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Isolation and antioxidative properties of phenolics-saponins rich fraction from defatted rice bran

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ABSTRACT

The study presents a protocol for the preparation of phenolics-saponins rich fraction (PSRF), a new active nutraceutical from defatted rice bran followed by the determination of its antioxidant properties. PSRF was prepared by employing a simple alcoholic fractionation procedure on the crude alcoholic extract (CAE) of defatted rice bran. PSRF was found to be significantly higher in the contents of total phenolic, saponin, and steroidal saponin than CAE and its counterpart, aqueous fraction (AqF) ($p < 0.05$). Except for iron chelating activity, PSRF exhibited notably higher activity than CAE and AqF in all antioxidant activity assays performed ($p < 0.05$). HPLC-DAD analysis revealed that PSRF contained substantially higher amounts of gallic acid, 4-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, and ferulic acid than CAE and AqF ($p < 0.05$). In conclusion, alcoholic fractionation of CAE simultaneously concentrated the phenolic compounds and saponins into PSRF, thus contributed to its higher antioxidant activity. Due to its elevated antioxidant properties, PSRF may be recommended for investigation as an active ingredient in the nutraceutical, functional food, and natural food preservative formulations. This is also the first report suggesting defatted rice bran as a potential and sustainable source of saponins.

1. Introduction

Rice is the staple food of almost all Asian countries and rice bran is the agricultural by-product of rice milling industry. In Malaysia, the annual production of rice paddies is about 2.4 million tons (Department of Statistics, Malaysia, 2011), out of which 56–58% is white rice, 10–12% broken rice, 18–20% husks, and 10–12% rice bran. Owing to rapidly increasing awareness and the corresponding mounting demand for rice bran and rice bran oil as healthy functional food ingredients, defatted rice bran has emerged as another potential by-product obtained as rice bran meal after the extraction of oil from rice bran. The production of defatted rice bran has proportionally increased with the rise of oil extraction from rice bran. Currently, defatted rice bran is still an underutilized secondary by-product and is generally disposed of or utilized as a low-cost animal feed ingredient. Due to the presence of substantial amounts of bioactive compounds including phenolics, defatted rice bran has strong antioxidative potentials (Devi and Arumughan, 2007; Mariod et al., 2010). The antioxidant properties of phenolics have been well-proven and investigations are in progress for the evaluation of their health-promoting, disease-preventative, and food protection properties (Gülçin, 2012; Saxena et al., 2012).

Besides phenolics, saponins are also of vital biochemical interest; they have been reported to possess appreciable antimicrobial (Hassan et al., 2010), hypocholesterolemic, and antiobesity properties (Zhao et al., 2005). Saponins are glycosides consisting of a steroidal or terpenoid aglycone, connected to one or more sugar moieties, and are potentially present in a number of plants such as beans, peas, lentils, and lupins (Guclu-Ustundag and Mazza, 2007). Saponins are of vital significance due to their ability to attach and bind proteins, which enhances protein stability against heat denaturation and decreases the susceptibility of proteins to proteases. These attributes have prompted researchers and health professionals to explore novel natural sources of saponins, preferably based on botanical origin. A number of plants have been successfully explored as potential sources of saponins, but it would be more economically advantageous if saponins could be obtained

Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid); AqF, aqueous fraction; BCB, β-carotene bleaching; BF, *n*-Butanol fraction; CAE, crude alcoholic extract; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EDTA, ethylenediaminetetraacetic acid; HPLC-DAD, high performance liquid chromatography with diode-array detection; PSRF, phenolics-saponins rich fraction; TPC, total phenolic content; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TSC, total saponin content; TSSC, total steroidal saponin content.

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from the discarded materials of high availability. The material under investigation in the present study, defatted rice bran, falls well within the focus of these researches. No reports investigating saponins from rice bran and defatted rice bran has been presented thus far. This report, for the first time, demonstrates that defatted rice bran is a potential source of saponins.

The preparation of bioactive-rich fractions with improved concentrations of certain bioactive compounds has conveniently gained momentum due to possible synergistic actions of different bioactives present in relatively higher concentrations compared to the individual functions of bioactive compounds. Phenolics and saponins, due to their signature antioxidant, antimicrobial, and hypocholesterolemic activities, possess significant food preservation, health promoting, and disease preventing properties. Their potentials in the control of food spoilage and rancidity is encouraging. In order to fulfill the industrial demands, development of some simple processing strategies for their simultaneous extraction and concentration is necessary. It would be time and cost effective if both of these valuable compounds could be concentrated simultaneously from a readily available source.

This study aimed to propose a simple protocol for the fractionation of defatted rice bran with different solvent systems for the preparation of a fraction containing simultaneously concentrated saponins and phenolics, followed by the determination of its antioxidant activity. It was hypothesized that the known bioactivities of defatted rice bran, attributed to phenolic compounds, could be further enhanced with the presence of saponins. Based on the findings from this study, the potential of the phenolics-saponins rich fraction (PSRF) from defatted rice bran in functional foods and nutraceuticals development is highly promising. The study is of significant worth in the context of exploring a highly valuable bioactives from secondary agrowaste material, i.e. defatted rice bran.

2. Materials and methods

2.1. Chemicals

All chemicals used were of analytical or HPLC grade. Methanol, chloroform, and Tween 20 were obtained from Fisher Scientific (Loughborough, Leicestershire, UK). Linoleic acid, gallic acid, β-carotene (Type I synthetic, 95%), anisaldehyde, sodium bicarbonate, diosgenin, vanillin, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), potassium persulfate, ferrous sulfate, hydrogen peroxide, ferrous chloride, ethylenediaminetetraacetic acid (EDTA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), α-tocopherol, Folin–Ciocalteu phenol reagent, and ferrozine were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). *n*-Hexane, *n*-butanol, absolute ethanol, sulfuric acid, ethyl acetate, acetonitrile and acetic acid were procured from Merck (Darmstadt, Germany).

2.2. Defatting of rice bran

Freshly generated rice bran was purchased from Padiberas Nasional Berhad (BERNAS, Kuala Lumpur, Malaysia). Samples were immediately subjected to microwave heating at 150 °C for up to 3 min for stabilization against microbial spoilage and hydrolytic rancidity. Samples were then cooled to room temperature, packed in ice bags, and transported to the laboratory. Dried samples were homogenized (Ultra-Turax T25 basic, IKA®-WERKE GmbH & Co. KG, Staufen, Germany) with *n*-hexane at a ratio of 1:2 (w/v) for 15 min at 9500 rpm. The homogenized mixture was filtered through Whatman No. 2 filter paper (Whatman Int. Ltd., England) and the residue was re-extracted twice following the same procedure to obtain defatted rice bran. Then, defatted rice bran was collected and dried in an oven at 50 °C for 3 h in order to remove any residual solvent left. Finally, defatted rice bran was passed through a 0.6 mm sieve and kept at −20 °C prior to the extraction process. The final moisture and fat contents of defatted rice bran were 9.53 ± 0.02% and 0.33 ± 0.03%, respectively.

2.3. Extraction and fractionation of defatted rice bran

A schematic representation of the extraction and fractionation procedures of defatted rice bran is depicted in Fig. 1. Briefly, defatted rice bran was homogenized and refluxed with 50% aqueous ethanol, at the ratio of 1:15 (w:v) for 3 h to obtain the crude alcoholic extract (CAE). The resulting mixture was filtered through Whatman No. 2 filter paper prior to solvent removal under reduced pressure (Rotavapor R210, Buchi, Postfach, Flawil, Switzerland) and lyophilization (Virtis Benchtop K Freeze Dryer, SP Industries, Warminster, PA, USA). Subsequently, 1 g of CAE was dispersed into 25 mL of distilled water and partitioned with 125 mL of *n*-hexane to remove any residual lipids. After separation from the *n*-hexane layer, the aqueous layer was homogenized (Ultra-Turax T25 basic, IKA®-WERKE GmbH & Co. KG, Staufen, Germany) with 125 mL of *n*-butanol at 9500 rpm for 15 min followed by sonication (PowerSonic 505, HwaShin Technology Co., Seoul, Korea) for 60 min at room temperature. Subsequently, the mixture was left at room temperature until both solvent layers were well-separated from each other. This partitioning procedure was repeated twice further and the *n*-butanol fraction (BF) was pooled and concentrated to dryness under reduced pressure (Rotavapor R210, Buchi, Postfach, Flawil, Switzerland). The aqueous fraction (AqF) left was subjected to lyophilization (Virtis Benchtop K Freeze Dryer, SP Industries, Warminster, PA, USA). The yield of CAE, BF, and AqF was measured and the fractions were stored at −80 °C until further analysis.

2.4. Determination of total phenolic, saponin and steroidal saponin contents

The total phenolic content (TPC) of CAE, BF, and AqF was determined using the Folin–Ciocalteu reagent assay. In brief, 5 mg of the extract/fraction were individually dissolved in 1 mL of

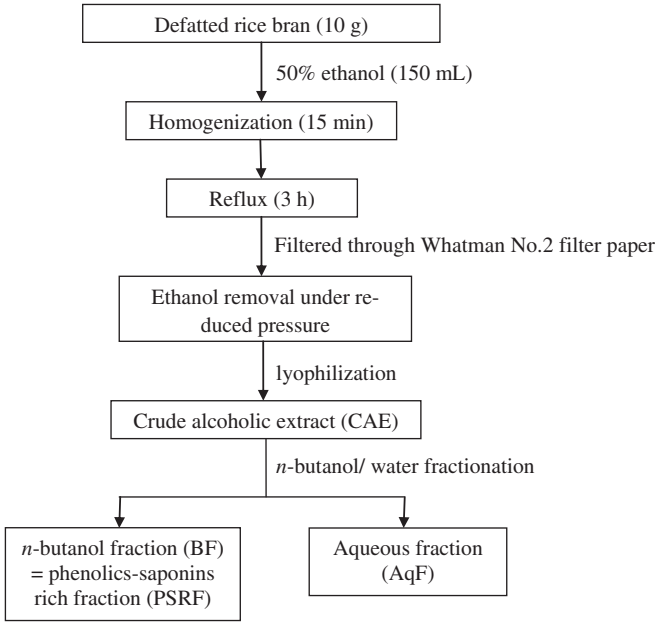

Fig. 1. Flow sheet diagram for the preparation of crude alcoholic extract (CAE), *n*-butanol fraction (BF), and aqueous fraction (AqF) from defatted rice bran.