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Appraisal of Thai glutinous rice husk for health promotion products

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ABSTRACT

Rice husks of the RD 6 variety were refluxed with water for 30, 60 and 120 min. The longest extraction time resulted in the greatest yield (0.089 ± 0.008%), radical scavenging activity (IC_{50} = 37.203 ± 0.421 μg/ml), and total phenolic and flavonoid contents (7.512 ± 0.008 g GAE/100 g extract and 36.886 ± 0.004 g QE/100 g extract). Fatty acids of cosmetic importance, such as hexanoic, heptatonic, octanoic, nonanoic, stearic, palmitic, oleic and linoleic acids, were quantified using the best antioxidant extract (166.004 mg/kg). The total phenolic content was related to the antioxidant activity and total fatty acid content (r = 0.997 and 0.864) in linoleic acid (r = 0.989), which displayed antioxidant properties (r = 0.996). The extract was non-cytotoxic in vero cells. Furthermore, it was compatible and stable in cosmetic formulations. Therefore, rice husks should be further studied for use in health promotion products.

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1. Introduction

Rice is a cereal responding as a rich source of fiber, vitamins, minerals, and bioactives of nutritional importance serving two-thirds of the world's population (Liu, 2007; Wang et al., 2011). Rice husks are an agricultural waste product from rice production, which is the principal cereal produced in Thailand, and there are more than 93 varieties. The increasing demand for domestic consumption and exportation of rice has increased the production of rice husk byproducts following the milling process. This fraction has low value because it is inedible (Butsat and Siriamornpun, 2010). There have been several attempts to recycle this agricultural residue for silica production (Chakraverty and Kaleemullah, 1991; Kalapathy et al., 2000; Kamath and Proctor, 1998), lightweight brick production for use in construction and electricity generation (Chatveera et al., 2006). However, the utilization of rice husks is limited. The rice husks are mainly eliminated by burning in Thailand, which causes environmental pollution. In addition, research and development of rice husk use for pharmaceuticals, dietary supplements and cosmetics has rarely been performed in Thailand, which is where an enormous amount of this agricultural waste is discarded, although a generation of phenolics from the KDML 105 variety cultivated in Thailand has been presented (Butsat and Siriamornpun, 2010).

Hydrothermal treatment of rice husks result in lignin-derived compounds, including caffeic acid and ferulic acid (Garrote et al., 2007), which are important compounds for pharmaceutical application due to their potential protective effects against photooxidative damage (Saija et al., 1999). In addition, fatty acids, such as linoleic, stearic, oleic and palmitic acids, were obtained (Garrote et al., 2007). These fatty acids are important in cosmetic formulas. Linoleic acid is a skin, hair and nail nourishing agent and has been approved as a hair growth promoter, skin moisturizer and effectively reduces surface lipid secretion (Barceló-Coblijn and Murphy, 2009; Darmstadt et al., 2002; Lautenschläger, 2003; Letawe et al., 1998;). This essential fatty acid exhibits anti-inflammatory effects and is used for acne treatment (Kanlayavattanakul and Lourith, 2011). Palmitic acid is commonly used in cleansing products in a cream or lotion form similar to stearic acid (Mitsui, 1997).

However, sustainable management of rice husks in Thailand, where a huge amount of rice is cultivated, has not been discussed. Therefore, concise, practical and safe extraction of pharmaceutically useful compounds from rice husks should be developed to promote efficient consumption of this cereal crop. The recovered biologically active compounds are potentially useful for pharmaceuticals, dietary supplements and cosmetics to achieve an increased quality of health using a low cost ingredient.

Abbreviations: DPPH, 1,1-Diphenyl-2-picrylhydrazyl; GAE, gallic acid equivalents; GC, Gas chromatography; FID, flame ionization detector; MS, mass spectrometry; QE, quercetin equivalents; TFC, total flavonoid content; TPC, total phenolic content.

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2. Materials and methods

2.1. Rice husk extract preparation

Husks of *Oryza sativa* cv. RD6, the main variety of rice cultivated in northern Thailand, was collected. Sample preparations were modified from Garrote et al. (2007). The husks were refluxed with deionized water at a 1:8 ratio of husks to water (w/v) for 30, 60 and 120 min. The extract was partitioned using CH_2Cl_2 (Fisher, UK) 3 times, and the organic phases were combined, washed in saturated NaCl, dried over anhydrous $MgSO_4$ (Carlo erba, Italy) and concentrated to dryness under *vacuo*. The extraction of each condition time was repeated 2 times. The samples were kept under 4 °C in a light protected vessels until used.

2.2. Radical scavenging activity

Antioxidant activity assessment was conducted based on 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay (Ardestani and Yazdanparast, 2007; Bamdad et al., 2011; Butsat and Siriamornpun, 2010). Briefly, DPPH (Fluka, USA) radicals (6×10^{-5} M) were prepared in absolute EtOH (Merck, Germany). Standard ascorbic acid (1, 2, 4, 6 and 8 μg/ml) in the same solvent was used to generate a calibration curve. Calibration curves with a linear coefficient (r) of more than 0.999 were compiled in this study. The scavenging activity of the sample concentrated to 5, 20, 50, 100 and 200 μg/ml against DPPH• was monitored at 517 nm using a microplate reader (ASYS, UVM340, UK). The inhibitory concentration at 50% (IC_{50}) of the extracts was calculated in comparison with ascorbic acid. The experiments were repeated 3 times.

2.3. Total phenolic content (TPC)

A serial dilution (5–30 μg/ml) of standard gallic acid purchased from Fluka (USA) was prepared in H_2O to generate a calibration curve ($r > 0.999$). The sample was mixed with water, Folin-Ciocalteu reagent (Carlo erba, Italy) and 2% Na_2CO_3 (Fisher, UK) prior to incubation for 1 h at ambient conditions. Absorbencies were recorded at 750 nm using a microplate reader. 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 (Tepe and Sokmen, 2007).

2.4. Total flavonoid content (TFC)

Quercetin (Fluka, USA) concentrated to 100–5000 μg/ml was prepared in MeOH to generate a calibration curve ($r > 0.999$). The sample solution was mixed with H_2O and 15% $NaNO_2$ (Fisher, UK), and the sample was incubated while shaking for 6 min. Thereafter, 10% $AlCl_3$ (Fisher, UK) was added, and the sample was incubated for 6 min. Prior to absorbance reading at 570 nm using the microplate reader, 4% NaOH (Fisher, UK) and water were added to adjust the final volume to 200 μl, and the sample was incubated for 15 min. The determination of each sample was repeated in triplicate. The TFC was calculated by comparing the sample with the standard curve and expressed as g of quercetin equivalents per 100 g of extract (g QE/100 g extract) (Ardestani and Yazdanparast, 2007).

2.5. Fatty acid analysis

Gas chromatography equipped with a flame ionization detector (Agilent, 6890N, USA) was performed using He as a carrier gas. Analyses were performed in splitless mode with an injection volume of 5 μl. Separation was conducted with an HP-Innowax capillary column (30 m × 0.32 mm, id. 0.25 μm). The injector temperature was maintained at 220 °C. The oven was programmed to increase from 80 to 250 °C at 7 °C min^{-1} and held at 250 °C for 5 min. Standard fatty acids in CH_2Cl_2, including valeric, hexanoic, octanoic and stearic acids (Acros, USA) nonanoic acid (Aldrich, Germany), palmitic acid (Riedel, Germany), heptanoic, oleic and linoleic acids (Fluka, France) concentrated to 2, 10, 50 and 100 μg/ml, were adopted for calibration curves ($r > 0.999$). Rice husk extracts (1 mg/ml) were directly analyzed in triplicate, and the contents were expressed in mg/kg of rice husk (Garrote et al., 2007).

2.6. Cytotoxicity against vero cells

Cytotoxicity against vero cells was conducted using the green fluorescent protein (GFP) detection method compared to Elliptine (Iwase and Tsutsui, 2007; Skehan et al., 1990). Toxicity was performed at the National Center for Genetic Engineering and Biotechnology of Thailand.

2.7. Solubility and stability of rice husk extracts

The solubility of the extract (10 mg) in glycerine and propylene glycol (1 g), which are commonly used in cosmetic formulations, was assessed. Miscibility was scored from 0 to 4 (insoluble to most soluble). The stability of the extract (3-fold of IC_{50}) was monitored at pH 4, 7 and 9 (Lourith et al., 2009).

2.8. Compatibility and stability in cosmetic base

Silicone base emulsions containing lauryl PEG/PPG-18/18 methicone and cyclomethicone (Summit chemical; Thailand), isopropyl myristate, cyclotetrasiloxane and dimethiconol (Namsiang; Thailand), deionized water, glycerine and sodium chloride were formulated based on the solubility and pH stability of the extract. Accelerated stability tests were conducted using the centrifugation assay (3000 rpm) at ambient temperature for 30 min in addition to heating and cooling (45 and 4 °C, 24 h each) for 6 cycles. Physicochemical characteristics were recorded, which are pH (at 25 °C) and viscosity, using spindle no. 5 at 20 rpm and 25 °C (Viscometer RVDV-II + Pro, Brookfield, USA). Variations of the ingredients were studied to obtain a stable physical base. Three stable bases were further evaluated in a preference test with 20 Thai female volunteers. The panelists were guided to choose the most preferred compound, which was calculated into overall preference (%). The silicone base emulsion with the best preference was selected to be incorporated with the rice husk extract. The sun protection factor (SPF) of the formulation was monitored using an SPF Analyzer and delineated using the manufacturer's recommended protocol (Optometric LLC, SPF 290S, USA). Each sample (0.136 ml) in propylene glycol (1% w/v) was separately spread on a transpore plate and left to dry for 15 min. The measurement was conducted and propylene glycol was used as a reference. Stability under an accelerated stability test was performed as described above. The physicochemical stability of the developed formula was monitored in addition to chemical stability in terms of SPF and TPC (Lourith et al., 2009).

2.9. Statistical analysis

Data were presented as the means ± SD and an ANOVA test was used to evaluate the difference between groups using the program SPSS version 16.0. The level of significance was at $p < 0.05$.

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