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Rice panicles: New promising unconventional cereal product for health benefits

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ABSTRACT

Historically, rice has been served as a food and as an herbal remedy. The claimed therapeutic properties of rice-derived products have attracted a considerable amount of attention, but these therapeutic properties are currently underestimated. Rice panicles of Phitsanulok 2 (PL), Suphan Buri 1 (SP) and San Pa Tong 1 (ST) varieties were compared based on their antioxidant activities and total phenolic content (TPC). The crude fiber, fat and mineral contents of PL were significantly ($p < 0.05$) greater than those of SP and ST. Phenolics were particularly found in PL, including insoluble phenolics in the PL_50%EtOH_EtOAc (Ins), PL_EtOAc_Direct (Ins) and PL_EtOAc (Ins) extracts. These extracts were comparable in their TPC, and ABTS and DPPH scavenging activities. The FRAP reducing power of PL_EtOAc_Direct (Ins) was significantly ($p < 0.05$) greater than those of the others, and it was similar to the anti-tyrosinase effect. All of the extracts were non-cytotoxic in fibroblasts and cultured neurons. The neuroprotective and neuritogenic activities were significant ($p < 0.05$) in PL_50%EtOH_EtOAc (Ins). The extracts contained health-promoting phenolics, particularly *p*-coumaric (2477–3153 μg/ml), ferulic (329–347 μg/ml) and caffeic (387–413 μg/ml) acids. Rice panicle extracts are therefore highlighted as potential antioxidants for health promotion products.

1. Introduction

Rice is used in several Asian traditional medicines for anti-diabetic, anti-inflammatory, anti-gastrointestinal disorder and anti-cardiovascular disease purposes, and it is also used as a diuretic (Ahuja et al., 2008; Umadevi et al., 2012). In addition, it is regarded as a potential medicinal herb for alcoholic liver disease prevention (Ding et al., 2012) and for skin diseases that are not limited to Asia. It is also used as a traditional Italian remedy (Pieroni et al., 2004; Saikia et al., 2006) and as a potential solution for malnutrition and chronic diseases (Dipti et al., 2012). Its high concentration of γ-aminobutyric acid (GABA) supports its benefit as a neuropromoter (Umadevi et al., 2012; Zhang et al., 2006). However, there is little scientific evidence confirming its traditional uses for skin and neurons.

Thailand is one of the world's major producers of rice, with more than 93 rice varieties. The phytonutrients in rice (Butsat and Siriamornpun, 2010; McKevith, 2004) are considered a main source of natural health promotion (Liu, 2007). Rice byproducts have been commonly underestimated, although high levels of bioactive products would be sufficient to serve as raw material for nutraceutical and pharmaceutical products (Esa et al., 2013; Lourith and Kanlayavattanakul, 2013).

Rice is largely cultivated in northern and northeastern Thailand in several varieties, particularly the *Oryza sativa* cv. *indica* variety. The varieties that are of particular economic importance, because

Abbreviations: ABTS, 2,2′–azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid; CA, caffeic acid; ChA, chlorogenic acid; pCA, *p*-coumaric acid; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EtOH, ethanol; EtOAc, ethyl acetate; FA, ferulic acid; FRAP, ferric reducing ability of plasma; GA, gallic acid; GAE, gallic acid equivalents; α-MEM, alpha-minimal essential medium; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PA, protocatechuic acid; PL, Phitsanulok 2; Q, quercetin; RA, rosmarinic acid; SiA, sinapic acid; SP, Suphan Buri; ST, San Pa Tong 1; SyA, syringic acid; TPC, total phenolic content; UPLC, ultra-high performance liquid chromatography; V, vanillin; XTT, (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide.

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they can be cultivated and harvested twice a year, are a glutinous rice of San Pa Tong 1 (ST), a longer grain white rice of Phitsanulok 2 (PL) and Suphan Buri 1 (SP). Profiles of biologically active rice phenolics differ during grain development and are particularly high during the flowering phase, i.e., rice panicle (Butsat et al., 2009; Lin and Lai, 2011), which is traditionally used as a cosmetic and dentifrice in Chinese remedies (Duke and Ayensu, 1985). Antioxidant phenolics of cereal, such as ferulic acid and *p*-coumaric acid, which are applicable in the food, cosmetics and/or pharmaceutical fields (Liu, 2007), are therefore examined in this industrial crop of Thailand. Thus, the three varieties of rice panicles that are of economic importance to northern Thailand were subjected to proximal comparative analysis. Then, the extracts were prepared and studied in terms of their antioxidant activities and total phenolic content. The best-performing extracts were also assessed based on their tyrosinase, neuroprotective and neuritogenic activities and phenolics.

2. Materials and methods

2.1. Chemicals

All chemicals used were analytical grade unless otherwise specified. The solvents for extraction, namely, ethanol (EtOH) and ethyl acetate (EtOAc), were purchased from Merck (Darmstadt, Germany). 2,2′−Azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing ability of plasma (FRAP) reagents for antioxidant activity assessments were supplied by Fluka (MO, USA), as were the ascorbic acid and $FeSO_4$ standards including those that were used for phenolic content analysis. Reference compounds for ultra-high performance liquid chromatography (UPLC) analysis were purchased from Fluka. The UPLC standards were diluted at various concentrations in AcCN (Labscan, Gliwice, Ireland). De-ionized water was prepared using a Milli-Q water purification system (Millipore, MA, USA). Chemicals and reagents for enzyme inhibitory activities were from Sigma−Aldrich (MO, USA). Media and supplements for cellular assessments were from Gibco (NY, USA). MTT and XTT were from USB (OH, USA), and DMSO (Sigma, MO, USA) was used to solubilize the cells.

2.2. Rice panicle collection

Rice panicles of three different varieties, PL, SP and ST, were harvested from the Chiang Rai rice research center during May to June 2012. The crop was loosely packed in the net bag and transported to our laboratory within 30 min of being harvested.

2.3. Proximal compositional analysis

Fiber, protein and fat contents were delineated by the AOAC (2000) protocol 985.29.

2.4. Rice panicle extraction

The harvested rice panicles were separately cleaned, dried and ground into powder (<1 mm). Soluble and insoluble or bound phenolics of rice panicles were extracted using the procedure reported by Butsat et al. (2009) with some modifications. The panicle was macerated in 95%, 70% and 50% EtOH, separately, for 1 h at 35 ± 5 °C, with continuous stirring at 150 rpm. The mixture was then filtered to separate the soluble phenolics (Sol). The residue was further hydrolyzed using 2 M NaOH. This mixture was filtered to separate the supernatant, which was then further neutralized using 2 M HCl and centrifuged to obtain the bound or insoluble phenolics (Ins). Soluble and bound phenolics were dried *in vacuo* using rotary evaporator. The yields of these crude extracts from each panicle variety were compared. Additionally, the crude extract was further partitioned using EtOAc to create the semi-purified EtOAc and aqueous (Aq.) extracts. Furthermore, the panicle was directly extracted with EtOAc, producing the soluble and insoluble phenolics, EtOAc (Sol) and EtOAc (Ins), respectively.

2.5. Total phenolic content (TPC)

A serial dilution of standard gallic acid was prepared to generate a calibration curve ($r > 0.999$). The sample was mixed with water, the Folin-Ciocalteu reagent, and 2% Na_2CO_3 and then incubated for 1 h at 35 ± 5 °C. Absorbances were recorded at 750 nm using a microplate reader (ASYS, UVM340, Cambridge, UK). The TPC was calculated and expressed as g of gallic acid equivalents per 100 g of extract (g GAE/100 g extract). All measurements were performed in triplicate (Butsat and Siriamornpun, 2010; Kanlayavattanakul et al., 2012).

2.6. $ABTS^{\bullet+}$ scavenging activity

Absorbance for ABTS containing potassium persulfate and EtOH mixed with samples was recorded following 5 min of incubation at 750 nm. The antioxidant efficiency (%) was compared to that of standard ascorbic acid in different concentrations ($r > 0.999$). Measurements were performed in triplicate (Butsat and Siriamornpun, 2010; Kanlayavattanakul et al., 2012).

2.7. $DPPH^{\bullet}$ scavenging activity

Antioxidant activity, on the basis of a DPPH assay, was assessed in parallel. DPPH in absolute EtOH was allowed to react with ascorbic acid to generate a calibration curve ($r > 0.999$). The scavenging activity of the sample against $DPPH^{\bullet}$ was monitored at 517 nm using a microplate reader. The radical terminating capability (%) was calculated in comparison with the standard values. The experiments were performed in triplicate (Butsat and Siriamornpun, 2010; Lourith and Kanlayavattanakul, 2013).

2.8. Ferric reducing ability of plasma (FRAP)

A FRAP reagent was prepared in a 2,4,6-tri(2-pyridyl)-*S*-triazine (TPTZ) solution with HCl, $FeCl_3$ (20 mM), and acetate buffer. The samples were reacted with FRAP reagent, and absorbance was recorded at 595 nm with the microplate reader. The reducing power of 1 mg of extract was expressed as an equivalent concentration (EC) of 1 μg $FeSO_4$. The assay was conducted in triplicate (Butsat and Siriamornpun, 2010; Kanlayavattanakul et al., 2012).

2.9. Tyrosinase inhibitory effect

The tyrosinase inhibitory activity was determined using the dopachrome method with L-Dopa as the substrate. A sample that was a mixture of a phosphate buffer and mushroom tyrosinase was incubated at 25 °C for 10 min. L-Dopa was then added to the mixture and incubated (20 min). The absorbance was measured at 490 nm using a serial dilution of kojic acid as a positive control ($r > 0.999$). The enzyme deactivation efficacy of each extract was monitored. The assay was performed in triplicate, and the inhibitory effect (%) was calculated in comparison with kojic acid (Kanlayavattanakul et al., 2012).