RESEARCH ARTICLE

Anti-invasive Activity against Cancer Cells of Phytochemicals in Red Jasmine Rice (*Oryza sativa* L.)

Komsak Pintha, Supachai Yodkeeree, Pornsirit Pitchakarn, Pornngarm Limtrakul*

Abstract

Red rice contains pharmacological substances including phenolics, oryzanol, tocotrienol and tocopherol. Recently, red rice extract has been employed as a source of antioxidants for inhibition of tumor growth. This study was carried out to evaluate the anti-invasion effects of red rice extract fractions on cancer cells. It was found that at 100 µg/ml of crude ethanolic extract (CEE), hexane fraction (Hex) and dichloromethane fraction (DCM) could reduce HT1080 and MDA-MB-231 cancer cell invasion. Hex and DCM revealed higher potency levels than CEE, whereas an ethyl acetate fraction (EtOAc) had no effect. Gelatin zymography revealed that Hex decreased the secretion and activity of matrix metalloproteinase-2 and -9 (MMP-2 and-9). In contrast, the DCM fraction exhibited slightly effect on MMPs secretion and had no effect on MMPs activity. Collagenase activity was significantly inhibited by the Hex and DCM fractions. High amounts of γ-oryzanol and γ-tocotrienol were found in the Hex and DCM fractions and demonstrated an anti-invasion property. On the other hand, proanthocyanidin was detected only in the CEE fraction and reduced MDA-MB-231 cells invasion property. These observations suggest that proanthocyanidin, γ-oryzanol and γ-tocotrienol in the red rice fractions might be responsible for the anti invasion activity. The red rice extract may have a potential to serve as a food-derived chemotherapeutic agent for cancer patients.

Keywords: Matrix metalloproteinase - red rice extract - invasion - phytochemicals

Asian Pac J Cancer Prev, **15 (11)**, 4601-4607

Introduction

Excess extracellular matrix (ECM) degradation by proteolytic enzymes is an important step in cancer cells metastasis. The key proteases involved in degrading the components of the ECM are matrix metalloproteinases (MMPs) (Yadav et al., 2014). Therefore, inhibition of ECM degradation was used as the target for anti cancer metastasis.

Many studies have shown that the pigmented rice, such as black and red rice, are the most nutritious of the rice and also a good source of bioactive phytochemicals, including tocopherols, tocotrienols, γ-oryzanol, and phenolic compounds (Zeng et al., 2013). Recently, several studies have shown that γ-oryzanol, γ-tocotrienol and anthocyanidin from pigmented rice exert anti-carcinogenesis and inhibit cancer cell growth (Banjerdpongchai et al., 2013; Zeng et al., 2013; Summart and Chewonarin, 2014). Among those phytochemicals, γ-tocotrienol, protocatechuic, ellagic acid, ferulic acid and chlorogenic acid have proven to exhibit anti invasion in cancer cells (Liu et al., 2010; Lin et al., 2011; Weng and Yen, 2012; Pitchakarn et al., 2013; Tsai et al., 2013;). However, there have been no reports on the anti-invasion activity of red rice extract in human cancer cells. Here, we investigated the anti invasion effect of red rice extract fractions on the HT1080 fibrosacroma and MDA-MB-231 breast human cancer cells.

Materials and Methods

Chemicals

Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin, and trypsin-EDTA were purchased from GIBCO-BRL (Grand Island, NY, USA). Fetal bovine serum (FBS) was purchased from Hyclone (Logan, UT, USA). MTT, gelatin, hydroxybenzoic acid, and catechins were purchased from Sigma Aldrich (St Louis, MO, USA). Matrigel was purchased from Becton Dickinson (Bedford, MA, USA). Protocatechuic acid, chlorogenic acid, p-coumaric acid, vanillic acid, ferulic acid, gallic acid and vitamin E analogs including tocopherol and tocotrienol (α-, β-, γ- and δ-) were purchased from ChromaDex (Irvine,CA,USA), and γ-Oryzanol was purchased from Wako Pure (Osaka, Japan). The solvents for red rice extraction and the mobile

*Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand *For correspondence: pornngarm.d@cmu.ac.th*

phase of HPLC, including hexane, dichloromethane, ethyl aceate, and methanol, were purchase from RCI Labscan (Samutsakorn, Thaliand).

Plant material

Whole grains of red rice (*Oryza sativa* L.) were harvested from Phayao Province, Thailand. A voucher specimen number was certified by the herbarium at the Flora of Thailand, Faculty of Pharmacy, Chiang Mai University (voucher specimen no. 023108) which was kept for future reference.

Preparation of red rice fractions

The air-dried and powdered specimens of red rice grains (1.0 kg) were extracted with 70% ethanol by being shaken at room temperature for 12 h. After evaporation and lyoplilization, the red rice crude ethanolic extract (CEE) was suspended in water and then sequentially extracted with n-hexane, dichloromethane and ethyl aceate to give hexane (Hex), dichloromethane (DCM) and ethyl acetate (EtOAc) fractions, respectively.

Determination of total proanthocyanidin content

Total proanthocyanidin concentration was determined using the vanillin assay (Gunaratne et al., 2013). The red rice extract was mixed with sulfuric acid/methanol solution and 1% vanillin in methanol (w/v). A control mixture of the sample was prepared by adding 100% methanol instead of the vanillin solution to correct the absorbance by the non-vanillin reactive compounds. After incubation for 15 min in a 30°C water bath, the absorbance of the sample and the control mixtures was measured at 490 nm against a reagent blank and their difference was used to determine total proanthocyanidin concentrations of the sample, which was expressed as milligram catechin/gram extract (mg CE/g extract).

HPLC analysis for phenolic, vitamin E analogs and γ-oryzanol in red rice fractions

The phenolic compounds and γ-oryzanol were determined by HPLC using an Inertsil ODS-3-C18 column (250 x 4.6 mm, 5 μm particle diameter, GL Science Inc., Japan). Gradient elution using two solvents; A (0.1% trifluoroacetic acid in water) and B (100% methanol) for detection phenolic compounds (Shao et al., 2014) and isocratic elution (methanol:acetonitrile; 65:35) were used for detection γ-oryzanol (Cho et al., 2012). Ten micro liters of the samples were injected in to the column with a flow rate 1.0 ml/ min. Standard phenolic compounds and γ-oryzanols were monitored at 280 and 325 nm, respectively. The vitamin E analogs were determined by HPLC C30 column (250 × 4.6 mm, 5 μm particle) (Pegg and Amarowicz, 2009) using isocratic elution with methanol:H_2O (93:7) as the mobile phase. An injection volume of 10 μl was used. The standards for the tocopherols and tocotrienols were monitored at 292 nm.

Cell lines and culture conditions

MDA-MB-231 human breast carcinoma cells, HT1080 human fibrosarcroma cells, and NIH3T3 fibroblasts cells were obtained from the American Type Culture Collection (ATCC). The cells were grown in DMEM supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10 % (v/v) of FBS. Cultures were maintained at 37°C in an atmosphere consisting of 5% CO_2. Before the cell treatment, the red rice extract fractions were dissolved in DMSO and were then diluted with the culture medium for the final concentration of DMSO at less than 0.1%.

Cell viability assay

Cytotoxicity of red rice extracts on HT1080 and MDA-MB-231 cells were determined by MTT assay as described previously (Banjerdpongchai et al., 2013). HT1080 and MDA-MB-231 cells were plated at 1.5x103 cells per well in 96-well plates and cultured in DMEM with 10% FBS. After being cultured for 24 h, various concentrations (0-200 μg/ml) of red rice extracts were added and the samples were incubated for 48 h. At the end of the treatment, 15 μl of MTT solution (5 mg/ml) were added and the samples were incubated for 4 h. The MTT formazan was dissolved with DMSO and absorbance was measured using a microplate reader at 540 nm with a reference wavelength of 630 nm.

Cell invasion assay

The invasive behavior of MDA-MB-231 and HT1080 cells was tested using the modified Boyden chamber assay as previous described (Pitchakarn et al., 2013). Polyvinylpyrrolidone-free polycarbonate filters (Millipore, Co Cork, Ireland) (8 μm pore sized) were coated with Matrigel (12 μg/filter). The lower chamber contained serum-free conditioned medium of NIH 3T3 fibroblast cells, which acted as a chemoattractant. MDA-MB-231 or HT1080 cells, at 1.25×10^5 cells, were plated onto the upper chamber with or without the tested compounds and the samples incubated for 24 h at 37°C, under 5% CO_2. After incubation, the non-invading cells were removed from the upper surface of the membrane. The invading cells on the lower surface of the membrane were fixed with methanol for 1 min and stained with toluidine blue for 5 min. The cells that had actively migrated to the underside surface of the filter were dissolved with 20% acetic acid and they were indirectly quantified by measuring the absorbance at 570 nm.

Gelatin zymography assay

The secretions of MMP-2 and MMP-9 of the treated cells were analyzed by gelatin zymography as previously described (Pitchakarn et al., 2013). HT1080 and MDA-MB-231 cells were treated with various concentrations (0-100 μg/ml) of the red rice fractions for 24 h in DMEM serum-free medium, and the culture supernatant was collected in equal amounts of the cells. The culture supernatant was separated by 10% polyacrylamide gels containing 0.1% w/v of gelatin under non-reducing conditions. After electrophoresis, gels were washed twice with 2.5% v/v of Triton X-100 for 30 min at room temperature to remove SDS. The gel was then incubated at 37°C for 18 h in an activating buffer (50 mM Tris-HCl, 200 mM NaCl, 10 mM $CaCl_2$, pH 7.4). Gels were stained with Coomassie Brillant Blue R (0.1%w/v) and destained in 30% methanol, 10% acetic acid. MMPs activity appeared

as a clear band against a blue background. Digestion bands were quantitated by Bio 1D software (Viber Lourmat).

To analyze the effect of red rice extract on MMP-2 and MMP-9 activities, the culture supernatant of HT1080, which contained MMP-2 and MMP-9, was subjected to 10% polyacrylamide gels containing 0.1% w/v of gelatin, as described above. After being washed with Triton X-100, the gel slab was cut into slices corresponding to the lanes and then put in to different tanks containing various concentrations of the red rice extract fractions suspended in activating buffer and were then incubated at 37ºC for 24 h. The strip of gel was stained with Coomassie Brillant Bule R and the MMPs activity was quantitated as described above.

Collagenase activity assay

The proteolytic activity of collagenase was measured using EnzChek Collagenase Assay kit (Molecular Probe). Briefly, 1 U/ml of collagenase was mixed with 10 μg/ml of fluorescein-conjugated gelatin (DQ gelatin) containing various concentrations of the red rice fractions suspended the in reaction buffer and put on 96 well microplates. The rate of proteolysis was determined by measuring the fluorescence intensity at 3 min intervals for 30 min with fluorometer. The fluorescence values were measured at an excitation wavelength of 485 nm and an emission wavelength of 528 nm. Enzyme inhibitory activity was estimated by linear regression of the fluorescent intensity recorded during that time.

Statistical analysis

All experiments were performed in duplicate. Quantifications are defined as mean±SD of three independent experiments and expressed as percentage of the control. Statistically significant differences throughout this study were calculated by Student's t-test. In all cases, a value of $p<0.01$ or $p<0.05$ was considered significant.

Results

Effect of red rice extract fractions on viability of HT1080 and MBA-MB-231 cells

The cytotoxicity of the red rice extract fractions on human cancer cells were evaluated by MTT assay. As shown in Figure 1A and 1B, at high concentrations (100 µg/ml) of the CEE, Hex, DCM and EtOAc fractions for 48 h had no affect on the MDA-MB-231 and HT1080 cell viabilities.

Effect of red rice extract fractions on HT1080 and MDA-MB-231 cell invasion

To investigate the anti-invasive property of red rice fractions on HT1080 and MDA-MB-231 cells, the Boyden chamber assay was used. The results displayed in Figure 2A and 2B demonstrate that the CEE, Hex and DCM fractions at 100 µg/ml significantly reduced the invasion of both HT1080 and MDA-MB-231 cells to 73%, 59%, 66% and 80%, 36%, 34%, respectively. However, the treatment with EtOAc fraction did not affect the cell invasion. Moreover, the invasion of the MDA-MB-231 cells was inhibited with the Hex and DCM fractions in a dose-dependent manner with IC_{50} of 50 and 45 µg/ml, respectively (Figure 2C and 2D).

Effect of red rice active fractions on MMPs secretion in HT1080 and MDA-MB-231 cells

MMP-2 and -9 are the important enzymes involved in ECM degradation and cancer metastasis. To explore

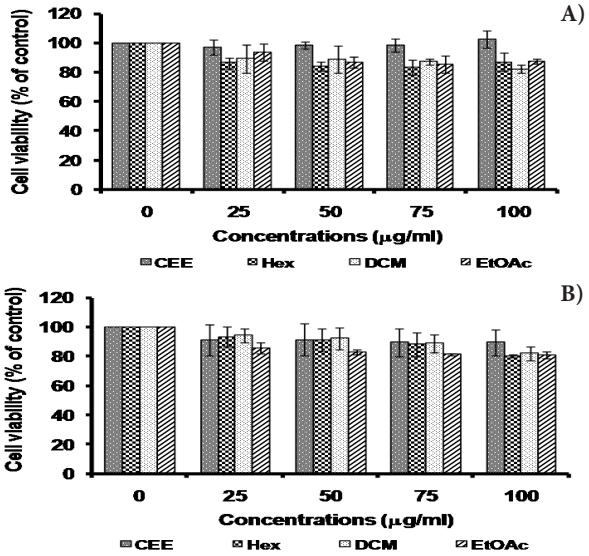

Figure 1. Cytotoxicity of Red Rice Fractions from Oryza Sativa L. on MDA-MB-231. A) and HT-1080 cells **B)**. Each point represents the mean of three independent experiments performed in triplicate

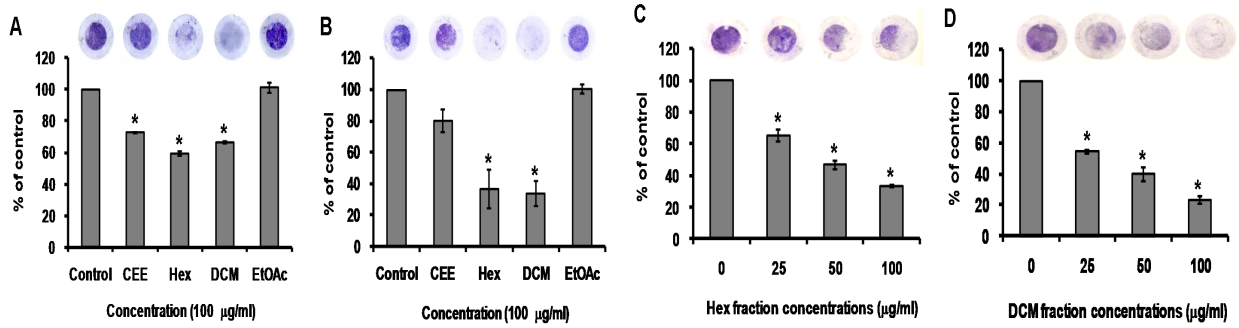

Figure 2. Effect of Red Rice Fractions at 100 µg/ml on HT1080. A) and MDA-MB-231 **B)** cells invasion and the inhibitory effect of various concentrations of the Hex **C)** and DCM **D)** fractions on MDA-MB-231 cell invasion. The cells (1.25×10^5) were seeded on a matrigel-coated filter of the upper chamber, which contained 100 µg/ml or 0-100 µg/ml of the red rice fractions and were incubated for 24 h in 37ºC. The cells that actively migrated to the lower surface of the filters were quantitated, as described in the previous method. The data represent the mean±SD with *$p<0.05$, when compared with the non treated cells

the suppressive potential of the active fractions that was associated with MMPs secretion, the HT1080 and MDA-MB-231 cells were treated with the Hex and DCM red rice fraction. The result from gelatin zymography indicate that treatment of the HT1080 cells with the Hex fraction at 100 μg/ml reduced MMP-2 and MMP-9 levels to 30% and 56%, respectively (Figure 3A). Moreover, DCM at 100 μg/ml also decreased the MMP-9 secretion from the cells to 61%, but had no effect on MMP-2 secretion (Figure 3B). In addition, at 100 μg/ml of the Hex and DMC fractions reduced the MMP-9 secretion from MDA-MB-231 cells to 65 and 70%, respectively when compared with the untreated cells (Figure 3C and 3D).

Inhibition of collagenase and MMPs activities by red rice active fractions

To determine whether red rice active fractions directly inhibit collagenase, MMP-2 and MMP-9 activities, the enzymes were directly incubated with the active fractions. As shown in Figure 4A, the Hex and DCM fractions reduced collagenase activity in a dose dependent manner with IC50 at 65 and 115 μg/ml, respectively. On the other hand, the Hex fraction reduced only the MMP-9 activity, but not the MMP-2 activity (Figure 4B). In contrast, the DCM fraction had no effect on MMPs activity.

Table 1. The Contents of Tocotrienols, Tocopherols and γ-Oryzanol in Red Rice Extract Fractions

Compounds	Fractions			
(mg/g extract)	CEE	Hex	DCM	EtOAc
γ- tocotrienol	1.05±0.42	2.78±0.02	1.97±0.23	nd
α- tocotrienol	nd	0.04±0.04	nd	nd
γ-tocopherol	nd	0.20±0.03	nd	nd
γ-oryzanol	70.82±1.63	127.55±2.57	94.85±6.49	16.13±0.76

*Values are mean±S.D. (n=3), nd not detectable

The contents of tocopherols, tocotrienols and oryzanol in red rice extract fractions and their activities on cancer cells invasion

Tocopherols, tocotrienols and γ-oryzanol in rice bran oil have been demonstrated to possess anti-cancer properties. From the results above, Hex and DCM exhibited anti invasion activity in cancer cells. The content of vitamin E analogs in red rice fractions revealed in Table 1 indicated that the Hex fraction had the highest concentration of γ-tocotrienol, followed by DCM and CEE. In contrast, vitamin E analogs could not be detected in the EtOAc fraction. In addition, tiny amounts α-tocotrienol and γ-tocopherol were only found in the Hex fraction. Gamma-oryzanol is the major sterol in rice bran. In this study, γ-oryzanol was identified in all fractions of red rice. However, the Hex fraction presented the highest amount of this sterol, followed by DCM, CEE and trace amounts were found in EtOAc. Next, we evaluated the anti-invasion of γ-oryzanol and γ-tocotrienol, which possessed the major phytochemical content in the non-

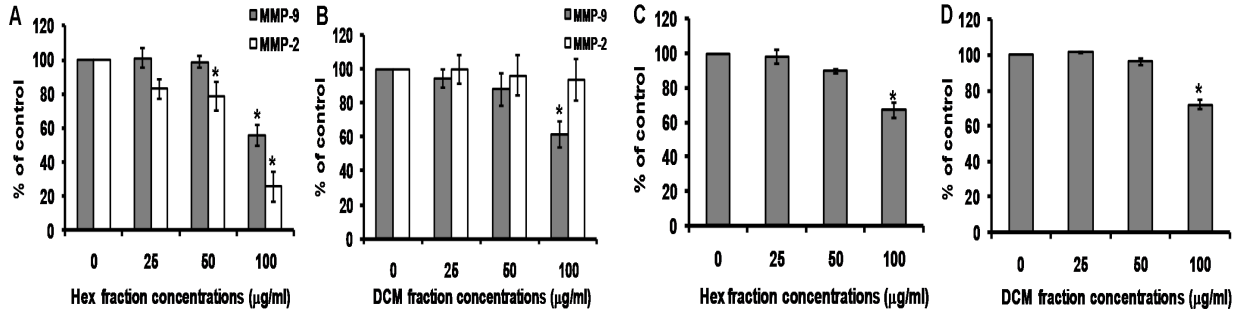

Figure 3. Effect of the Hex and DCM Fractions on MMPs Secretion from HT-1080 Cells (MMP-2&-9) (A, B) and MDA-MB-231 Cells (MMP-9) (C, D). A sub-confluent of the cancer cells was incubated in the absence and presence of various concentrations (0-100 μg/ml) of the Hex and DCM fractions in a serum-free medium for 24 h. Equal amounts of proteins were loaded and the gelatinolytic activity of MMPs in the conditioned medium was determined by gelatin zymography. The band intensities of MMPs were quantitated by densitometry. The data represent the mean±SD with *P<0.01, when compared with the non treated cells

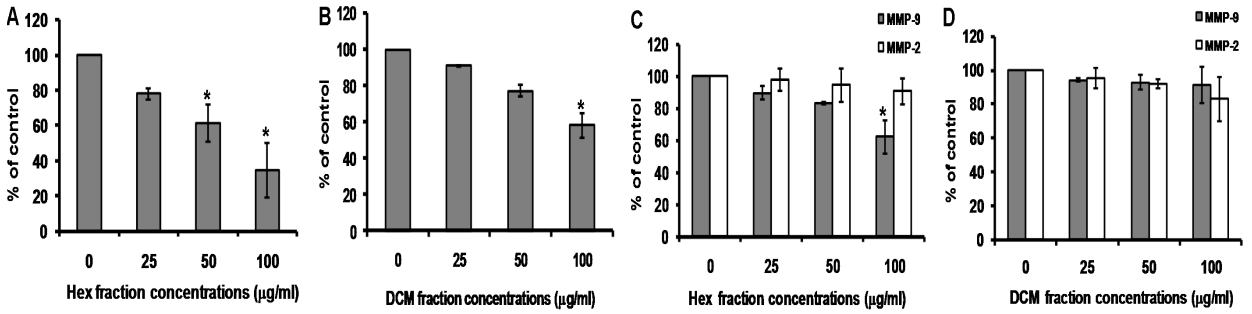

Figure 4. Effect of the Hex and DCM Fractions on Collagenase Activity (A,B) and MMPs activity (C,D). The inhibitory effect of various concentrations (0-100 μg/ml) of the Hex and DCM fractions on the proteolytic activity of collagenase was measured using a gelatin fluorescent substrate. To determine the effect of the red rice fractions on MMP-2 and MMP-9 activity, equal amounts of HT1080 cell condition media were subjected to electophoresis in PAGE containing gelatin. The Hex and DCM fractions (0-100 μg/ml) were directly solubilized in activation buffer. The gel slab was cut into slices corresponding to the lanes and then put in different tanks containing different concentrations of the fraction and then incubated 37°C for 24 h. The band intensities of MMPs were quantitated by densitometry. The data represent the mean ±SD with *p<0.01, when compared with non treated cells

polar fraction. Our result show that γ-oryzanol at 50 μg/ml and γ-tocotrienol at 10 μg/ml reduced MDA-MB-231 invasion to 38 and 50 % of the control, respectively (Figure 5A).

The contents of phenolic compounds and proanthocyanindin in red rice extract fractions and their activities on cancer cells invasion

Phenolic compounds have been reported to inhibit invasion of cancer cells. The results from Table 2 indicated that EtOAc had high content of protocatechuic acid and catechins, followed by ferulic acid and vanillic acid. The DCM fraction contained high content of catechins, vanillic acid and ferulic acid and low amount of chlorogenic acid and protocatechuic acid. In contrast, low concentrations of these phenolic compounds were detected in the CEE and Hex fractions.

Next, we determined the anti-invasion properties of the phenolic compounds represented in the red rice extracts. The results showed that 50 μg/ml of ferulic acid and vanillic acid, but not protocatechuic acid, coumaric acid and catechins could reduced MDA-MB-231 cell invasion to 60 and 65% (Figure 5B), respectively. Although, vanillic and ferulic acid were detected in the EtOAc fraction, 1 μg/ml of vanillic acid and ferulic acid, which were present in 100 μg/ml of the EtOAc fraction, were not sufficient to promote anti-invasion (Figure 5C).

On the other hand, high amounts of proanthocyanidin were only observed in the CEE fraction, as shown in Table 2. In contrast, the level of proanthocyanidin in the Hex, DCM and EtOAc fractions could not be detected. Our findings show that standard proanthocyanidin derived from grape seed extract could reduce cancer cell invasion in a dose dependent manner, for which 5 μg/ml of proanthocyanindin reduced cancer invasion to 70% of the control (Figure 5D).

Table 2. The Contents of Phenolic Compounds and Proanthocyanidin in Red Rice Extract Fractions

Phenolic compounds	Fractions			
(mg/g extract)	CEE	Hex	DCM	EtOAc
Photocatecheuic	0.45±0.011	0.17±0.01	0.64±0.06	56.68±7.85
Catechin	0.48±0.16	0.49±0.23	21.22±0.36	65.75±4.33
Chlorogenic	1.18±0.141	0.40±0.09	0.15±0.003	0.91±0.64
Vanillic	0.14±0.003	0.09±0.01	4.57±0.14	12.18±2.07
Coumaric	0.03±0.001	nd	0.11±0.07	7.76±0.63
Ferulic	0.12±0.006	nd	1.48±0.18	13.73±2.38
Proanthocyanidin	59.56±5.08	nd	nd	nd

*Values are mean±S.D. (n=3), nd not detectable

Discussion

Several studies have shown that pigmented rice extracts exhibit a number of beneficial bioactivities. Specifically, red rice extract has exhibited potent antioxidative, anti-inflammatory, and anti-cancer cell proliferation. The biological properties of the red rice extract were attributed, in part, to their phytochemical content, mainly found in phenolics, flavonoids, vitamin E analogs and γ-oryzanol (Tammasakchai et al., 2012; Gunaratne et al., 2013; Niu et al., 2013). To date, there is no evidence to show that red rice extract exerts an effect on cancer cell metastasis. Here, we investigated the anti-metastasis potential of red rice extract fractions on human cancer cells and identified the bioactive constituents in their fractions.

The metastatic progression of malignant tumors requires the proteolytic degradation of ECM components in the basement membrane, which allowed cancer cells to invade the blood or the lymphatic vessel and travel to other tissues or organs. In this study report for the first time that the CEE, Hex and DCM fractions of red rice extract reduced HT1080 and MDA-MB-231 human cancer cell invasion, for which the DCM and Hex fractions showed a higher potential than the CEE fractions.

Considerable evidence has accumulated which suggests that the inhibition of MMPs expression or enzyme activity can be used as an early target in preventing cancer metastasis.

Our results from the gelatin zymography showed that the Hex fraction had the highest potential to reduce the secretion of MMP-2 and-9 from HT1080 cells. However, the cells that were treated with the DCM fraction significantly decreased only the MMP-9 secretion, but not that of the MMP-2. On the other hand, the secretions of MMP-9 from MDA-MB-231 cells were reduced by Hex and DCM fractions. Moreover, the effects of red rice extract fractions on MMP-2 and-9 activity were examined by gelatin zymogaphy. The Hex fraction reduced MMP-9 activity, but not MMP-2. In addition, the Hex fraction strongly inhibited collagenase activity better than the DCM fraction. From these results, it is suggested that the Hex and DCM fractions reduced cancer cell invasion, at least partly in the inhibition of the ECM degradation enzymes.

Red rice contains a variety of bioactive components with chemopreventive and chemotherapeutic activities, including phenolic compounds, γ-oryzanol, tocopherols and tocotrienols (Henderson et al., 2012; Tammasakchai

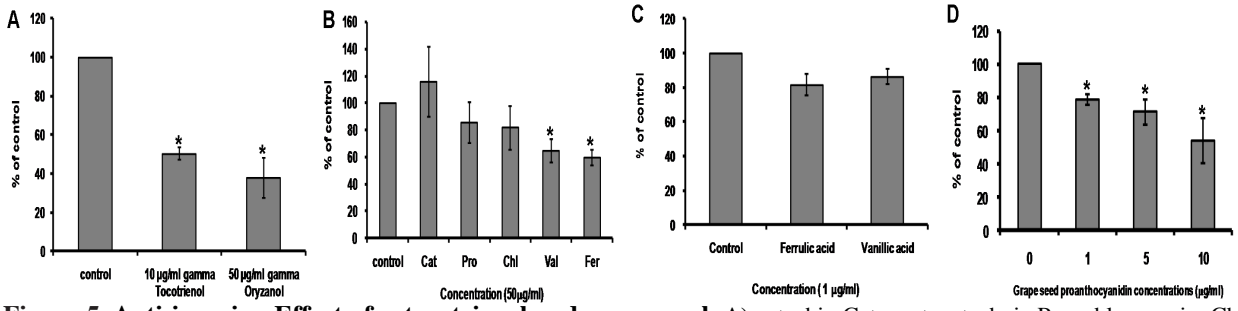

Figure 5. Anti-invasion Effect of γ-tocotrienol and γ-oryzanol. A) catechin;Cat, protocatechuic;Pro, chlorogenic; Chl, vanillic;Val and ferulic; Fer **B)**, 1 μg/ml of ferulic and vanillic **C)** and grape seed proanthocyanidin on MDA-MB-231 cell invasion **D)**. The data represent the mean ±SD with *p<0.01

et al., 2012).

The contents of γ-oryzanol and γ-tocotrienol in red rice extract fractions appeared highest in the Hex fraction, followed by the DCM fraction, while fewer amounts or none at all were detected in the EtOAc fraction. Specifically, γ-oryzanol is the major component contained in the Hex and DCM fractions. Gamma oryzanol is a mixture of phytosteryl ferulates found in rice bran. It is particularly important to note that γ-oryzanol exhibits antioxidant, anti-carcinogenesis and anti-inflammatory properties (Henderson et al., 2012; Klongpityapong et al., 2013). Here, we report for the first time that γ-oryzanol reduced MDA-MB-231 cell invasion, which corresponded with the finding that Hex and DCM fractions reduced cancer cell invasion. On the other hand, several studies have reported that γ-tocotrienol showed anti-tumor effects, as well as the potential to induce apoptosis, and to inhibit angiogenesis and metastasis (Henderson et al., 2012; Manu et al., 2012). Our results confirm that at 10 μg/ml of γ-tocotrienol reduced MDA-MB-231 cell invasion. This result is consistent with the findings of Liu et al in 2010. They reported that γ-tocotrienol reduced SGC-7901 gastric adenocarcinoma invasion via a down regulation of MMP-2 and -9 expressions (Liu et al., 2010). These data collectively support the acknowledgement of the inhibitory effect of the Hex and DCM fractions on cancer invasion, which might be the effect of γ-oryzanol and γ-tocotrienol.

Our data indicates that catechins are the major phenolic found in the EtOAc and DCM fractions. Moreover, the EtOAc fraction contained high concentrations of photocatecheuic acid, and the minor phenolic constituents were vanillic acid, ferulic acid and coumaric acid. In this report, we demonstrated that vanillic acid and ferulic acid at a concentration of 50 μg/ml, reduced MDA-MB-231 cell invasion. Whereas, the other phenolic compounds in the EtOAc fraction could not reduce cancer cell invasion. These results are similar to those of several studies, which have reported on the anti-metastasis property of vanillic and ferulic acid (Lirdprapamongkol et al., 2009; Tsai et al., 2013). However, the concentration of the vanillic acid and ferulic acid were present in 100 μg/ml of the EtOAc fraction did not to be sufficient doses to promote anti-invasion.

Proanthocyanindin is a class of polymeric phenolic compound consisting mainly of catechin, epicatechin, gallocatechin, and epigallocatechin units. The degree of polymerization and galloylation affects their bioactivity and proanthocyanidin profiles differently depending on the food sources (Akagi et al., 2011; Praphasawat et al., 2011; Feng et al., 2014). Our results showed that proanthocyanidin was detected only in the CEE fraction, but not in the Hex and DCM fractions. We also demonstrated that proanthocyanidin reduced cancer cell invasion, which was similar to previous findings which have stated that proanthocyanidins derived from various kinds of medicinal plants, including grape seeds, have been known to exert an anti-metastaisis effect on cancer cells (Sun et al., 2011). Thus, it is likely that the CEE fraction reduced cancer cell invasion, which is part of the proanthocyanidin.

In conclusion, our study demonstrated that the Hex and DCM fractions of red rice extract highly exhibited anti-invasion potential via the inhibition of MMPs secretion and activity which could be attributed to the high content of γ-oryzanol and γ-tocotrienol. On the other hand, the anti-invasion activity of CEE fraction may be partly due to the effect of the proanthocyanidin. This suggested that red rice extract containing γ-oryzanol, γ-tocotrienol and proanthocyanidin possess anti-invasion potential in cancer cells and might be used as a food supplement for cancer patients.

Acknowledgements

This work was supported by the Agricultural Research Development Agency (Public Organization) (ARDA) and the Faculty of Medicine, Chiang Mai University, Thailand.

References

Akagi T, Katayama-Ikegami A,Yonemori K (2011). Proanthocyanidin biosynthesis of persimmon (Diospyros kaki Thunb.) fruit. *Scientia Horticulturae*, **130**, 373-80.

Banjerdpongchai R, Wudtiwai B,Sringarm K (2013). Cytotoxic and apoptotic-inducing effects of purple rice extracts and chemotherapeutic drugs on human cancer cell lines. *Asian Pac J Cancer Prev*, **14**, 6541-8.

Cho JY, Lee HJ, Kim GA, et al (2012). Quantitative analyses of individual gamma-Oryzanol (Steryl Ferulates) in conventional and organic brown rice (*Oryza sativa* L.). *J Cereal Science*, **55**, 337-43.

Feng LL, Liu BX, Zhong JY, et al (2014). Effect of grape procyanidins on tumor angiogenesis in liver cancer xenograft models. *Asian Pac J Cancer Prev*, **15**, 737-41.

Gunaratne A, Wu K, Li D, et al (2013). Antioxidant activity and nutritional quality of traditional red-grained rice varieties containing proanthocyanidins. *Food Chem*, **138**, 1153-61.

Henderson AJ, Ollila CA, Kumar A, et al (2012). Chemopreventive properties of dietary rice bran: current status and future prospects. *Adv Nutr*, **3**, 643-53.

Klongpityapong P, Supabphol R, Supabphol A (2013). Antioxidant effects of gamma-oryzanol on human prostate cancer cells. *Asian Pac J Cancer Prev*, **14**, 5421-5.

Lin HH, Chen JH, Chou FP,Wang CJ (2011). Protocatechuic acid inhibits cancer cell metastasis involving the down-regulation of Ras/Akt/NF-kappaB pathway and MMP-2 production by targeting RhoB activation. *Br J Pharmacol*, **162**, 237-54.

Lirdprapamongkol K, Kramb JP, Suthiphongchai T, et al (2009). Vanillin Suppresses Metastatic Potential of Human Cancer Cells through PI3K Inhibition and Decreases Angiogenesis *in vivo*. *J Agric Food Chemistry*, **57**, 3055-63.

Liu HK, Wang Q, Li Y, et al (2010). Inhibitory effects of gamma-tocotrienol on invasion and metastasis of human gastric adenocarcinoma SGC-7901 cells. *J Nutr Biochem*, **21**, 206-13.

Manu KA, Shanmugam MK, Ramachandran L, et al (2012). First evidence that gamma-tocotrienol inhibits the growth of human gastric cancer and chemosensitizes it to capecitabine in a xenograft mouse model through the modulation of NF-kappaB pathway. *Clin Cancer Res*, **18**, 2220-9.

Niu YG, Gao BY, Slavin M, et al (2013). Phytochemical compositions, and antioxidant and anti-inflammatory properties of twenty-two red rice samples grown in Zhejiang. *Lwt-Food Science and Technology*, **54**, 521-7.

Pegg RB, Amarowicz R (2009). Content of tocopherol isomers

in oilseed radish cultivars - a short report. *Pol J Food Nutr Sci*, **59**, 129-33.

Pitchakarn P, Chewonarin T, Ogawa K, et al (2013). Ellagic acid inhibits migration and invasion by prostate cancer cell lines. *Asian Pac J Cancer Prev*, **14**, 2859-63.

Praphasawat R, Klungsupya P, Muangman T, et al (2011). Antimutagenicity and antioxidative DNA damage properties of oligomeric proanthocyanidins from Thai grape seeds in TK6 cells. *Asian Pac J Cancer Prev*, **12**, 1317-21.

Shao YF, Xu FF, Sun X, et al (2014). Phenolic acids, anthocyanins, and antioxidant capacity in rice (*Oryza sativa* L.) grains at four stages of development after flowering. *Food Chemistry*, **143**, 90-6.

Summart R, Chewonarin T (2014). Purple rice extract supplemented diet reduces DMH- induced aberrant crypt foci in the rat colon by inhibition of bacterial beta-glucuronidase. *Asian Pac J Cancer Prev*, **15**, 749-55.

Sun Q, Prasad R, Rosenthal E, Katiyar SK (2011). Grape seed proanthocyanidins inhibit the invasive potential of head and neck cutaneous squamous cell carcinoma cells by targeting EGFR expression and epithelial-to-mesenchymal transition. *BMC Complement Altern Med*, **11**, 134.

Tammasakchai A, Reungpatthanaphong S, Chaiyasut C, et al (2012). Red strain oryza sativa-unpolished thai rice prevents oxidative stress and colorectal aberrant crypt foci formation in rats. *Asian Pac J Cancer Prev*, **13**, 1929-33.

Tsai CM, Yen GC, Sun FM, et al (2013). Assessment of the anti-invasion potential and mechanism of select cinnamic acid derivatives on human lung adenocarcinoma cells. *Mol Pharm*, **10**, 1890-900.

Weng CJ, Yen GC (2012). Chemopreventive effects of dietary phytochemicals against cancer invasion and metastasis: phenolic acids, monophenol, polyphenol, and their derivatives. *Cancer Treat Rev*, **38**, 76-87.

Yadav L, Puri N, Rastogi V, et al (2014). Matrix metalloproteinases and cancer - roles in threat and therapy. *Asian Pac J Cancer Prev*, **15**, 1085-91.

Zeng YW, Yang JZ, Pu XY, et al (2013). Strategies of functional food for cancer prevention in human beings. *Asian Pac J Cancer Prev*, **14**, 1585-92.