JOURNAL OF HEALTH SCIENCE AND ALTERNATIVE MEDICINE

Vol.04 Issue 02 MAY-AUG 2022

ISSN 2673-0294 (online)

J Health Sci Altern Med (2022)
DOI: 10.14456/jhsam.2022.2

Journal of
Health Science and Alternative Medicine

Original Article Open Access

Effects of the local purple sticky rice bran extract on antioxidant activity and calcium oxalate crystal formation and aggregation *in vitro*

Witoon Khawsuk, Tistaya Semangeon, Parinyaporn Nuurai*

[1]*Faculty of Allied Health Sciences, Burapha University, Chonburi, Thailand, 20131*

Received June 6, 2022
Accepted July 1, 2022
Published August 30, 2022

*Corresponding author**:** Parinyaporn Nuurai, Faculty of Allied Health Sciences, Burapha University, Chonburi, Thailand

E-mail: parinyaporn@go.buu.ac.th

ABSTRACT

Introduction: Kidney stone disease is a common urological disorder affecting human health. Inhibition of calcium oxalate (CaOx) formation and aggregation can prevent the recurrence of kidney stones. Pigmented rice has been studied for various functions including antioxidant activity, anti-inflammation, antidiabetic, anticancer, and antiaging. However, the function of purple rice bran on the antioxidant activity related to the kidney stone disease is limited.

Objective: To investigate the effect of the purple sticky rice bran (PSB) extract obtained from the local strain at Songkhla province on its antioxidant activity and subsequent *in vitro* inhibition of CaOx crystal formation and aggregation.

Methodology: The PSB ethanol extract was prepared at different concentrations (0.025–1.4 mg/mL). The levels of anthocyanin and total phenolic compounds were analyzed using pH differential and the Folin–Ciocalteu reagent methods. The antioxidant activity of PSB extract was determined using the DPPH assay and FRAP. The formed CaOx crystals were incubated with the PSB extract at different concentrations (20 - 400 µg/mL). The number of CaOx crystals and their aggregation forms were determined and compared with the control.

Results: The levels of anthocyanin and total phenolic compounds in PSB extract were 18.67 ± 1.50 µg/g and 34.70 ± 1.64 mg gallic acid equivalent/g of rice bran, respectively. The DPPH free radical scavenging antioxidant activity and ferric-reducing antioxidant power increased after the concentration of the PSB extract was increased. The IC50 of ascorbic acid and PSB extract were 0.016 and 0.07 mg/ml, respectively. The PSB extract significantly decreased the formation and the number of CaOx crystals. Moreover, the high concentration of PSB extract augmented the calcium oxalate dihydrate (COD) crystal formation rather than the monohydrate crystal formation. Furthermore, it inhibited the CaOx crystal aggregation in a dose-dependent manner.

Conclusion: PSB extract contained substances with high antioxidative activity and could suppress the CaOx crystal formation and aggregation. This study can be helpful to the researchers in the development of strategies for the prevention of kidney stones**.**

Keywords: *purple sticky rice bran; antioxidant activity; calcium oxalate; crystal aggregation*

Introduction

Kidney stone or nephrolithiasis is a global disease caused by an etiology of multifactorial genetic and environmental factors [1]. The most commonly found kidney stones are calcium-containing, particularly the calcium oxalate (CaOx) stones, which account for ~70%–75% of urinary stones [2]. Calcium oxalate monohydrate (COM) crystals are the core crystalline crystals found in urinary stones; however, calcium oxalate dihydrate (COD) crystals exist in smaller quantities [3]. COM crystals have been previously reported to be more harmful to renal epithelial cells than COD crystals [4]. The pathogenesis mechanism underlying the crystallization of the calcium oxalate ions in the renal tubules is a complex process, followed by the growth and aggregation to a size that can obstruct the tubular lumen [5]. The crystal adhesion to the renal epithelial cells can induce toxicity and several changes in protein expression, which play an essential role in various biological processes [6-7]. The retention of these crystals within the kidney or renal-collecting system can cause severe pain, nausea,

vomiting, fever, and blood in the urine. Small renal stones are usually treated with medications that can cause several side effects. Traditionally, many natural plants have been used to treat urinary stones and were found to be effective. Several reports have shown that dietary plants can inhibit and prevent kidney stone pathology both *in vitro* and *in vivo* [8-9]. Green tea, raspberry, parsley, pomegranate, yellow-fruit nightshade, *Hibiscus sabdariffa* (roselle), *Origanum vulgare* (oregano), *Herniaria hirsuta* L., and *Phyllantus niruri* L., have received considerable attention based on their scientific analyses [8-9]. These medicinal plants are helpful for the treatment of nephrolithiasis due to their exceptional properties like diuretic with antioxidant activity and exhibit inhibitory effects on crystallization, nucleation, and crystal aggregation. Furthermore, they contain phytochemicals, such as catechin, epicatechin, diosmin, rutin, quercetin, epigallocatechin-3-gallate, hyperoside, and curcumin [8].

Pigmented rice with purple, red, or black bran layer have been widely cultivated and consumed in Asia for a very long time [10]. The bran layers are rich sources of phytochemicals and antioxidants (e.g., tocopherols, tocotrienols, ɣ-oryzanol, vitamin B, and inositol) and raw materials for others (e.g., rice oil, ferulic acid, and phenolic compounds) [11-12]. These natural color substances belong to the flavonoid family, especially anthocyanins, which benefit human health. Typically, anthocyanin in colored rice is known as acetylated procyanidins, showing a free radical scavenging activity [13]. They have various bioactive capabilities, including antioxidant activity, anti-inflammatory, antiviral, antidiabetic, anticancer, and antiaging effects. However, only little is known about their efficacy in kidney stone disease. Interestingly, Semangoen et al. reported that the Sang-Yod sticky rice extract decreased the pathological effects in the kidneys of an experimental model of ethylene glycol-induced nephrotoxicity in rats, including tubular dilation, shrinkage of the glomerulus, and the flattened renal tubular cells. Ethylene glycol can cause toxicity in the kidneys via the formation of CaOx crystals in humans. The expression of antioxidant enzymes, superoxide dismutase, and catalase were increased in the Sang-Yod sticky rice extract-treated group [14]. Furthermore, a study on the black-purple Riceberry extract showed significant inhibition of the growth and aggregation of CaOx crystals, especially the COM crystals, *in vitro* [15]. Additionally, rice bran therapy can prevent urinary stone disease in hypercalciuria patients [16]. This study aims to investigate the antioxidant activity of the PSB extract and its role in the CaOx crystal formation and aggregation *in vitro*. These findings may be helpful in the treatment of urolithiasis and could lead to the development of safe products.

Methodology

Preparation of the PSB extract

The 400 grams of the PSB was ground to powder. The 800 ml of 75% ethanol was added. The mixture was then incubated at 37°C for 24 hours with gentle shaking. The extracted solutions were collected and filtered. The sample was re-extracted once and then evaporated in oven at 60°C for a few days. Thereafter, the sample was dried in lyophilizer and kept at -20°C. The percentage of extraction yield was calculated as follows: Weight of dry extracted powder/Weight of raw material x 100.

Determination of total monomeric anthocyanin

Total monomeric anthocyanin was determined using pH differential method according Semangoen et al. [14] protocol. After determination of appropriate dilution factor, the test sample was prepared in two dilutions with different pH buffer, one with 0.025M Potassium Chloride pH 1.0 buffer and the other with 0.4M Sodium acetate pH 4.5 buffer. After incubation at 20 min, the absorbance was analyzed at 510 and 700 nm. The concentration of total monomeric anthocyanin was calculated and determined as cyanidin-3-glucoside equivalents.

Determination of total phenolic compound

Total phenolic compound was analyzed by Follin Ciocalteu reagent method following Khawsuk et al. [15] protocol with some modification. Briefly, the gallic acid standard solutions were prepared at the concentration of 0.04, 0.08, 0.12, 0.16, 0.20, 0.24, and 0.28 mg/ml. The PSB extract solutions were diluted at the concentration of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mg/ml. Each sample was mixed with Follin Ciocalteu reagent with gentle agitation for 1 min. After that, the 5% Sodium carbonate solution was added to each sample. The mixture was incubated for 60 minutes at room temperature in the dark room. The absorbance was then determined at 760 nm with spectrophotometer. The equation of gallic acid standard curve was plot and determined. The linear curve of total phenolic compound of PSB extract was calculated from the equation of gallic acid standard. Total phenolic compound was determined as mg gallic acid equivalents (GAE) per gram of rice bran.

1,1-Diphenyl-2-picryl-hydrazyl radical (DPPH) scavenging activity

The antioxidant activity of PSB extract was determined by DPPH scavenging method following Jun et al. [17] with some modification. Firstly, the standard solutions of ascorbic acid were prepared at the concentration of 0.01, 0.02, 0.03, 0.04, 0.05, and 0.06 mg/ml. The PSB extract solutions were diluted at the concentration of 0.025, 0.05, 0.1, 0.2, and 0.3 mg/ml. Each sample was then mixed with 0.2mM DPPH solution in 40% acetone and incubated for 30 minutes at room temperature in the dark room. At 517 nm

absorbance was measured by spectrophotometer. The reaction was calculated as follow: the percentages of scavenging activity = [(absorbance$_{517\ nm\ of\ control}$ - absorbance$_{517\ nm\ of\ sample}$)/ absorbance$_{517\ nm\ of\ control}$] x 100. The half maximal inhibitory concentration (IC_{50}) of both ascorbic acid and PSB extract were also determined

Ferric reducing antioxidant power (FRAP) assay

Ferric reducing power of PSB extract was determined by the method following Vijayalakshmi and Ruckmani [18] with some modification. Briefly, the standard solutions of ascorbic acid were prepared at the concentration of 0.02, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, and 0.18 mg/ml. The PSB extract solutions were diluted at the concentration of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mg/ml. Each sample was then mixed with 0.2M Sodium phosphate buffer, pH 6.6 and 1% (v/v) Potassium ferricyanide. The reaction was incubated for 20 min at 50 ºC. After that, a 10% (v/v) trichloroacetic acid was mixed to the reaction and centrifuged at 2,200 g for 5 minutes. The supernatant was collected and incubated with deionized water, and 0.1% Ferric chloride. At 700 nm absorbance was then measured by spectrophotometer. The equation of ascorbic acid standard curve was plot and determined. The linear curve of Ferric reducing power of PSB extract was calculated from the equation of ascorbic acid standard. The Ferric reducing power of PSB extract was expressed as mg of ascorbic equivalents.

In vitro experimental design

The effect of PSB extract on CaOX crystal formation

The CaOX crystal was performed by the method following Thongboonkerd et al. [7]. Firstly, 10 mM calcium chloride and 10 mM sodium oxalate were added to the 24 well plate. The final concentration of 5 mM calcium chloride and 0.5 mM sodium oxalate were performed by placing them with 10 mM Tris buffer containing 90 mM sodium chloride (pH 7.3). The PSB extract solutions were diluted at the concentration of 20, 50, 100, 200, and 400 µg/ml and then added in to the well. The solution without the PSB extract was used as a control group. The reaction was incubated at room temperature overnight and then observed under high power field (40X) of a phase contrast microscope. The characteristic of CaOX crystal was observed into two hydrate forms including a hexagonal shape of COM and a tetragonal bipyramidal shape of COD [19]. The calculated number of CaOX crystals were represent as COM and COD crystal formation which random counted from 10 areas/sample.

The effect of PSB extract on CaOX crystal aggregation

The CaOX crystal was performed by the method following Khawsuk et al. [15] protocols. Firstly, 10 mM calcium chloride and 10 mM sodium oxalate were added to the 24 well plate. The final concentration of 5 mM calcium chloride and 1 mM sodium oxalate were performed by placing them with artificial urine ($MgSO_4$-$7H_2O$ and KCl) pH 6.5. The PSB extract solutions were diluted at the concentration of 20, 50, 100, 200, and 400 µg/ml and then added in to the well. The solution without the PSB extract was used as a control group. The reaction was incubated at room temperature overnight and then observed under high power field (40X) of a phase contrast microscope. The CaOX crystal aggregation were represented as COM agglomerates which random counted from 10 areas/sample. The percentage of CaOX crystal aggregation inhibition was calculated using the formula: [(number of COM agglomerates of control - number of COM agglomerates of sample)/number of COM agglomerates of control] x 100.

Statistical analysis

The mean and standard deviation ($\bar{x} + SD$) were calculated from the relevant data in triplicate experiments. Statistical significance was evaluated by Turkey's Post-Hoc test using One-Way Analysis of Variance (ANOVA) procedure (IBM SPSS statistics version.22). A significant difference was considered if $p<0.05$.

Results

1. Yield and total monomeric anthocyanin of PSB extract

The extraction yield of PSB extract was 19.33% of dry weight. The amount of total monomeric anthocyanin was 200.5 µg/ml. In addition, the calculated amount of total monomeric anthocyanin was 18.67+1.50 µg/g of rice bran.

2. Total phenolic compound

The total phenolic compound of PSB extracts at the concentration of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mg/ml were 0.035+0.002, 0.08+0.003, 0.117+0.008, 0.152+0.005, and 0.191+0.003 mg gallic acid, respectively (Figure 1).

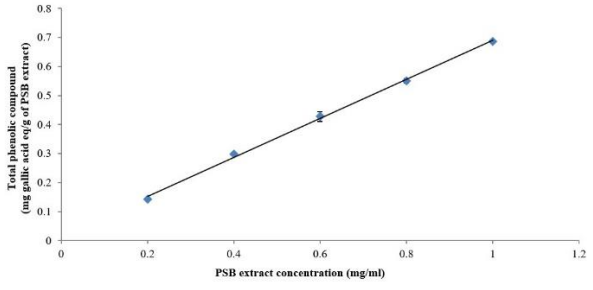

Figure 1 The total phenolic compound of PSB extract at various concentrations calculated from the equation of gallic standard

The amount of total phenolic compound was 189.6+8.99 mg gallic acid per gram of PSB extract. In addition, the calculated amount of total phenolic compound was 34.70+1.64 mg gallic acid per gram of rice bran.

3. Antioxidant activity

The DPPH free radical scavenging capability of ascorbic acid standard at the concentration of 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 mg/ml were 27.22+3.62, 65.54+3.81, 73.99+2.37, 89.76+2.85, 91.44+0.77, and 92.16+0.43, respectively whereas the DPPH free radical scavenging capability of PSB extracts at the concentration of 0.025, 0.05, 0.1, 0.2, and 0.3 mg/ml were 23.64+1.53, 36.90+2.23, 63.97+1.56, 77.83+1.23, and 78.91+2.58, respectively (Figure 2).

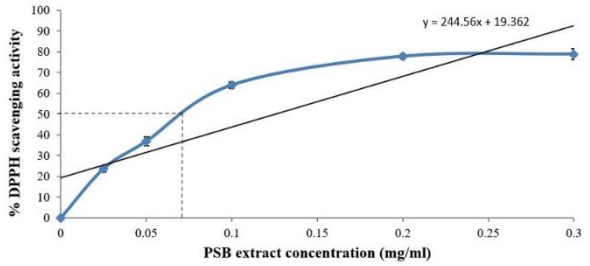

Figure 2 The percentage of DPPH scavenging activity of PSB extract at various concentrations. The dash line indicated IC50.

In addition, the IC50 of ascorbic acid and PSB extract were 0.016 and 0.07 mg/ml, respectively.

Ferric reducing antioxidant activity of PSB extracts at the concentration of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mg/ml were 0.027 ± 0.004, 0.042 ± 0.008, 0.067 ± 0.007, 0.075 ± 0.003, 0.097 ± 0.0005, 0.109 ± 0.003 and 0.122 ± 0.003, respectively (Figure 3).

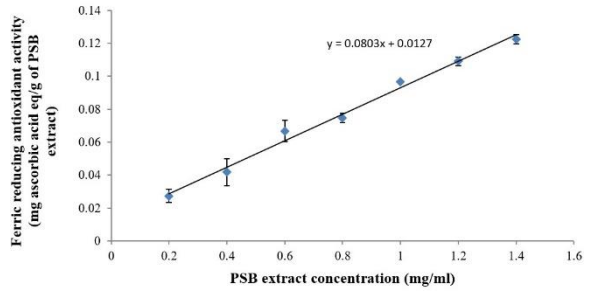

Figure 3 The Ferric reducing antioxidant activity of PSB extract at various concentrations calculated from the equation of ascorbic acid standard.

In addition, the calculated Ferric reducing power was 102.91+16.45 mg ascorbic acid per gram of PSB extract and 18.83+2.79 mg ascorbic acid per gram of rice bran.

4. CaOX crystal formation and aggregation *in vitro*

4.1 The effect of PSB extract on CaOX crystal formation

The CaOX crystals were formed after the incubation of Ca2+ and oxalate ions and were predominantly composed of COM and COD crystals in the control group (Figure 4).

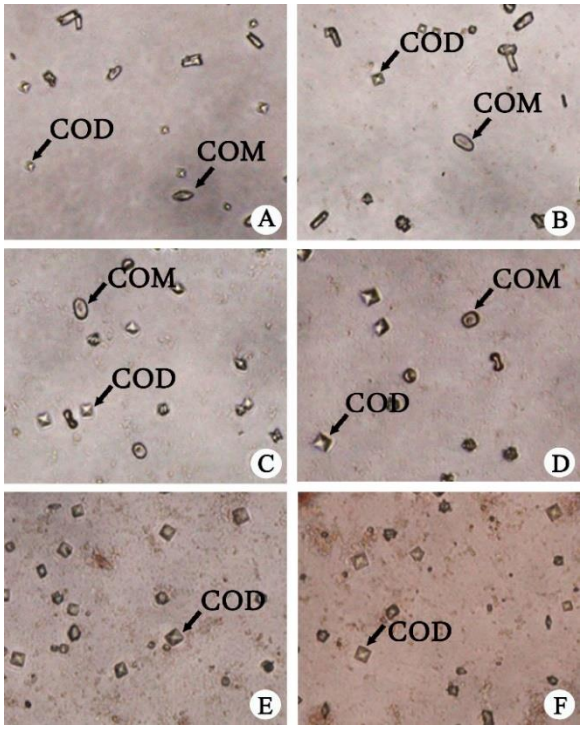

Figure 4 The photograph showing CaOX crystal formation in the control group (A) and treated group (B – F) with PSB extract at concentrations of 20, 50, 100, 200, and 400 µg/ml, respectively. COM; calcium oxalate monohydrate, COD; calcium oxalate dihydrate.

After incubation of PSB extract at different doses (20, 50, 100, 200, and 400 µg/mL) with the CaOX crystals, a significant decline was noted in the number of CaOX crystals compared to the control. Interestingly, the number of CaOX crystals was noticeably reduced upon increasing the concentration of PSB extracts to 100 µg/mL. However, upon incubation of PSB extract at a higher dose of 200 and 400 µg/mL with the CaOX crystals, only a slight decrease was noted in the number and formation of crystals compared to control (Figure 5).

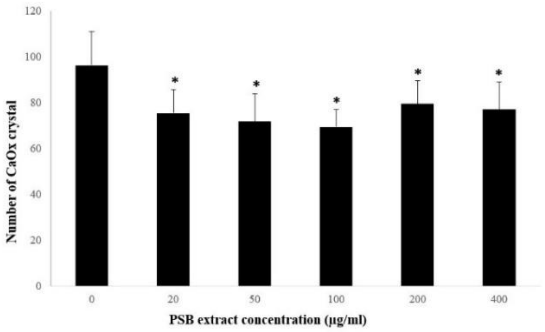

Figure 5 The number of CaOX crystal formation after incubation with PSB extract at various concentrations. Asterisk indicated significant different at $p<0.05$.

Moreover, the CaOX crystal formation appeared in COD rather than the COM form at a higher dose of 200 and 400 µg/mL (Figures 4E and F).

5.2 The effect of PSB extract on CaOX crystal aggregation

Crystal aggregation in all groups was identified as the COM agglomerates (Figure 6A).

After incubation with PSB extract, a significant decrease was noted in the number of CaOX crystal aggregation compared to control. Furthermore, the number of CaOX crystal aggregation greatly decreased upon increasing the concentration of PSB extract (Figure 6B).

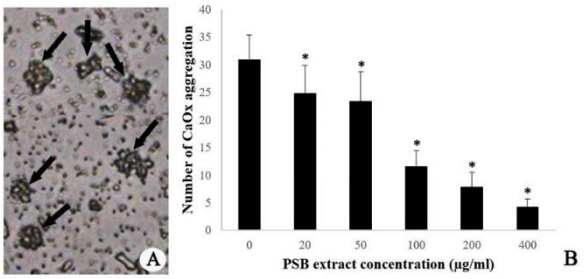

Figure 6 A. The photograph showing CaOX crystal aggregation (arrows; agglomerate form). B. The number of CaOX crystal aggregation after incubation with PSB extract at various concentrations. Asterisk indicated significant different at $p<0.05$.

In addition, the inhibition in CaOX crystal aggregation after treatment with PSB extract at a dose of 20, 50, 100, 200, and 400 µg/mL was 19.61%, 24.46%, 62.39%, 74.68%, and 86.31%, respectively.

Discussion

Previous studies with differential extraction systems demonstrated the antioxidative substances and their antioxidant activity in the extracted rice [14], [15], [17], [20], [21]. In this study, the total anthocyanin, phenolic compound, and the scavenging activity of purple sticky rice bran were higher than previously demonstrated in Riceberry and Sung-Yod sticky rice using 75% ethanol extraction [14], [15]. In addition, the extraction yield and total phenolic compounds in PSB extract were similarly present in the 40% acetone extract obtained from the black rice bran which were higher than those of red, brown, and green pigmented rice brans [17].

Using the DPPH free radical scavenging and ferric-reducing power assay, an increase in the antioxidant activity was found by increasing the concentration of the PSB extract, suggesting that the level of anthocyanin and total phenolic compound in PSB extract may be associated with the DPPH free radical scavenging capability. The anthocyanin can act as a reducing agent that can chelate metal ions such as Cu, Fe, or Ca present in the environment [15]. Previously, three different anthocyanin components, including the cyanidin-3-glucoside, peonidin 3-glucoside, and cyanidin 3-galactoside, were identified in purple rice bran extract [20]. The antioxidant activity of cyanidin-3-glucoside and peonidin 3-glucoside can suppress the production of reactive oxygen species and nitric oxide in the activated macrophages and prevent DNA damage and LDL deterioration *in vitro* [22]. In addition, the proposed active compounds containing anthocyanin and polyphenol in the H. sabdariffa extract reduced the deposition of stone-forming substances in the kidney and serum of urolithic rats [8].

The PSB extract was shown in this study to inhibit the CaOx crystal formation, as previously demonstrated in Riceberry [15]. Furthermore, it induced the formation of COD rather than COM crystals at high concentrations. This result is consistent with the previous studies reported in the rupturewort (Herniaria hirsuta) [23], rhizome (Bergenia ligulata) [24], flowering plant (O. vulgare) [25] and Riceberry (Oryza sativa) [15], suggesting that their extracts contain substances that can cause a morphological change in COM crystals, which appear to be tetragonal bipyramidal shape of COD crystals. This morphological change could interfere with the sequential step of crystal deposition along the urinary tract because the COD crystals have a lower affinity toward the renal epithelial cells than COM crystals [26]. In contrast, the formation of COM crystals induced by urinary supersaturation is a crucial step for subsequent crystal aggregation [27].

This study shows that the PSB extract dose-dependently inhibited CaOx crystal aggregation, similar to the previous studies in B. ligulata [24], O. vulgare [25] and Riceberry [15]. The COM crystals are attached to the renal tubular cell to form aggregates which is a critical step leading to their retention in urinary stone formation. [28]. Using substances that interfere with crystal formation and aggregation mechanisms is essential for preventing recurrent kidney stone formation [25]. Previous studies reported the relation between antioxidant activity and the kidney stone disease that antioxidants (e.g., selenium, vitamin E, and catechin) can protect against oxidative injury by inducing calcium oxalate-crystal deposition, which could prevent the development of kidney stones [29], [30], [31]. Moreover, a study on the extracted B. ciliata leaves showed that a group of phenolic compounds could dissolve CaOx and calcium phosphate urinary stones [32]. Study on the Riceberry extract, the proposed mechanism that anthocyanin might chelate Calcium ions during the CaOx crystal formation. The low amount of Ca concentration might not be enough to form CaOx crystals which leads to the reduction of crystal number and crystal aggregation [15]. This study demonstrated that the purple sticky rice bran extract contained substances with high antioxidative capacity and could protect against the CaOx crystal formation and aggregation *in vitro*. Further studies underlying the mechanistic insights are required to elucidate the potential of the purple sticky rice bran extract in kidney stone prevention.

Acknowledgements

This study was supported by grants from the Faculty of Allied Health Sciences, Burapha University, Chonburi, Thailand.

References

[1] Romero V, Akpinar H, Assimos DG. Kidney stones: a global picture of prevalence, incidence, and associated risk factors. Rev Urol. 2010 Spring;12(2-3):e86-96. PMID: 20811557.

[2] Coe F, Evan A, Worcester E. Pathogenesis and Treatment of Nephrolithiasis, Seldin and Geibisch's. The Kidney. 2013, 2311-2349, 2.

[3] Kok DJ, Khan SR. Calcium oxalate nephrolithiasis, a free or fixed particle disease. Kidney Int. 1994 Sep;46(3):847-54. doi:10.1038/ki.1994.341.

[4] Wiessner JH, Mandel GS, Mandel NS. Membrane interactions with calcium oxalate crystals: variation in hemolytic potentials with crystal morphology. J Urol. 1986 Apr;135(4):835-9. doi:10.1016/s0022-5347(17)45871-x.

[5] Ratkalkar VN, Kleinman JG. Mechanisms of Stone Formation. Clin. Rev. Bone Miner. Metab. 2011 Dec; 9(3-4), 187–197. doi: 10.1007/s12018-011-9104-8.

[6] Semangoen T, Sinchaikul S, Chen ST, Thongboonkerd V. Proteomic analysis of altered proteins in distal renal tubular cells in response to calcium oxalate monohydrate crystal adhesion: Implications for kidney stone disease. Proteomics Clin Appl. 2008 Jul;2(7-8):1099-109. doi:10.1002/prca.200780136.

[7] Thongboonkerd V, Semangoen T, Sinchaikul S, Chen ST. Proteomic analysis of calcium oxalate monohydrate crystal-induced cytotoxicity in distal renal tubular cells. J Proteome Res. 2008 Nov;7(11):4689-700. doi:10.1021/pr8002408.

[8] Nirumand MC, Hajialyani M, Rahimi R, Farzaei MH, Zingue, S, Nabavi SM, et al. Dietary Plants for the Prevention and Management of Kidney Stones: Preclinical and Clinical Evidence and Molecular Mechanisms. Int J Mol Sci. 2018 Mar;19(3):765. doi: 10.3390/ijms19030765.

[9] Butterweck V, Khan SR. Herbal medicines in the management of urolithiasis: alternative or complementary? Planta Med. 2009 Aug;75(10),1095–1103. doi:10.1055/s-0029-1185719.

[10] Ahuja U, Ahuja SC, Chaudhary, N, Thakrar R. 2007. Red rices – past, present and future. Asian Agri-History. 2007;11:291–304.

[11] Sookwong P, Nakagawa K, Murata K, Kojima Y, Miyazawa T. Quantitation of tocotrienol and tocopherol in various rice brans. J Agric Food Chem. 2007 Jan;55(2):461-466. doi:10.1021/jf0621572.

[12] Jariwalla RJ. Rice-bran products: phytonutrients with potential applications in preventive and clinical medicine. Drugs Exp Clin Res. 2001;27(1):17-26. PMID: 11276826.

[13] Oki T, Masuda M, Kobayashi M, Nishiba Y, Furuta S, Suda I, et al. Polymeric procyanidins as radical-scavenging components in red-hulled rice. J Agric Food Chem. 2002 Dec;50(26):7524-7529. doi:10.1021/jf025841z.

[14] Semangoen T, Khawsuk W, Simanon N, Soiphet T, Homjai W, Boonchaleaw B, et al. Antioxidant effects of unpolished Sung-Yod sticky rice on prevention of ethylene glycol-induced renal pathology in rats. Chula Med J. 2018 May – Jun; 62(3):451 – 64.

[15] Khawsuk W, Semangoen T, Nuurai P, Mepan W, Wingworn K. Antioxidant activity of unpolished Riceberry (Oryza sativa) and the inhibition of calcium oxalate crystal growth and aggregation. Chula Med J. 2018 May - Jun; 62(3):419 – 34.

[16] Ebisuno S, Morimoto S, Yasukawa S, Ohkawa T. Results of long-term rice bran treatment on stone recurrence in hypercalciuric patients. Br J Urol. 1991 Mar;67(3):237-40. doi:10.1111/j.1464-410x.1991.tb15125.x.

[17] Jun HI, Song GS, Yang EI, Youn Y, Kim YS. Phenolic compounds of pigmented rice bran extracts. J Food Sci. 2012 Jul; 77:759-64. doi:10.1111/j.1750-3841.2012.02763.x.

[18] Vijayalakshmi M, Ruckmani K. Ferric reducing anti-oxidant power assay in plant extract. Bangladesh J Pharmacol. 2016 Jun;11:570-2. doi:10.3329/bjp.v11i3.27663.

[19] Thongboonkerd V, Semangoen T, Chutipongtanate S. Factors determining types and morphologies of calcium oxalate crystals: molar concentrations, buffering, pH, stirring and temperature. Clin Chim Acta. 2006 May;367(1-2):120-31. doi:10.1016/j.cca.2005.11.033.

[20] Jang S, Xu Z. Lipophilic and Hydrophilic Antioxidants and Their Antioxidant Activities in Purple Rice Bran. J. Agric. Food Chem. 2009 Jan;57(3):858–62. doi: 10.1021/jf803113c.

[21] Srisawat U, Panunto W, Kaendee N, Tanuchit S, Itharat A, Lerdvuthisopon N, et al. 2010. Determination of Phenolic Compounds, Flavonoids, and Antioxidant Activities in Water Extracts of Thai Red and White Rice Cultivars. J Med Assoc Thai. 2010;93(Suppl.7):S83-S91.

[22] Hu C, Zawistowski J, Ling W, Kitts DD. Black rice (Oryza sativa L. indica) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. J Agric Food Chem. 2003 Aug 27;51(18):5271-7. doi:10.1021/jf034466n.

[23] Atmani F, Khan SR Effects of an extract from Herniaria hirsuta on calcium oxalate crystallization *in vitro*. BJU Int. 2000 Apr;85(6):621-5. doi: 10.1046/j.1464-410x.2000.00485.x.
[24] Bashir S, Gilani AH. Antiurolithic effect of Bergenia ligulata rhizome: an explanation of the underlying mechanisms J Ethnopharmacol. 2009 Feb 25;122(1):106-16. doi:10.1016/j.jep.2008.12.004.
[25] Khan A, Bashir S, Khan SR, Gilani AH. Antiurolithic activity of Origanum vulgare is mediated through multiple pathways. BMC Complement Altern Med. 2011 Oct 17;11:96. doi: 10.1186/1472-6882-11-96.
[26] Wesson JA, Worcester EM, Wiessner JH, Mandel NS, Kleinman JG. Control of calcium oxalate crystal structure and cell adherence by urinary macromolecules. Kidney Int. 1998 Apr;53(4):952-7. doi:10.1111/j.1523-1755.1998.00839.x.
[27] Hess, B., Kok, D.J., 1996. Nucleation growth and aggregation of crystals. In: Coe, F.L., Favus, M.J., Pak, C.Y., Parks, J.H., Preminger, G.M. (Eds.), Kidney Stones, Medical and Surgical Management. Lippincott-Raven, Philadelphia, PA, USA, pp. 3–32.
[28] Kok DJ, Papapoulos SE, Bijvoet OLM. Crystal agglomeration is a major element in calcium oxalate urinary stone formation. Kidney Int. 1990 Jan;37(1):51-6. doi:10.1038/ki.1990.7.
[29] Kumar MS, Selvam R. Supplementation of vitamin E and selenium prevents hyperoxaluria in experimental urolithic rats. J Nutr Biochem. 2003 Jun;14(6):306-13. doi:10.1016/s0955-2863(03)00033-0.
[30] Thamilselvan S, Menon M. Vitamin E therapy prevents hyperoxaluria-induced calcium oxalate crystal deposition in the kidney by improving renal tissue antioxidant status. BJU Int. 2005 Jul;96(1):117-26. doi:10.1111/j.1464-410X.2005.05579.x.
[31] Itoh Y, Yasui T, Okada A, Tozawa K, Hayashi Y, Kohri K. Preventive effects of green tea on renal stone formation and the role of oxidative stress in nephrolithiasis. J Urol. 2005 Jan;173(1):271-5. doi:10.1097/01.ju.0000141311.51003.87.
[32] Byahatti VV, Pat KV, D'Souza MG. Effect of Phenolic Compounds from Bergenia ciliata (Haw.) Sternb.leaves on Experimental kidney stones. Anc Sci Life. 2010 Jul;30(1):14-7. PMID: 22557418.