Anthocyanins in Thai rice varieties: distribution and pharmacological significance

Sivamaruthi, B.S., Kesika, P. and "Chaiyasut, C.

Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand

Abstract

Anthocyanins are phenolic, water-soluble, predominant flavonoids of plants, and are known for its wide distribution and its pharmacological importance. Almost all the plant sources like vegetables, fruits, cereals, grains are residing with anthocyanins. The type and quantity of the anthocyanins differ based on the species, varieties, cultivars, even the growth stage of the same plant, part of the plant, ethnic and environmental factors. Rice is one of the regular food sources for more than half of the people in the world. The rice cultivars and strains vary among the countries. Apart from the typical, polished, white rice, some of the colored rice varieties are in use. Anthocyanin present in the rice outer layer contributes the color of the rice. The nature, concentration, and distribution of anthocyanins are found to be varied among the rice cultivars. The current review focused on the anthocyanins content of Thai rice varieties and its reported pharmacological significance.

Introduction

Rice commonly consumed food worldwide especially among Asian peoples. The nutritional value and the phytochemical contents were found to be differed among the rice varieties (Goufo and Trindade, 2014; Ujjawal, 2016). The quality of the rice depends on their phytochemical content, which is highly influenced by several factors like geography, irrigation, quality of the fertilizers used, and cultivars, etc. The majority of the chemical constituents of the rice were residing in the rice bran (RB), and endosperm (Pengkumsri et al., 2015a). All components of rice have specific chemopreventive activity (Henderson et al., 2012). The occurrence of γ-oryzanol and phenolic acids, ferulic acid ester, sterol, phytosterols, and carotenoids contributes the RB oil as an effective chemopreventive agent (Lamberts and Delcour, 2008). Rice comprised of the high content of tocols (tocotrienols and tocopherols) and γ-oryzanol than that of the other common cereal grains. It is well known that rice consists of anthocyanins, cellulose, lignin, vitamin B, amino acid, and some minerals (Ryan et al., 2011). Anthocyanins are the most important water-soluble pigments that belong to the flavonoid group and are accountable for the different color in plant tissues (Glover and Martin, 2012; Trouillas et al., 2016; Cortez et al., 2017). Anthocyanins extensively occur in several plants, fruits, vegetables, and are documented by a recent study (Chaiyavat et al., 2016). The fruits, especially berries, are well-known source of anthocyanin. However, it is not affordable to all the people in their daily life. Rice is one of the commonly used stable foods around the world. The distribution of the rice anthocyanins depends on the rice cultivars. The consumption of colored rice varieties is increasing among the people, because of its health benefits. Moreover, rice bran (by-product of rice milling) is considered as an agricultural waste, which are rich in phytochemicals especially anthocyanins. The recent developments in extraction methods of anthocyanins in rice bran, and clinical studies have proven that the rice anthocyanins are cheap and abundant source of potent bioactive compound with antioxidant, anti-inflammatory, and other health promoting properties. The present review focused on the content, and distribution of anthocyanins, specifically in Thai rice varieties. Moreover, the review also glances about the general property of anthocyanins with a particular reference to pharmaceutical values.

Structure of anthocyanins

Anthocyanins are glycosylated polyphenolic compound of anthocyanidins, byproducts of polyhydroxy and polymethoxy 2-phenylbenzopyrylium linked by anthocyanidin attached with variable glycosidic moieties like

Keywords

Anthocyanins
Thai rice
Pharmacological importance

© All Rights Reserved
arabinose, fructose, galactose, glucose, rhamnose, and xylose at the C3, C5 or C7 locations (Smeriglio et al., 2014). Anthocyanins consist of two benzyl rings and a heterocyclic ring and linked via carbon (3n of C) bridge. Cyanidin (Cy), petunidin (Pt), peonidin (Pn), malvidin (Mv), delphinidin (Dp), and pelargonidin (Pg) are widely present in plants. About seventy percentage of anthocyanidins are Cy, Dp, and Pg, which are non-methylated form of three glycosylated anthocyanidins, and Cy is present in common edible plants (Kong et al., 2003; Castañeda-Ovando et al., 2009; Lucioli, 2012). The structure of the commonly occurring rice anthocyanins was represented in Figure 1.

Stability of anthocyanins

The anthocyanins are more vulnerable to degradation, and are easily affected by several physical and biological parameters like pH, temperature, oxygen, light, and enzymes. The stability of the anthocyanins is also attributed by its structure (Figure 2).

The glycone molecules are relatively stable when compared with its aglycone form (with glycosyl units and acyl groups). The hydroxyl and methoxyl groups, and acylation also influence the stability. Glycone forms (anthocyanins) are more stable than its respective aglycone forms (anthocyanidins) (Andersen, 2001; Stintzing and Carle, 2004). In nature, they commonly exist as glycone forms (anthocyanins) for stability purpose (Timberlake and Bridle, 1966).

The pH is one of the most influencing factors of stability, and color of the anthocyanins. The anthocyanins are more stable in acidic condition than in the alkaline condition. The impact of pH on the color of anthocyanins (chromophore) has been reported from pH 1 to 14. It is known that the anthocyanins are exhibiting four different ionic form in aqueous condition with respective pH such as flavylium cation (pH 1-3), carbinol pseudobases or hemiketals (pH 4-5), quinonoidal base (pH 6-8), and chalcone (pH 7-8). The carbinol form is colorless, whereas chalcone form is more unsteady, and easily breaks into phenolic acid and aldehydes (Fleschhut et al., 2006). The increase in pH reduces the bonding of conjugated heterocyclic ring that leads to open ring formation, and facilitates the degradation. Thus, flavylium cation in acidic condition is more stable, whereas, in alkaline condition, anthocyanins are prevailing as chalcone form, which is certainly susceptible to the degradation.

Oxygen is one of the factors in promoting the degradation of anthocyanins. In the combination with temperature, oxygen fastens the anthocyanins degradation, whereas anaerobic condition protects the anthocyanins from decomposition. It is reported that the presence of spare oxygen stimulate the depigmentation in berry juices (Nebesky et al., 1949), and the oxidation of anthocyanins lead to the browning of fruits and vegetables (Jackman et al., 1987).

Although light is an essential factor required for the biosynthesis of pigments, it triggers the anthocyanin degradation faster than oxygen due to
the UV protective nature of anthocyanins. The light activates the flavylium cation to an excited state and leads to degradation (Furtado et al., 1993). Thus, it is advisable to store the anthocyanin-rich food materials, and other formulations in dark at acidic condition (Kearsley and Rodriguez, 1981; Markakis, 1982).

Apart from physical factors, enzymes are the major biological factor that affects the stability of anthocyanins. For example, glycosidases are the destructive force of covalent bond of glycosyl residue in the aglycones (anthocyanidins) and sugar links of glycones (anthocyanin) (Huang, 1956). Moreover, phenolases (phenol oxidases and polyphenol oxidases) and peroxidases could react directly at the phenolic links of anthocyanins, possibly by the oxidation of phenolics and formation of quinines and browning of anthocyanins (Yokotsuka and Singleton, 1997; Garcia-Palazon et al., 2004).

Distribution of anthocyanins in Thai rice cultivars

Thai rice cultivars

The survey of rice varieties in Thailand by the Rice Research Institute (1982-86) documented about 1,500 rice varieties in northeast Thailand (Chaidee and Thongpitak, 1992). Another survey revealed that only 18% of the rice land were occupied by the modern rice varieties during 1990’s in Thailand (Rerkasem, 2007). In general, white rice, Hom Mali, Pathumthani fragrant rice, glutinous rice, husked rice, parboiled rice, broken parboiled rice are cultivated in all the part of Thailand and also exporting them to many places around the world (Office of Agricultural Economics, 2010). Only, the traditional rice cultivar, KDM 105, was widely cultivated along with the mutant varieties such as RD 6 and RD 15 (Rerkasem, 2007). In Thailand, many people are consuming glutinous sticky rice as their daily food, especially north and northeast Thai people. Several glutinous rice cultivars are being cultivated and consumed in large quantity, among which following are the most popular cultivars such as Sanpatong, RD6, and RD10 (Keeratipibula et al., 2008).

Major phytochemicals of Thai rice

Several studies have been reported about the phytochemical content of Thai rice cultivars. The major phytochemical constituents of rice are phenolic acids, anthocyanins, tocols, and γ-oryzanol. The individual phenolic acids like caffeic acid, chlorogenic acid, protocatechuic acid, p-hydroxybenzoic acid, p-coumaric acid, and syringic acid were studied and recorded in Thai cultivars. The concentration and the presence of phenolic acid were found to be varied depends on the rice strain. Pengkumsri et al. (2015a) reported the detailed phenolic acid, flavonoids, and anthocyanin content of Chiang Mai Black rice, Mali Red rice, and Suphanburi-1 Brown rice varieties of northern Thailand. The Chiang Mai black rice contains caffeic acid, protocatechuic acid, p-coumaric acid, and syringic acid, whereas, Mali red rice and Suphanburi-1 brown rice varieties have not been reported for the caffeic acid, and p-coumaric acid content, but these varieties were accounted for the presence of either p-hydroxybenzoic acid or chlorogenic acid (Pengkumsri et al., 2015a). About 17.54 ± 0.75, 17.54 ± 0.88, and 18.49 ± 1.52mg of γ-oryzanol per gram of Chiang Mai Black rice, Mali Red rice, and Suphanburi-1 Brown rice bran oil, respectively, were reported (Pengkumsri et al., 2015b). The changes in the phenolic acid content, especially protocatechuic acid, and vanillic acid, of Thai purple rice by different cooking methods were reported (Chatthongpisut et al., 2015).

Anthocyanins content

Black rice (Oryza sativa L. indica) is one of the anthocyanins rich rice cultivar, characterized with heavy pigmented outer layer. There are six commonly foundaglycones, that covers 95% of total anthocyanins content, such as delphinidin (Dp), pelargonidin (Pg), peonidin (Pn), cyanidin (Cy), malvidin (Mv), and petunidin (Pt) found in rice (Eder, 2000; Kong et al., 2003). The summary of the reported anthocyanin content in Thai rice varieties were tabulated (Table 1). Maisuthisakul and Changchub (2014) reported the anthocyanins content of nine Thai rice varieties namely Kum, Hawm kanya, Hawm nil, Sang yod, Red Jasmine, Hawm Ubon, Jasmine rice 105, Lao tek, and Sin lek. The impact of extraction methods on the yield of the phytochemicals was also reported. The Kum rice cultivar (black rice) noted to possess high anthocyanins content among the other tested samples, and acid hydrolysis method was best in terms of yield.

The anthocyanin content and antioxidant ability of three thai rice varieties, namely, Phitsanulok 2 (non-pigmented rice), Niew Dam (glutinous black rice), and Hom Nil (black non-waxy rice) were reported with respect to soaking and germination time. The content of anthocyanins varied from 1.09 ± 0.75 to 10.80 ± 5.20, 17.89 ± 12.20 to 103.45 ± 10.03, and 3.22 ± 1.75 to 10.89 ± 3.64 mg/100g of Phitsanulok 2, Niew Dam, and Hom Nil rice, respectively (Sutharut and Sudarat, 2012). Another study also suggested that the Niew Dam rice contains the maximum amount of naturally occurring anthocyanin (109 mg/100g rice) (Sompong et al., 2011).

Riceberry is a cross breed of Hom Nil rice
Table 1. Summary of reported anthocyanins content in Thai rice cultivars.

<table>
<thead>
<tr>
<th>Cultivar/strain</th>
<th>Anthocyanins content</th>
<th>Remarks</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2027</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57x807</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sivamaruthi et al./IFRJ 25(5): 2024-2032</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2027</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57x807</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Table 1. Summary of reported anthocyanins content in Thai rice cultivars.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ND: Not detected; S/AH: Soaking / acid hydrolysis method; db: Dry basis; Cy-3-glu: Cyanidin-3-glucoside; Pn-3-glu: Peonidin-3-glucoside; mg Mal. Eq.: mg malvidin equivalent; DM: dry matter; #average of anthocyanin contents in rice cultivated at different environmental conditions</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
and Khao Dawk Mali 105, and it is recognized for possessing high content of peonidin and cyanidin (66.76 and 150.81 mg/100g of rice, respectively) (Jittorntrum et al., 2009). The impact of salt stress on the anthocyanins content of Riceberry, Kham, Khamdoiakdet, KDML 105, Sinlek, and BC2F7#62-56 varieties were reported. The concentration of cyanidin-3-glucoside of Khamdoiakdet and KDML105 increased considerably after 60mM of salt treatment, whereas slight or no significant changes were witnessed in Riceberry, Kham and Sinlek, and BC2F7#62-56 (Daiponnak et al., 2010).

The ethanolic extract of some of the Thai rice varieties (Mun Poo Phayao, Mun Poo Pink, Mun Poo HPM, Sang Yod Phattalung, Sang Yod Songkla, Niew Dam Phayao, Niew Dam Doi Hang, Hom Nil Ngok, Hom Nil Tai Tai, Hom Nil Thinnakorn, Hom Nil Phayao) were studied for determining the anthocyanin content, and the average concentration of anthocyanins were found to be varied from 0.636 ± 0.393 to 1.428 ± 0.985 mg GAE/mL extract. To the maximum of 2.849 ± 0.104 mg GAE/mL extract of total anthocyanins was recorded in Mun Poo Pink (Kitisin et al., 2015).

The influence of cooking strategies and changes in the anthocyanin content of the Thai purple rice was reported. The total anthocyanins content was 64.5 ± 0.2 mg/100g of raw rice, 40.9 ± 0.3, 20.3 ± 0.1, and 11.0 ± 0.1 mg/100g of rice cooked using rice cooker, autoclave and microwave, respectively. The major anthocyanins in Thai purple rice were found as 3-glucosides of peonidin and cyanidin (Chatthongpisut et al., 2015). The results revealed that the quality of the rice regarding anthocyanins was demolished by the regular cooking processes, especially by microwaves. The 3-glucosides of peonidin and cyanidin content of Mali Nil Surin No.6 rice was about 200µg/g dry weight and 492 µg/g dry weight, respectively. The Mali Nil Surin No.6 rice strain was superior to black rice, *O. sativa* L. japonica var. SBR (Hiemori et al., 2009) and Korean black rice cultivars namely, Sinnongheugchal and Sintoheugmi (Surn and Koh, 2014) regarding peonidin-3-glucoside content. About 41.7% and 74.2% of degradation of 3-glucosides of peonidin and cyanidin were reported in Thai purple rice upon cooking processes. The studies suggested that peonidin-3-glucoside is relatively stable than cyanidin-3-glucoside against temperature mediated damages (Hiemori et al., 2009; Chatthongpisut et al., 2015).

Chakuton et al. (2012) documented the anthocyanin content of commonly used eight colored local Thai rice cultivars such as Neawdan53, Parnia97, Wongwan98, Neawdan1-202, Neawdan2-203, Malii-dang2-206, Jaowdam208, and Jaowdam209. The Leum Phua, Klam, Hawn Nil, and Black Rose cultivars were subjected to the phytochemical analysis, and the study found that Leum Phua has a high concentration of anthocyanin, phenolic compounds than that of the other tested varieties (Suwannalert and Rattanachithhawat, 2011).

Some of the genetically related Thai black rice varieties (Khao Hom Nin BT, Khao Hom Nin BD, Khao Hom Nin BT No. 3, and 1000-11-2-26.) were assessed for the anthocyanins content, and the results indicated that the major anthocyanins and its levels were ranging from 16.01–34.40 µg/mL (cy-3-O-gluc) and 2.43–7.36 (pe-3-O-gluc) µg/mL. The strain Khao Hom Nin BT exhibited the high content of anthocyanin than that of the other tested samples (Pitija et al., 2013).

Sompong et al. (2011) detailed about the anthocyanins distribution among the Thai black, and red rice varieties such as Niaw Dam Pleuak Dam, Niaw Dam Pleuak Khaob, Bahng Gaw, Niaw Dawk Yong, Niaw Look Pueng, Haek Yah, Niaw Lan Tan and Sung Yod Phatthalung. Obviously, the black rice varieties Niaw Dam Pleuak Khaob, and Niaw Dam Pleuak Dam were recognized for possessing high content of anthocyanins (19.39 ± 0.09 - 137.41 ± 16.66 mg/100g of rice).

Seven promising Thai black indigenous rice varieties such as ULR012, ULR017, ULR038, ULR046, ULR238, ULR239, and ULR291 were studied in detail for determining the anthocyanin content upon changes in environmental conditions. The results indicated that the average anthocyanins concentration were recorded in ULR012, ULR017, ULR038, ULR046, ULR238, ULR239, and ULR291, respectively with tested environmental influences (Somsana et al., 2013). We have reported the phytochemical and anthocyanin content of three Thai rice strains namely Mali Red rice, Chiang Mai Black rice, and Suphanburi-1 Brown rice cultivars with different extraction methods. The results indicated that Chiang Mai Black rice have the maximum amount of phytochemicals, anthocyanins, and bioactivities than that of the other tested samples (Pengkumsri et al., 2015a).

**Pharmacological importance of anthocyanins**

Health promoting properties of anthocyanins from dietary sources are beneficial for human health (Figure 3, Table 2). The anthocyanins are recognized for its several health benefits such as free radical scavenger, anti-inflammatory, anti-cancer, anti-obesity, anti-diabetes candidate, and also for the cardio protective nature, antimicrobial property,
etc. (He and Giusti, 2010; Pojer et al., 2013; Reis et al., 2016). Yao et al. (2013) investigated the role of anthocyanins of black rice in controlling the cholesterol levels in vitro conditions. They revealed that 3-glucosides of cyanidin and peonidin in the black rice extract effectively reduced the absorption of cholesterol in Caco-2 cells (Yao et al., 2013). Yu group studied the protective role of anthocyanins of black rice against the breast cancer using the human breast cancer cells that are positive for human epidermal growth factor receptor 2. They evidenced that the anthocyanins of black rice exhibited metastasis inhibition ability both in vitro and in vivo models (Luo et al., 2014). Yu group also revealed that anthocyanins of black rice suppress the activation of RAS/RAF/MAPK signaling pathway players to inhibit the metastasis in the human breast cancer cells (Chen et al., 2015). Another study by Chung group showed that anthocyanins of black rice exhibited anti-metastasis ability by inhibiting the activation of NF-kB and PI3K/Akt pathway players in human oral cancer cells (Fan et al., 2015). Shimoda group have reported that intake of anthocyanin rich purple rice extract (single dose: 25 mg) effectively reduced the raise of postprandial blood sugar that occurs after the consumption of 200g rice ball by the healthy human subjects (Shimoda et al., 2015).

Many studies have been reported on the benefits of anthocyanins isolated from the rice varieties of Thailand (Table 1). Sangkitikomol et al. (2010) has reported the health promoting properties (antioxidant and anti-hyperlipidemic activity) of Thai black sticky rice using HepG2 cells. The Thai black sticky rice extract that is rich in anthocyanins reduced the oxidative stress and regulated the expression of low-density lipoprotein receptor gene in HepG2 cells (Sangkitikomol et al., 2010).

Leardkamolkarn et al. (2011) reported the chemopreventive properties of the Thai rice (Riceberry) bran using Caco-2, MCF-7, and HL-60 cells. The methanolic extract of Riceberry bran showed anti-cancer properties, which was mainly due to the presence of peonidin-3-glucoside and cyanidin-3-glucoside (Leardkamolkarn et al., 2011). Similarly, the 3-O-glucosides of peonidin and cyanidin was reported as the major anthocyanins of Thai black rice, and the extract of Thai black rice bran showed high antioxidant activity (Pitija, 2013). Leum Phua (unpolished Thai rice) exhibited a high content of total phenolic content, anthocyanin content, and antioxidant activity when compared with the other varieties of Thai rice namely, Black Rose, Hawm Nil, and Klam (Suwannalert and Rattanachitthawat, 2011).

Banjerdpongchai et al. (2013) reported the anti-carcinogenic activity of purple rice of Payao, Thailand. The methanolic extract of Payao purple rice showed the cytotoxic effect on human HepG2 cells by inducing apoptosis via the mitochondrial pathway. The extract also exhibited synergetic effect by increasing the cytotoxic effect of vinblastine (Banjerdpongchai et al., 2013). Chatthongpisut et al. (2015) reported the anticancer activity of Thai purple rice using human colon cancer cells. The methanolic extract of cooked (sterilization) Thai purple rice showed high antioxidant and anti-proliferative activity on Caco-2 cells (Chatthongpisut et al., 2015). Suttajit group investigated the health promoting property of anthocyanin extracted from black sticky rice of Payao, Thailand and revealed that the anthocyanin of Thai black rice extract effectively enhanced the learning and memory in cerebral-
ischaemia mice model (Kangwan et al., 2015).

**Recommended dosage of anthocyanins**

The recommended daily dosage for the consumption of anthocyanins by humans differ among the countries. Italy, New Zealand, and Korea have been prescribing the anthocyanins as medicine. Whereas in Japan and U.S.A, anthocyanins are recognized as a health supplement. Based on the in vivo studies, acceptable daily consumption of anthocyanins is 2.5 mg/kg of body weight (Clifford 2000; He and Giusti, 2010). As per the Japan Health Food and Nutrition Food Association, 29 mg of anthocyanins per day is the recommended dose for human consumption (Yamamoto et al., 2013).

**Conclusion**

Several studies have been reported about the phytochemical content of Thai rice cultivars and its changes upon different processing conditions, and environmental changes. Whereas, the biological activity of Thai rice extract, especially anthocyanins, are poorly reported when compared to other rice varieties. Recent studies have revealed the health beneficial effects of Thai rice anthocyanins like antioxidant, anti-carcinogenic and anti-hyperlipidemic activity. Anthocyanin of Thai black glutinous rice has also been reported for its role in effectively improving the learning and memory impairment. Further detailed study on anti-inflammatory, anti-diabetes, and anti-obesity properties of Thai rice and its active phytochemicals are required to explore the natural remedies for the diseases, and metabolic disorders.

**Acknowledgments**

All the authors thankfully acknowledge the Faculty of Pharmacy and Chiang Mai University, Chiang Mai for the essential support. BSS also gratefully acknowledges the CMU Post-Doctoral Fellowship.

**References**


Sivamaruthi et al./IFRJ 25(5): 2024-2032

Technology (Campinas) 35(2): 331-338.


