish)	0.46 ± 0.02	7	3.67	0.02*
5-fluorouracil (5FU)	0.33 ± 0.10	6	3.23	0.03*

Data represent the mean ± SD of three independent experiments. One-way ANOVA test, *p<0.05.

The finding from Quagliariello *et al*. (2016) shows that extracts from brown rice observed as the potential of anti-inflammatory or anticancer properties at concentrations 1 and 3 mg/mL. The extracts showed significant toxicity for HT-29 cells after 24 hours of exposure, with an IC50 value at a concentration of 1.3 mg/mL (p<0.05). Additionally, pulsed electric field extracts of brown rice significantly reduced interleukin production and gene expression in colon cancer cells, indicating that brown rice has natural anti-inflammatory substances.

In comparison with different varieties of rice, unpolished rice is categorized as coloured rice. Examples of coloured rice include brown rice, black rice, red rice, purple rice and many more (Priya *et al*., 2019). In a similar method of this study, Thanuja *et al.* (2022) observed the raw black rice that had been ground until it became flour and known as native black rice. Meanwhile, the modified method of native black rice used a buffer of 0.1M sodium acetate and added 0.2 g of alpha-amylase enzyme. This study indicates that by increasing the concentration of black rice from 25–400 µg/mL, the cell viability of HCT-116 clines decreased range from 96.69% to 32.72%. Overall, native black rice flour date-haven the highest anticancer activity concentration centration compared to modified native black rice flour.

A study by Wattayagrn *et al.* (2022) examined the cytotoxic effects of hydrolyzed rice berry rice bran extract on two distinct colon cancer cell lines, SW-620 and HT-29, which are both metastatic cancer cell lines (HRBE). As a result, the hydrolyzed rice berry rice bran extract caused senescence in non-metastatic cancer cells and induced apoptosis in metastatic cancer cells. This finding was similar to this study due to the same method of using ultrapure water at a concentration of 20g/L and concentrated by freeze drying and keeping 20 ℃ until further use. As the results, on HT-29, the half-maximal inhibitory concentrations (IC50) of HRBE were 43,677 µg/mL, 11,164 µg/mL, and 6053 µg/mL, and on SW-620, the IC50 values were 9880 µg/mL, 6870 µg/mL, and 5468 µg/mL during 24 hours, 48 hours and 72 hours, respectively. This can conclude that hydrolyzed rice berry rice bran extract had higher cytotoxic efficiency on SW-620 and HT-29 colon cancer cell lines.

Moreover, a study by Phannasorn *et al.* (2017) examined the impact of purple rice extract on colon cancer cell growth induced by the treatment of proinflammatory cytokines, interleukin (IL-6). This study observed that IL-6 has the power to stimulate the growth and proliferation of HT-29 cells at both 24 and 48 hours. This finding showed that purple rice extract had anti-proliferation effects on HT-29 colon cancer cells that were inflamed. According to a study

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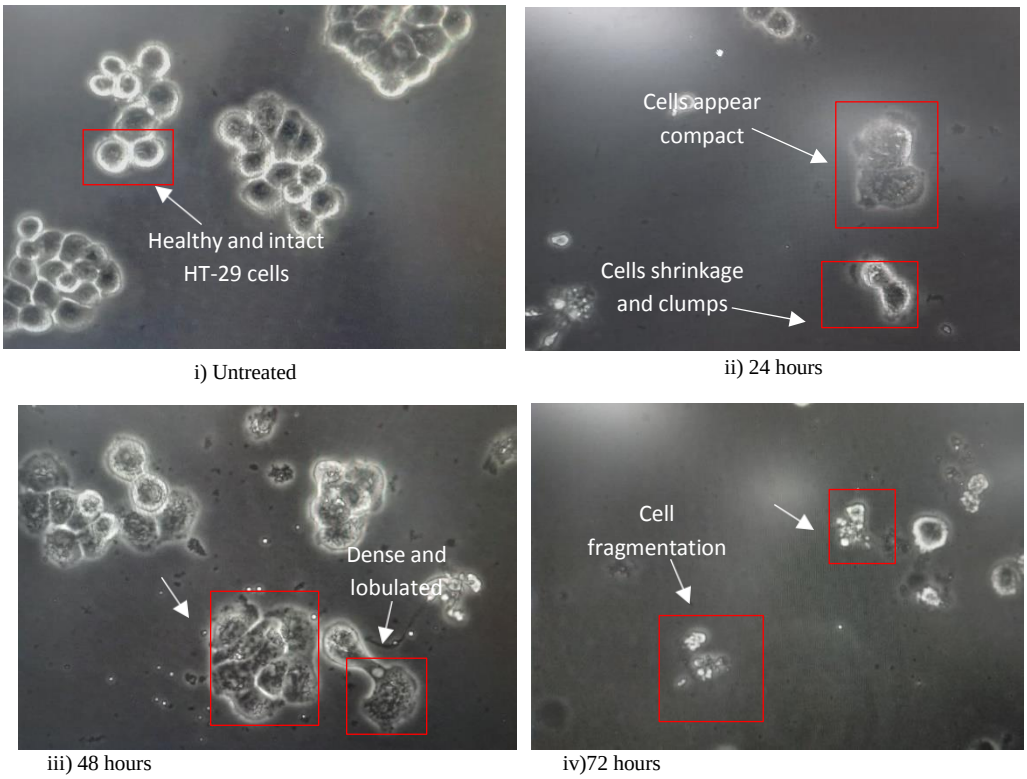
conducted by Rao *et al.* (2019), on the capacity of phenolic extracts from coloured rice to induce apoptosis in colorectal cancer, SW480, phenolic compounds found in purple rice may inhibit cancer cell proliferation by activating apoptosis.

A study observed by Moirangthem *et al.* (2021) using human colorectal cancer cells (HCT-116) showed a significant reduction of more than 50% when treated with 200 g/mL of bran extract ($p = 0.04$). In another study by Takashima *et al.* (2013), the rice bran water extract (RBWE) at 1.0 mg/mL, the proliferation of LS174T human colon cancer cells was significantly reduced and showed 37.2% of IC50 values. This showed that rice bran has convincing outcomes for colon cancer cells.

Cells Morphology Observation and Fluorescence Staining Apoptosis of Fragrant Rice MRQ 74 Extract on Colon Cancer Cells Lines (HT-29)

Figure 1, 2 and 3 shows the inhibition of HT-29 by polished rice extract, unpolished rice extract and 5-fluorouracil (5FU) extract. Morphological changes were starting from 0 hours, 24 hours, 48 hours and 72 hours. During 0 hours (untreated cell), HT-29 cells were round, shiny and intact. **[Figure 1,2,3, (i)]**. During 24 hours, HT-29 cells treated with rice extract and 5FU extract observed that the cells appeared compact and started to shrink and clump **[Figure 1,2,3, (ii)].** Then, at 48 hours, the cells become dense and lobulated due to loss of normal cell shape. During this phase, some cells are partially detached from the surface, and some of the organelles become too compact. The volume of the cells decreased as a result of the rounding off of the cells **[Figure 1,2,3, (iii)].** The formation of apoptosis bodies, cell fragmentation, and a lot of debris membrane blebbing occur during the 72 hours, indicating the final stage of apoptosis **[Figure 1,2,3, (iv)]**



i) Untreated ii) 24 hours

iii) 48 hours iv)72 hours

Figure 1. Inhibition of HT-29 cell line by polished fragrant rice MRQ 74 extract for 3 days. Cells morphology of HT-29 was examined after being treated with IC50 at (i) untreated, (ii) 24 hours, (iii) 48 hours and (iv) 72 hours. The photographs were taken at 20x magnification with inverted microscope (Nikon, Japan).

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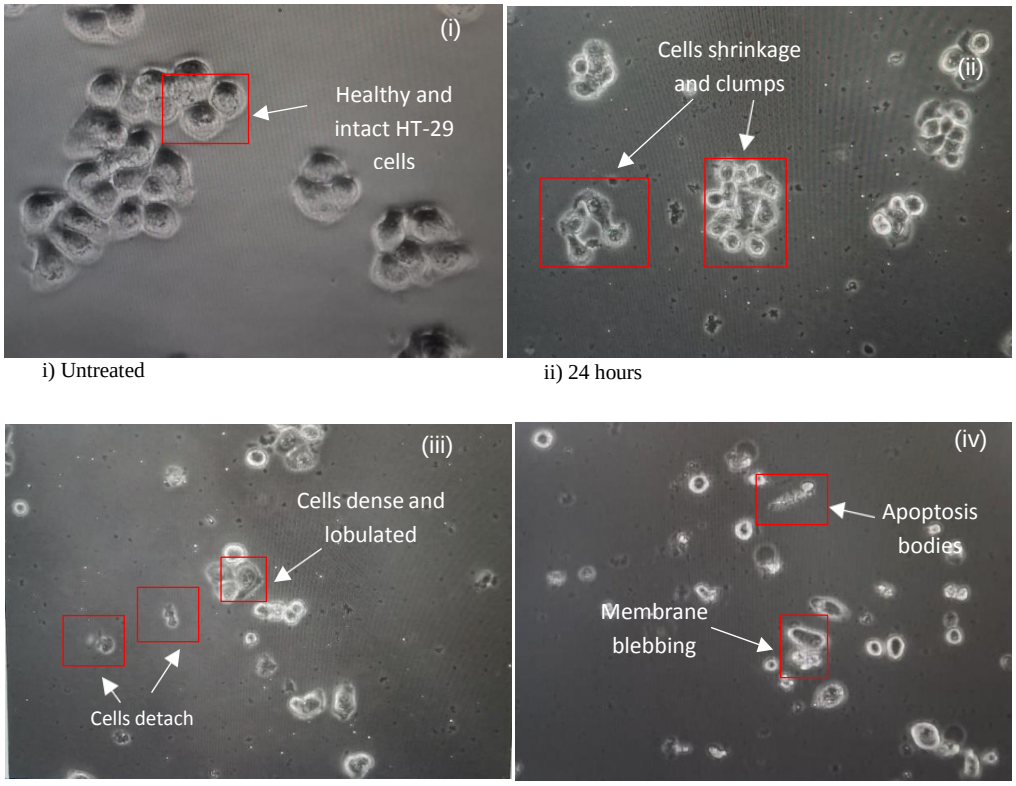
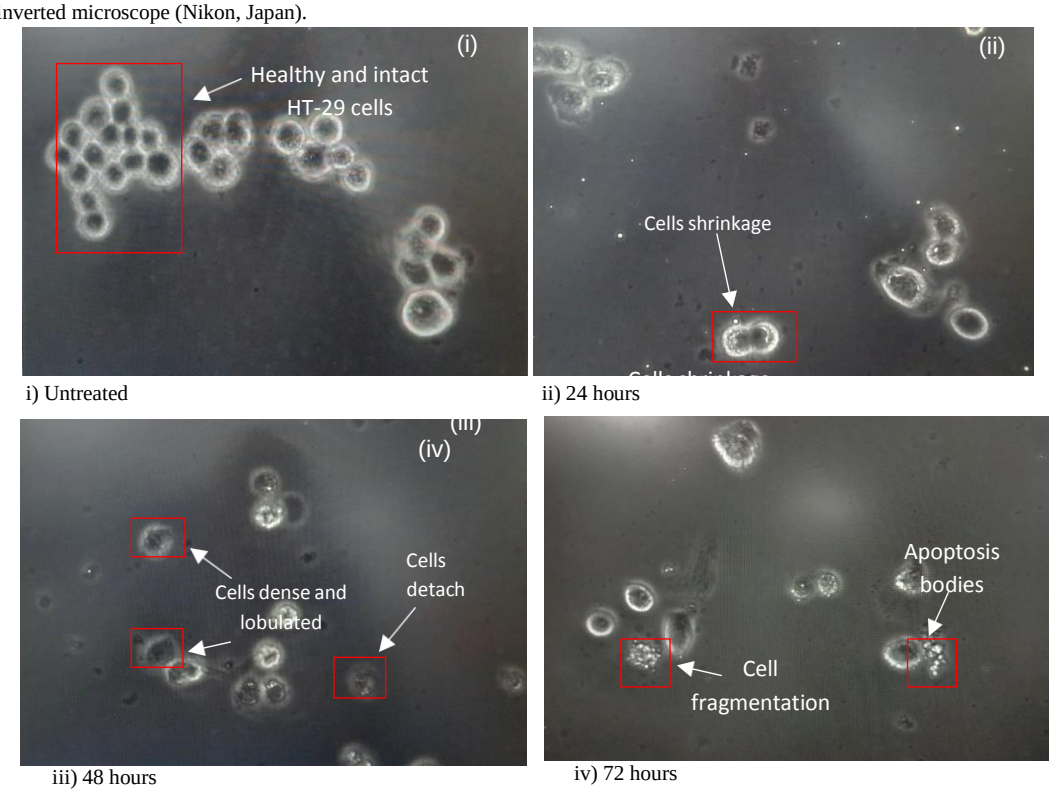


i) Untreated ii) 24 hours

iii) 48 hours iv)72 hours

Figure 2. Inhibition of HT-29 cell line by unpolished fragrant rice MRQ 74 extract for 3 days. Cells morphology of HT-29 was examined after being treated with IC50 at (i) untreated, (ii) 24 hours, (iii) 48 hours and (iv) 72 hours. The photographs were taken at 20x magnification with inverted microscope (Nikon, Japan).



i) Untreated ii) 24 hours

iii) 48 hours iv) 72 hours

Figure 3. Inhibition of HT-29 cell lines by 5FU extract for 3 days. Cells morphology of HT-29 was examined after being treated with IC50 at (i) untreated, (ii) 24 hours, (iii) 48 hours and (iv) 72 hours. The photographs were taken at 20x magnification with inverted microscope (Nikon, Japan).

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Based on **the figure 4,** fluorescence micrographs of HT-29 were observed in polished fragrant rice extract, unpolished rice extract, and 5-Fluorouracil extract after being treated with IC50 values at 24 hours, 48 hours and 72 hours. For untreated cells, the cells are viable and displayed round and have green fluorescence colour at all hours of treatment **[Figure 4 (a,b,c (i)].** Yellow stain was detected when polished rice extract, unpolished rice extract and 5FU extracts were stained with AOPI at 24 hours and 48 hours **[Figure 4 (a, b (ii,iii,iv)].** At 72 hours, the red fluorescence colour appears **[Figure 4 (c (ii,iii,iv)].**

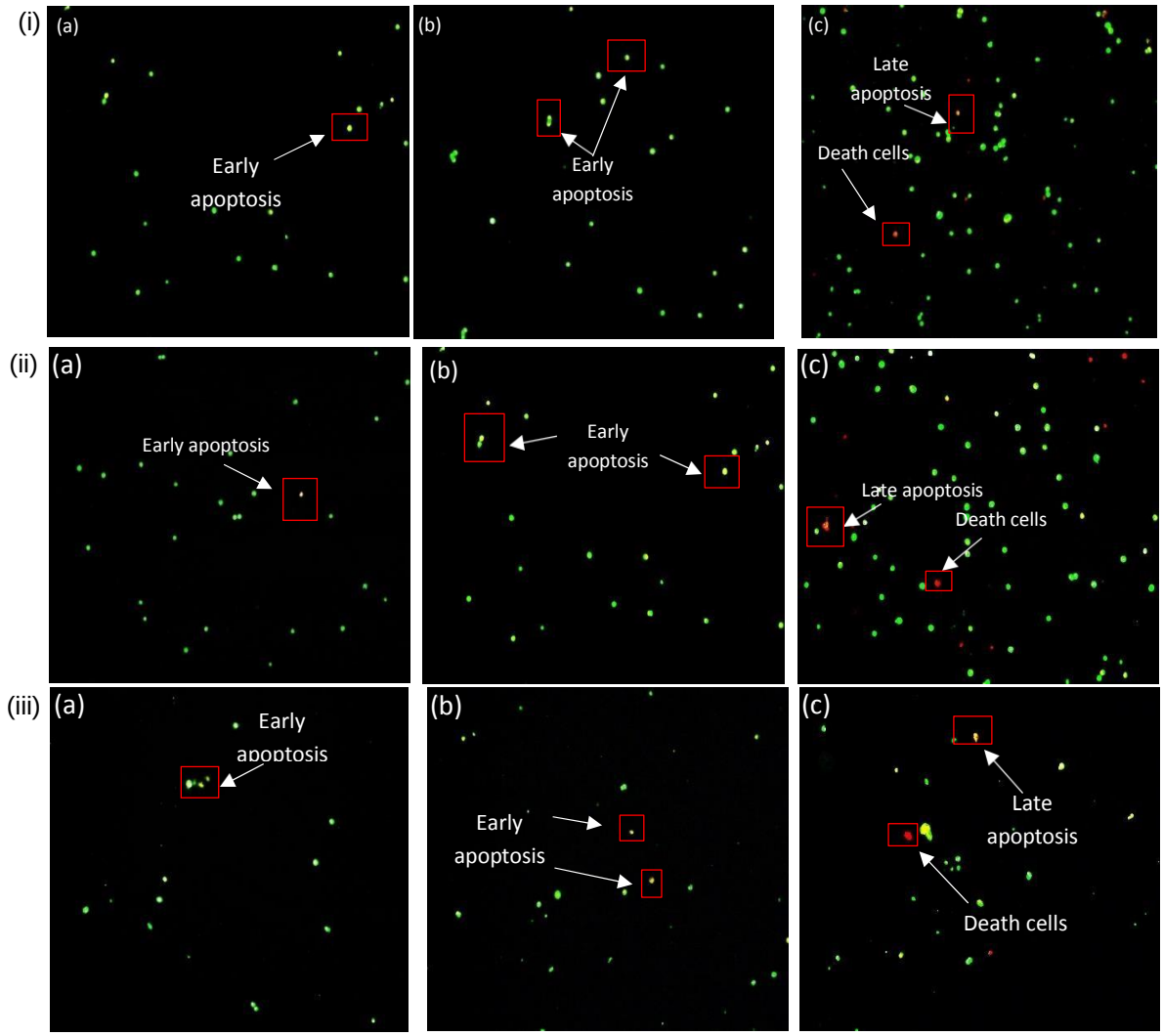

Figure 4. Fluorescence micrographs of rice extracts stained with AOPI. The micrographs of HT-29 were examined after each extract were treated with IC50 values at (a) 24 hours, (b) 48 hours and (c) 72 hours; (i) untreated cells; (ii) fragrant rice MRQ 74 aged (polished); (iii) fragrant rice MRQ 74 aged (unpolished); (iv) 5FU. Magnification 10x were taken with fluorescence microscope (Nikon, Japan).

Cell morphology observation of fragrant rice MRQ 74 extracts on colon cancer cell lines (HT-29) was observed by using the light inverted microscope with a magnification of 10x at three interval times, which are 24 hours, 48 hours and 72 hours. Meanwhile, in determining the apoptotic effect of rice extract on colon cancer cell line, fluorescence staining apoptosis was observed using 10 g/mL concentration of Acridine orange (AO)/ propidium iodide (PI) staining. An Olympus-BX51 fluorescence microscope was used to observe apoptosis or cell viability at a 10x magnification.

Apoptosis is a type of programmed cell death with specific characteristics of morphological and biochemical changes in cells. Apoptosis, or programmed cell death, is essential for the maintenance of tissues, the physiological state of organs, and heritably controlled cell death for the stability of cell proliferation (Majtnerová *et al.*, 2018). Apoptosis has five stages that begin with chromatin condensation, shrinkage and rounding off, nuclear fragmentation, membrane blebbing, the presence of apoptosis bodies, and cell debris (Abou-Ghali *et al.*, 2015). AO is a membrane-permeable cationic colour that reaches living cells' nucleic acids and redirects a green fluorescence. Meanwhile, red fluorescence is produced by all dead nucleated cells after PI enters dead cells with compromised membranes.

Based on **Figure 1,2,3, (i)** of morphological changes, during 0 hours, which was categorized as untreated cells, HT-29 cell lines were round, shiny and intact in polished rice extract, unpolished rice extract and 5-fluorouracil extract.

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At this phase, the cells were justified as healthy cells because the cells grew normally for a colon cancer cell line and cell confluence up to 90%. For untreated cells, the cells are viable and displayed round and have green fluorescence colour at all hours of treatment **(Figure 4.4 (a,b,c (i)).**

During 24 hours of treatment, HT-29 cells treated with rice extract and 5-Fluorouracil extract observed that cells appeared compact and started to shrinkage and clumps **(Figure 1,2,3 (ii)).** Cells undergo shrinkage, resulting in a smaller size, and the cytoplasm is denser and tightly packed (Baharara *et al*., 2015). Then, at 48 hours, the cells become too compact, dense and lobulated due to loss of normal cell shape. **(Figure 1,2,3 (iii)).** This indicates the phase before the cell completely detached and has a mass with dark eosinophilic cytoplasm (Rahim et al., 2021). Early apoptosis, which indicates a yellow stain, was detected when polished rice, unpolished rice and 5-Fluorouracil extracts were stained with AOPI at 24 hours and 48 hours of treatment **(Figure 4 (a, b, ii, iii, iv).**

During 72 hours, the formation of apoptosis bodies, cell fragmentation and a lot of debris membrane blebbing indicate the final stage of apoptosis. **(Figure 1,2,3 (iv)).** This is consistent with a previous study from Tan *et al.* (2016), using water brewers' rice extract, which displayed apoptotic characteristics such as membrane blebbing, nuclear fragmentation and apoptotic bodies. Based on **Figure 4 (c, ii, iii, iv),** red fluorescence colour and cell undergo late apoptosis and death cell after being treated with polished rice, unpolished rice and 5FU extract.

During 72 hours of treatment, the cell nuclei stained with orange and light red showed cells with an intact shape, indicating that the cells were in the late stages of apoptosis but were not dead (Kntayya *et al.*, (2018). The cells demonstrated as death cells due to the PI dye making cells appear fluorescence red. A recent study from Wattayagorn *et al*. (2022) showed the morphology changes of SW-620, including apoptotic-related features such as membrane blebbing and chromatin condensation. The red colour from PI staining was also observed to indicate late apoptosis.

Conclusions

The results of this study show the IC50 values that indicate anti-proliferative activity against colon cancer cell line (HT-29) after being treated with fragrant rice MRQ 74 water extract. In comparison between the two categories of fragrant rice, unpolished rice extract has promising potential as an anticancer as the IC50 values shows are higher than polished rice after 72 hours of treatment. Moreover, HT-29 cell lines also showed morphological changes after being treated with fragrant rice MRQ 74 water extract. HT-29 cell line shows morphological changes from cell shrinkage to apoptosis of cell in 24 hours, 48 hours and 72 hours. HT-29 also reduce cell viability in AO/PI staining from viable cell to early apoptosis. Orange and red fluorescence colour appeared when the cells were in the phase of late apoptosis and death. It can be concluded that fragrant rice MRQ 74 had an anti-proliferative effect against HT-29 colon cancer cells. Hence, this shows that the fragrant rice MRQ 74 has potential as an anticancer and could deliver new data as well as evidence regarding research on cytotoxic activity on fragrant rice using water extraction.

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