RESEARCH ARTICLE

Editorial Process: Submission:04/04/2025 Acceptance:10/03/2025 Published:10/17/2025

Effective Anti-Cancer Activity of Red Rice Bran Extract on Cervical Cancer and Breast Cancer Cells

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Abstract

Objective: Many researchers have shown that red rice bran is a rich source of antioxidants and has potential anticancer properties. This research aims to evaluate the cytotoxicity and inhibitory effects of Red Rice Bran Extract (RRBE) on the growth and migration of human cervical and breast cancer cells. **Methods:** HeLa and MCF-7 cancer cells were treated with various concentrations of RRBE for 24 and 48 h. Cytotoxicity was assessed using the MTT assay. Then, the half-inhibitory concentration (IC_{50}) value was determined. The anti-proliferative effects were evaluated using the MTS assay, and the anti-cell migration activity was assessed using the wound healing assay. **Result:** It was found that the RRBE exhibited cytotoxicity to both HeLa and MCF-7 cancer cells. As the concentration level increased (100 - 1000 µg/ml), the survival rate of the cells significantly decreased, especially at 48 h, corresponding to the IC_{50} values of 818.67 µg/ml and >1000 µg/ml for HeLa and MCF-7, respectively. Then, concentrations of RRBE with lower cytotoxic effects were selected for further experiments, which revealed that treatment with RRBE at concentrations of 100, 250, 500, and 1000 µg/ml significantly suppressed the proliferation and reduced the migration of those cancer cells. **Conclusion:** These results demonstrate that RRBE has potent anti-cancer activity in HeLa and MCF-7 cancer cells, making it a suitable candidate for in vivo studies and development as an anti-cancer agent.

Keywords: Red Rice Bran Extract- Anti-call proliferation- Anti-cell migration- cervical cancer cells- breast cancer cells

Asian Pac J Cancer Prev, **26 (10)**, 3719-3725

Introduction

Cancer is a disease in which abnormal cells grow out of control and form tumors and/or spread to other organs. The latter case is called metastasis, a life-threatening process that is often fatal. For this reason, cancer is a major problem of public health worldwide. The World Health Organization estimated that cancer accounted for nearly 10 million deaths in 2020 [1, 2]. Among the cancers that develop in women, breast cancer and cervical cancer are the most common type of malignancy in both developed and less developed countries [3–5]. Several factors may bring about the onset of these cancers. It was found that persistent infection of the cervix by human papilloma virus accompanied by activation of cell proliferation in tumor cells is responsible for 95% of cervical cancer cases [6]. Breast cancer, meanwhile, is a complex disease based on the molecular subtype of the cancer cells. The most prevalent breast cancer is the luminal A subtype (HR+/HER-) which leads to increased cell proliferation and/or the moving out of cancer cells [7, 8]. Due to the prevalence and nature of breast cancer and cervical cancer, inhibiting these two types of cancer is an important but challenging task.

Rice (Oryza sativa L.) is the main food staple in many cultures, especially in Asia. During the milling process of rice production, rice bran is one of the byproducts but it is considered valuable due to the fact that it contain a highly bioactive compound. Specifically, rice bran is rich in phytochemicals [9, 10]. In particular, several types of polyphenolic compounds have been identified, especially in red rice bran. These compounds have potent pharmaceutical activity such as scavenging property and antioxidant activity [11, 12]. Our previous research reported that red rice bran extract (RRBE) could suppress the proliferation of colon cancer cells. The cellular mechanism underlying the proliferative suppression is the induction of apoptosis and the cell cycle arrest in those cancer cell lines (HT29 and HCT116), indicating that RRBE may have an anti-cancer action [13]. The aim of this study was to evaluate the efficacy of RRBE in inhibiting breast cancer and cervical cancer cells.

Materials and Methods

Preparation of RRBE solution

RRBE was provided by Assist. Prof. Dr. Narongsak Munkong (Department of Pathology, University of

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Phayao, Thailand). The extract was initially dissolved in dimethyl sulfoxide (DMSO) to achieve a concentration of 10 mg/ml as the stock solution. The stock solution was further diluted with culture medium and stored at a temperature of 8°C and protected from light.

Cell culture

HeLa (Cervical) and MCF-7 (Breast) cancer cell lines were provided by Dr. Prapaipat Klungsupya (Thailand Institute of Science and Technological Research, TISTR). These were then cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), and streptomycin/penicillin (1x). The cells were incubated in an incubator at 37°C with a 5% CO_2 atmosphere. The culture medium was changed every 2-3 days during the cultivation process.

Cell viability assessment using MTT test

The HeLa and MCF-7 cells were plated at a density of 5 x 10^3 cells per well in a 96-well plate. The cells were then incubated in a cell culture incubator and allowed to adhere to the surface. The old culture medium was replaced by RRBE at concentrations ranging from 100 to 1,000 µg/ml and the cells were then incubated for a further 24 and 48 hours. After the designated incubation period, the culture media containing RRBE were aspirated, and then 50 µl of a 5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution was added into each well of a 96-well plate, which was subsequently placed in an incubator with 5% CO_2 at 37°C for 3 hours. Following this, MTT solution was carefully removed and then replaced with 100 µl of DMSO. The plate was checked and measured at 550 nm using a microplate reader. The percentage of cell viability (% Viability) was calculated using the following formula [14]:

% Viability = [ODSample/ ODControl] x 100
ODSample = OD value of the RRBE group
ODControl = OD value of the untreated control group

The cell viability graph was generated from percentage viability data, and concentrations of RRBE and the half inhibitory concentration (IC_{50}) were calculated from the linear regression equation.

Anti-proliferative evaluation

The inhibitory effect of cell proliferation was assessed using the MTS assay with the Cell Titer 96 Aqueous One Solution Cell Proliferation Assay (Promega, USA). The HeLa and MCF-7 cancer cells were cultured at a density of 5 x 10^3 cells/well in a 96-well plate and incubated in a cell culture incubator for 24 hours. Subsequently, the cells were treated with RRBE at concentrations of 100, 250, 500, and 1000 µg/ml. The cells were then incubated at 37°C with 5% CO_2 for 48 hours. After the incubation period, 20 µl of the MTS reagent was added to each well and these were then incubated for 2-3 hours in the cell culture incubator. The absorbance was measured at a wavelength of 492 nm using a microplate reader. The percentage of relative proliferation was calculated using the same method as described previously [15].

Anti-cell migration activity using a wound healing assay

The HeLa and MCF-7 cancer cells were plated at a concentration of 6×10^5 cells/ml in a 96-well plate and then incubated for 24 hours. The old culture medium was replaced by DMEM without serum and the cells were then incubate in a CO_2 incubator at 37°C for 24 hours. A wound was then created by scraping the cell monolayer using a sterile 200 µL tip. Any detached cells were removed by washing with PBS. The cells were then treated with various concentrations of RRBE extract, followed by incubation in a CO_2 incubator at 37°C with 5% CO_2. The wound width was measured and captured at the initial time point and again after 24 hours. The percentage of wound closure was calculated using the following formula [16].

Wound Closure% =$(A_{t=0h} - A_{t=\Delta t} / A_{t=0h})$ x100%

$A_{t=0h}$ is the area of the wound measured immediately after scratching (t=0h)

$A_{t=\Delta t}$ is the area of the wound measured h hours after the scratch was performed

Statistical analysis

The experiments were conducted at least three times for each test and the results were presented as mean ± standard deviation (mean ± SD). Statistical analysis was performed using analysis of variance (ANOVA) to determine the differences among group means. The least significant difference (LSD) method was used for multiple comparisons between group means. A t-test was used to find the differences between the means of two independent groups. A significance level of 0.05 ($p < 0.05$) was used for all experiments.

Results

Effect of RRBE on the viability of cervical cancer and breast cancer cells

HeLa and MCF-7 cells were treated with RRBE at various concentrations and exposure times. It was observed that the cell populations were reduced with treatments at increasing concentrations of RRBE, and the morphology of those cells changed when compared to the control group, especially at 48 hours after incubation (with no marked difference after 24 hours). Treatment with RRBE exhibited increased cell shrinkage and detachment from the culture surface, which was found in both cancer cell lines, as depicted in Figure 1. In the HeLa cells, cell viability of 60.38% to 50.87% was shown in the concentration range of 100 to 1000 µg/ml (Figure 2) at 48 hours of RRBE treatment.

Similarly, the percentage of viability was 94.39% to 59.42% for MCF-7 at the same concentrations (Figure 3). Based on the equation of the straight linear regression fitted line, the IC_{50} values at 48 hours were 818.67 µg/ml and >1000 µg/ml for HeLa and MCF-7, respectively. These results suggests that RRBE has the potential to inhibit HeLa and MCF-7 cells.

Effect of RRBE on proliferative suppression in cervical cancer and breast cancer cells

To confirm the inhibitory effects of RRBE on cancer

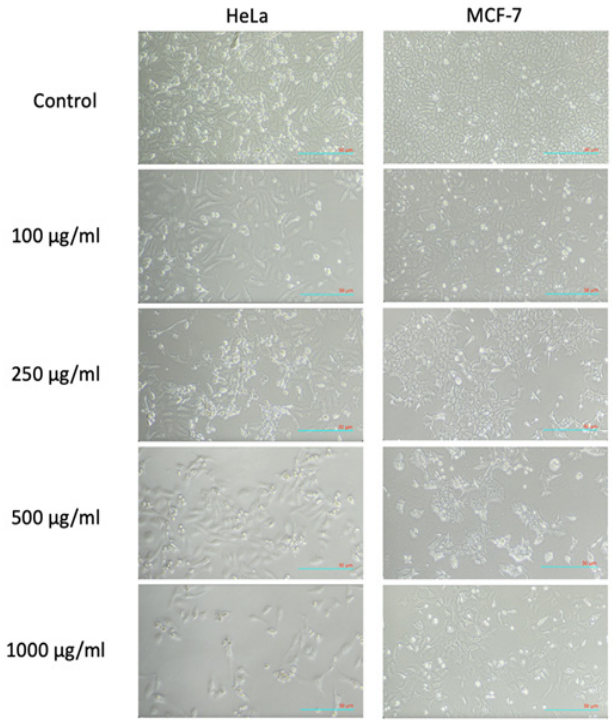

Figure 1. The Effect of RRBE on HeLa and MCF-7 Cells. Morphological change following treating with RRBE at concentrations of 100, 250, 500, and 1000 µg/ml for 48 hours.

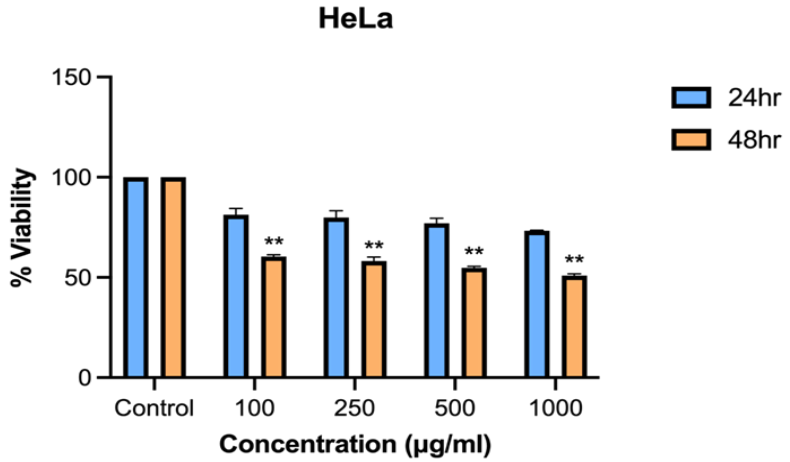

Figure 2. The Viability of HeLa Cells after RRBE Treatment. Cells were incubated with RRBE at 100 to 1000 µg/ml for 24 and 48 hours. **P<0.01

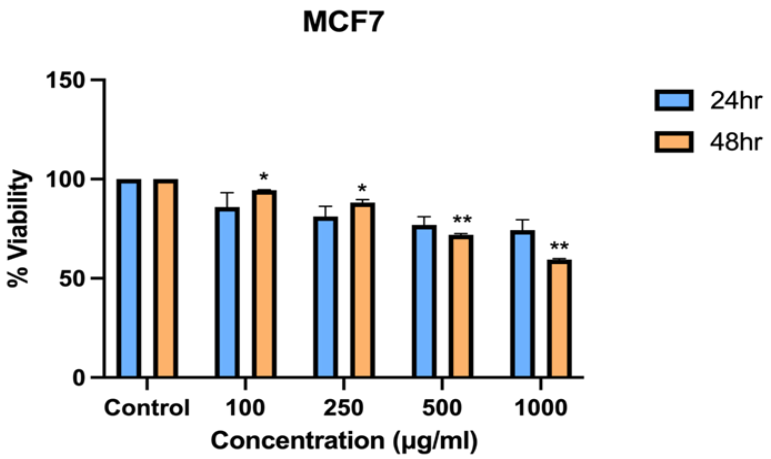

Figure 3. The Viability of MCF-7 Cells after RRBE Treatment. Cells were incubated with RRBE at 100 to 1000 µg/ml for 24 and 48 hours. * P < 0.05, ** P < 0.01

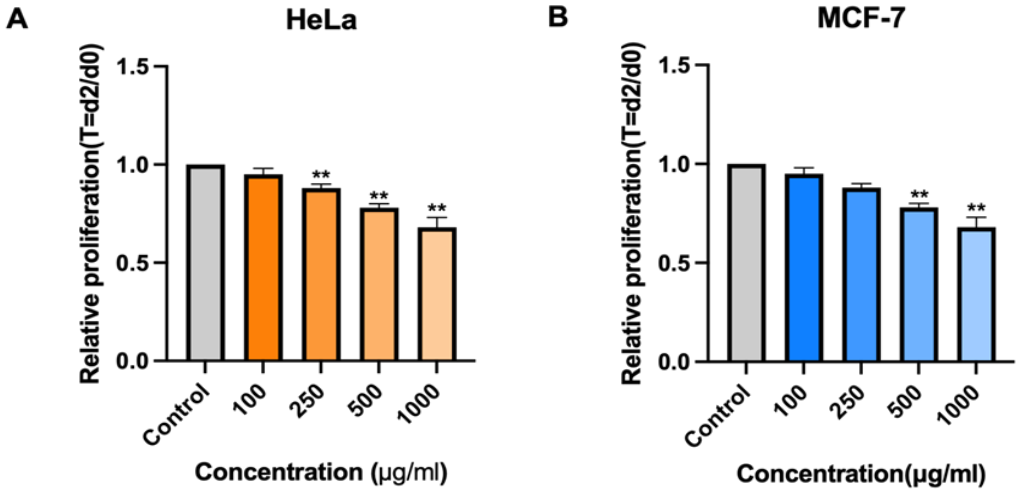

Figure 4. The Effect of RRBE on Proliferation of HeLa (A) and MCF-7 (B) cells at concentrations of 100, 250, 500 and 1000 µg/ml. ** $P < 0.01$

cells, we evaluated the effect of RRBE on the proliferative suppression of HeLa and MCF-7 cells, with RRBE concentrations being chosen for the experiment due to their nontoxicity. The results revealed that both cell lines exhibited a statistically significant decrease in proliferation ($p < 0.05$) at concentrations of 100, 250, 500, and 1000 µg/ml after 48 hours of treatments with RRBE. These results show that RRBE significantly inhibits the proliferation

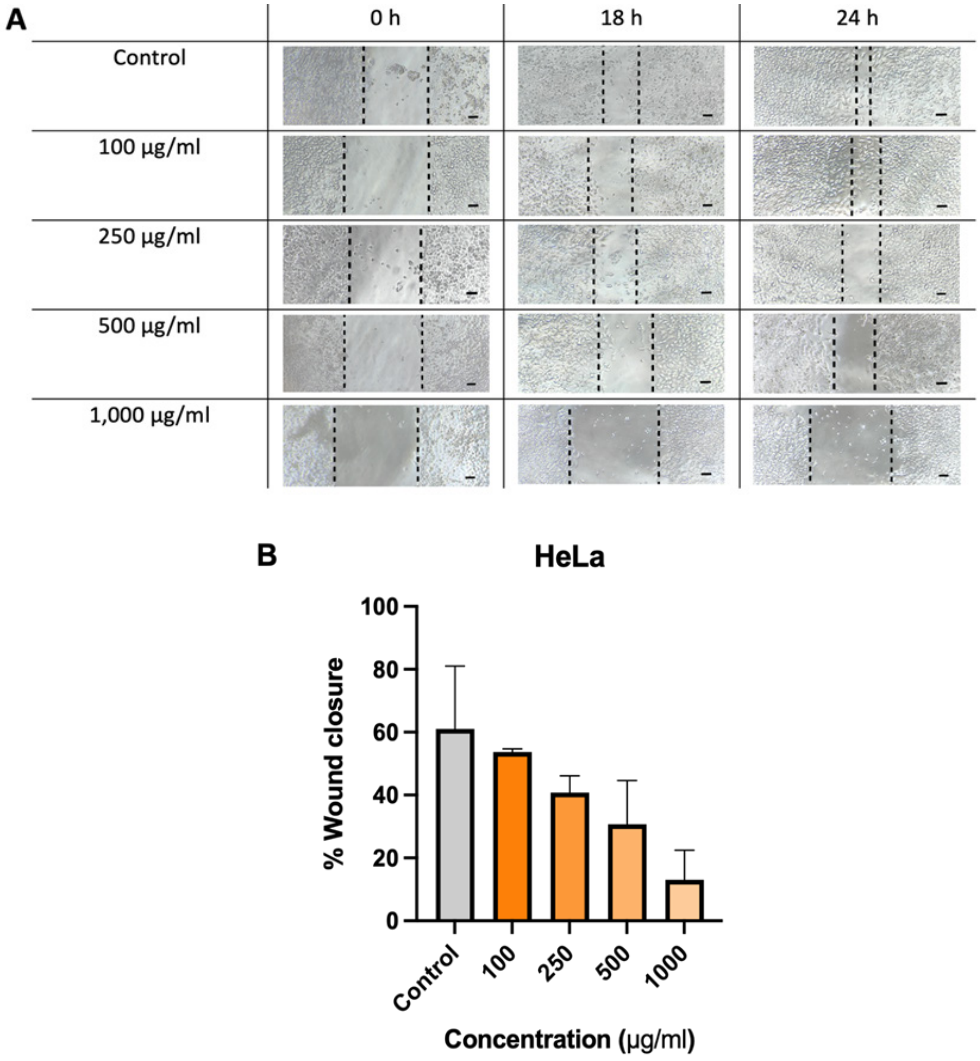

Figure 5. The Inhibitory Effect on Migration of HeLa Cells with RRBE Treatment. Cells were treated with RRBE at concentrations of 100, 250, 500 and 1000 µg/ml. The wound gap was measured at 0, 18 and 24 hours.

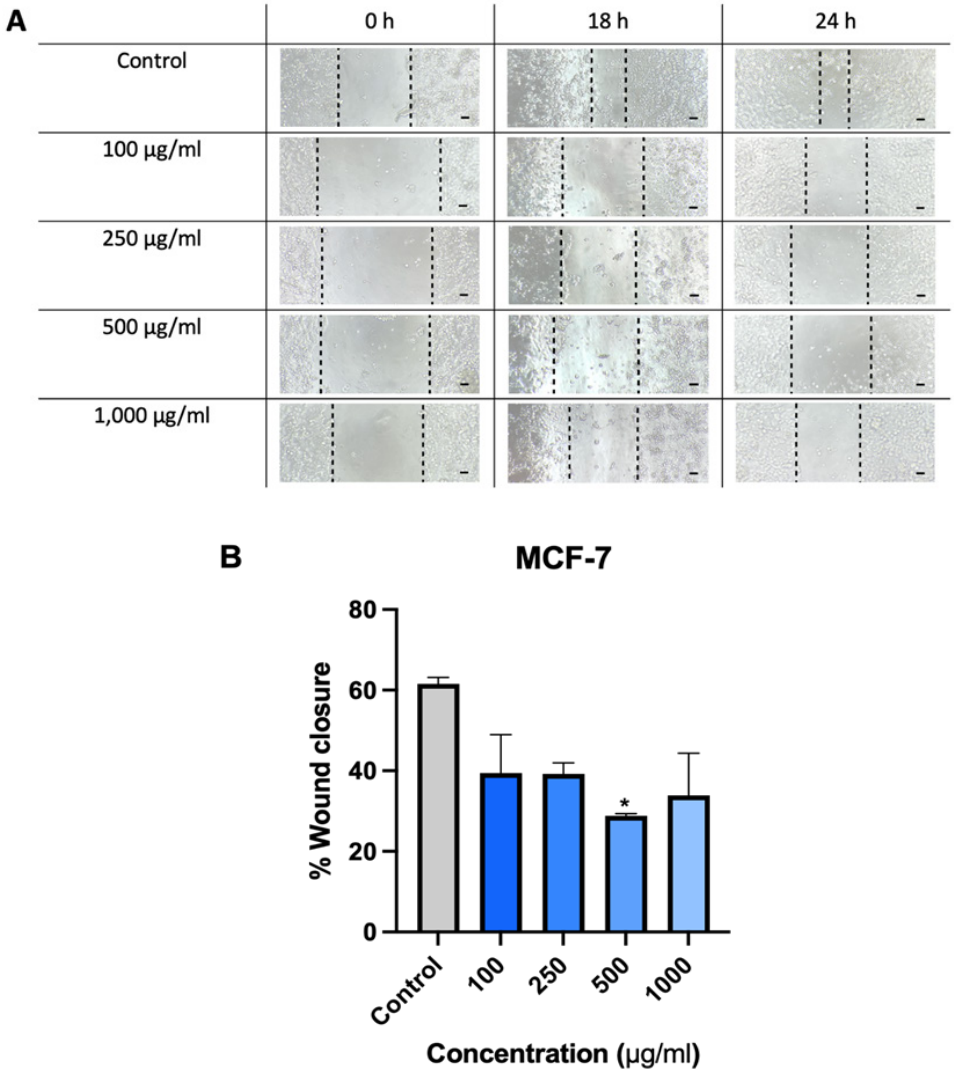

Figure 6. The Inhibitory Effect on Migration of MCF-7 Cells with RRBE Treatment. Cells were treated with RRBE at concentrations of 100, 250, 500 and 1000 µg/ml. The wound gap was measured at 0, 18 and 24 hours. * $P < 0.05$

of HeLa and MCF-7 cells in a concentration-dependent manner. At concentrations of 100, 250, 500, and 1000 µg/mL, the relative proliferation of HeLa cells was 0.96, 0.85, 0.68, and 0.66, respectively (Figure 4A). For the MCF-7 cells at concentrations of 100, 250, 500, and 1000 µg/mL, the relative proliferation was 0.95, 0.88, 0.78, and 0.68, respectively (Figure 4B). Collectively, these results indicate that RRBE effectively inhibits the proliferation of HeLa and MCF-7 cells.

Effects on migration of cervical cancer and breast cancer cells

The inhibitory effects of RRBE on the migration of HeLa and MCF-7 cells were assessed using the wound healing assay. The cells were treated with RRBE at concentrations of 100, 250, 500 and 1,000 µg/mL and then incubated for 24 hours. For the HeLa cells, the wound observed in the control group, which was not treated with RRBE, showed a reduction in the distance between the edges of the wound (Figure 5). The degree of wound closure was 61.08% indicating that the cells continued to migrate into the wound. In the groups treated with RRBE at concentrations of 100, 250, 500 and 1000 µg/mL, the wound closure was 53.72%, 40.78%, 30.71%, and 13.01% respectively. These results suggest that RRBE inhibits the migration of HeLa cells.

The effect of RRBE on the migration of MCF-7 cells was evaluated at concentrations of 100, 250, 500 and 1000 µg/mL. The wound closure rate in the control group was 61.59% (Figure 6). In the groups treated with RRBE, the wound healing rate showed a decrease as the concentration increased, with wound closure of 39.42%, 39.23%, 28.80%, and 33.92% for RRBE at concentrations of 100, 250, 500 and 1000 µg/mL, respectively. These results suggest that RRBE reduces the migration of MCF-7 cells, highlighting its potential as an anti-metastatic agent.

Discussion

This study investigated the effects of red rice bran extract (RRBE) on HeLa and MCF-7 cancer cells. It was found that RRBE exhibited cytotoxicity against cancer cells and inhibited their proliferation, both in HeLa and MCF-7 cells. Moreover, RRBE was shown to inhibit

the migration ability of both types of cancer cells. The inhibition of HeLa and MCF-7 cell migration observed in this study suggests that RRBE extract may possess anti-metastatic potential. These findings are in accordance with recent research (Ming-Hsuan Chen et al. 2012), which reported that the health benefits of rice bran include its anti-oxidative, anti-inflammatory, and anti-cancer properties [17]. The same study also reported that rice bran contains high levels of phytochemicals, for example, total phenolic, flavonoid, and anthocyanin content, and thus has potent pharmaceutical activity [12, 18] Ming-Hsuan Chen also revealed in their study that the extract from red rice bran which was rich in proanthocyanidins content; had the greatest activity against leukemia, cervical cancer, and stomach cancer cells. Similarly, in our own previous research, conducted on HCT116 and HT29 cells, we found that RRBE did not have cytotoxic or mutagenic properties in non-cancerous cell lines but has anti-cancer potential against colorectal cancer cells. Moreover, we revealed that RRBE's antiproliferative activity was due to its induced apoptosis and cell cycle arrest [13]. Previous research by Supranee Upanan et al. [19] revealed the mechanism underlying the proliferative suppression by RRBE. They reported that the anti-cancer activity of the proanthocyanidin-rich fraction obtained from RRBE on HepG2 cancer cells was due to the induction of cell death via apoptosis cascade through the activation of PARP-1, caspase-8 and caspase-3 cleavage. Furthermore, they also discovered that the HepG2 cells could not pass the G2/M phase and stopped growing due to a reduction in cyclin B1 and cdc25 proteins caused by treatment with RRBE [19]. Interestingly, our results are consistent with those previously reported by Patamapan Tamvapee et al. [20], who found that the growth of colorectal LoVo cancer cells was inhibited by apoptosis induction through MAPK signaling pathway and p53 activation after the fatty acid fraction which was isolated from treatment with RRBE. The MAPK signaling pathway is the most important pathway affecting cell proliferation and survival [21, 22], thus suggesting that RRBE might play a role in anti-cancer ability.

Furthermore, our findings aligned with those from previous research on the anti-migration activity of RRBE. In this study, we showed that RRBE not only inhibited cell proliferation, but also attenuated the migration effect in cervical and breast cancer cells. This is consistent with the findings of Nguyen et al. [23], who reported that compounds A6 and A15 from the indole-based benzene sulfonamide group exhibited significant inhibitory effects on the proliferation and migration of breast cancer cells under hypoxic conditions. These effects were mediated through the inhibition of carbonic anhydrase IX (CA IX), an enzyme involved in regulating intracellular pH in cancer cells. Furthermore, a synergistic effect was observed when these compounds were combined with doxorubicin, indicating a specific mechanism of metastasis suppression via the disruption of cellular homeostasis. In addition the study by Lee et al. [24] reported that Arctiin, a lignan glycoside isolated from Arctium lappa L., exhibited significant inhibitory effects on the migration and invasion of cervical cancer cells. The underlying mechanism involves the downregulation of S100A4, a key protein that promotes cancer cell metastasis, as well as the suppression of the PI3K/Akt signaling pathway, which plays a crucial role in regulating cell survival and motility. These findings highlight the potential of Arctiin as an anti-metastatic agent through specific mechanisms targeting cancer cell migration. Collectively, it can be concluded that the of red rice bran extract appears to be a viable candidate for a cancer therapy strategy. However, further research is warranted to elucidate the molecular mechanisms underlying its effects and to evaluate its efficacy and safety through in vivo models.

Author Contribution Statement

K.S.- conception or design of the work, analysis of data, drafting the work, and final approval of the manuscript, P.P. and P.J.- reviewing it critically for important intellectual content or analysis of data, R.P.- designed the research methodology and editing the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgements

General

We appreciate Associate Professor Dr. Prasit Suwannalert from the Faculty of Science, Mahidol University who provided cervical cancer cells (HeLa) and breast cancer cells (MCF-7) for our study. The Red Rice Bran Extract (RRBE) was provided by Assistant Professor Dr. Narongsak Munkong from the Faculty of Medicine, University of Phayao.

Data Availability

The data for this study are available from P.P. upon request.

Ethical Declaration

This study used available cervical cancer cells (HeLa) and breast cancer cells (MCF-7) which were collected from patients who consented the collection of their tissues.

Conflict of Interest

There is no conflict of interest.

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