Protocol of protein concentration

Briefly, Proteins were extracted with water (flour: water = 1: 10) at pH 11.0 using a solution 2 M NaOH and a continuous agitation speed of 500 rpm for 2 h. Flour suspension was centrifuged at 9000 × g for 30 min and the clarified supernatants were pooled and adjusted to pH 4.0 using 2 M HCl. Protein precipitate was centrifuged at 9000 × g for 30 min. phytochemicals were extracted using 1:20 (w/v) solid-to-liquid ratio using ethanol as solvent for 2 h at room temperature in the dark. Phenolic compounds were determined in alcoholic extracts by Folin-Ciocalteau method. The extraction procedure was repeated until phenolic compounds were not detected in alcoholic extracts. The mixture was filtered using filter paper. Finally, the pellet was freeze-dried and stored at −20 °C until analysis.

ABTS scavenging ability

A stock solution of ABTS radicals included 20 mL of ABTS solution (7 mM) and 10 mL of potassium persulphate solution (88 mM) and kept overnight in dark at room temperature for 12 h to generate $ABTS^+$ cation. Working solution of $ABTS^+$ was than prepared, by diluting stock solution with 0.1M PBS to an absorbance of 0.7 ± 0.05 at 734 nm. Fraction (20 µL) was allowed to react with 200 µL of $ABTS^+$ working solution, followed by the incubation in dark for 6 min at room temperature. Absorbance was performed at 734 nm using a micro-plate reader. A standard calibration curve with Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acid) was used ($y = 0.37x - 0.72$, $r^2 = 0.9995$, where y = ABTS scavenging ablitiy (%)

and x = Trolox concentration (μM)) and ABTS radical-scavenging capacity was evaluated by using the equation:

ABTS scavenging ablitiy (%) = ($A_{blank} - A_{Sample}$) / A_{Blank} x 100,

Where A_{sample} is absorbance of mixture containing sample and A_{blank} is absorbance of mixture containing water instead of sample.

Ferrous ion (Fe^{2+}) chelating ability

Fraction (50 μL) was added with 50 μL of ferrous chloride (1 mM) and 100 μL of ferrozine (5 mM), followed by the incubation in dark for 10 min at room temperature. Absorbance was performed at 580 nm using a micro-plate reader. A standard calibration curve with EDTA-2Na ($y = 22.858\ln(x) - 65.964$, $r^2 = 0.9961$, where y = Fe^{2+} chelating ability (%) and x = EDTA-2Na concentration (μg/mL)) was used and ferrous ion (Fe^{2+}) chelating ability was evaluated by using the equation:

Ferrous ion (Fe^{2+}) chelating ability (%) = ($A_{blank} - A_{Sample}$) / A_{Blank} x 100,

Where A_{sample} is absorbance of mixture containing sample and A_{blank} is absorbance of mixture containing water instead of sample.

Ferric reducing antioxidant power (FRAP)

Freshly prepared FRAP reagent (200 μL) was mixed with 20 μL of sample. The FRAP reagent was prepared by mixing 0.1 M sodium acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-Tri(2-pyridyl)-1,3,5-triazine) in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ in the ratio of 10:1:1. After 10 min of incubation at room temperature, the absorbance

was measured at 595 nm. A freshly prepared standard solution of Trolox was used for preparation of calibration curve ($y = 0.0027x + 0.0016$, $r^2 = 0.9999$, where y = Absorance and x = Trolox concentration (μM)).

Oxygen radical absorbance capacity (ORAC) assay

Briefly, 120 μL fluorescein disodium (100 nM in 73 mM PBS with pH 7.4) and 20 μL sample were transferred into a 96-well microplate and incubated at 37 °C for 15 min. After mixing with 60 μL of AAPH (2,2'-Azobis(2-methylpropionamide) dihydrochloride) (80 mM), the plate was automatically shaken before the first reading, and the fluorescence was recorded at 2 min intervals for 120 min. A freshly prepared standard solution of Trolox was used for preparation of calibration curve ($y = 0.0801x + 0.8005$, $r^2 = 0.9916$, where y = AUC and x = Trolox concentration (μM)). The area under the fluorescence decay curve (AUC) was calculated as followed:

$AUC = 1 + \sum F_i/F_{0,}$

where F_0 is the initial fluorescence reading at 0 min and F_i is the fluorescence reading at time I (i = 1–120).

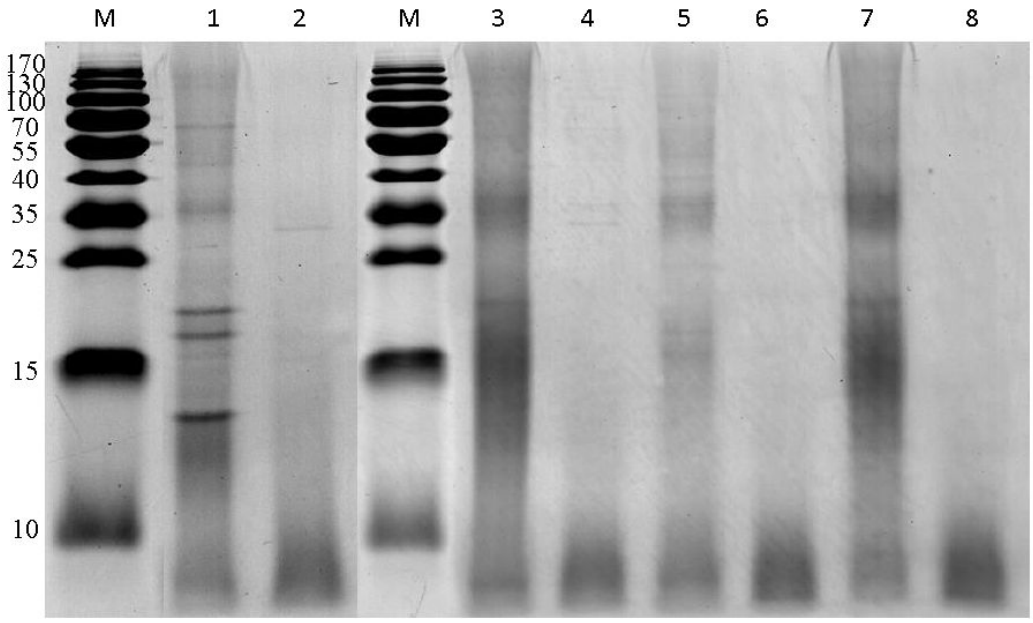

Fig. S1. SDS-PAGE of foxtail millet protein extracts and protein hydrolysates. M = marker, 1 = RAW protein, 2 = RAW protein digest, 3 = GR protein, 4 = GR protein digest, 5 = GRB protein, 6 = GRB protein digest, 7 = GRM protein, 8 = GRM protein digst

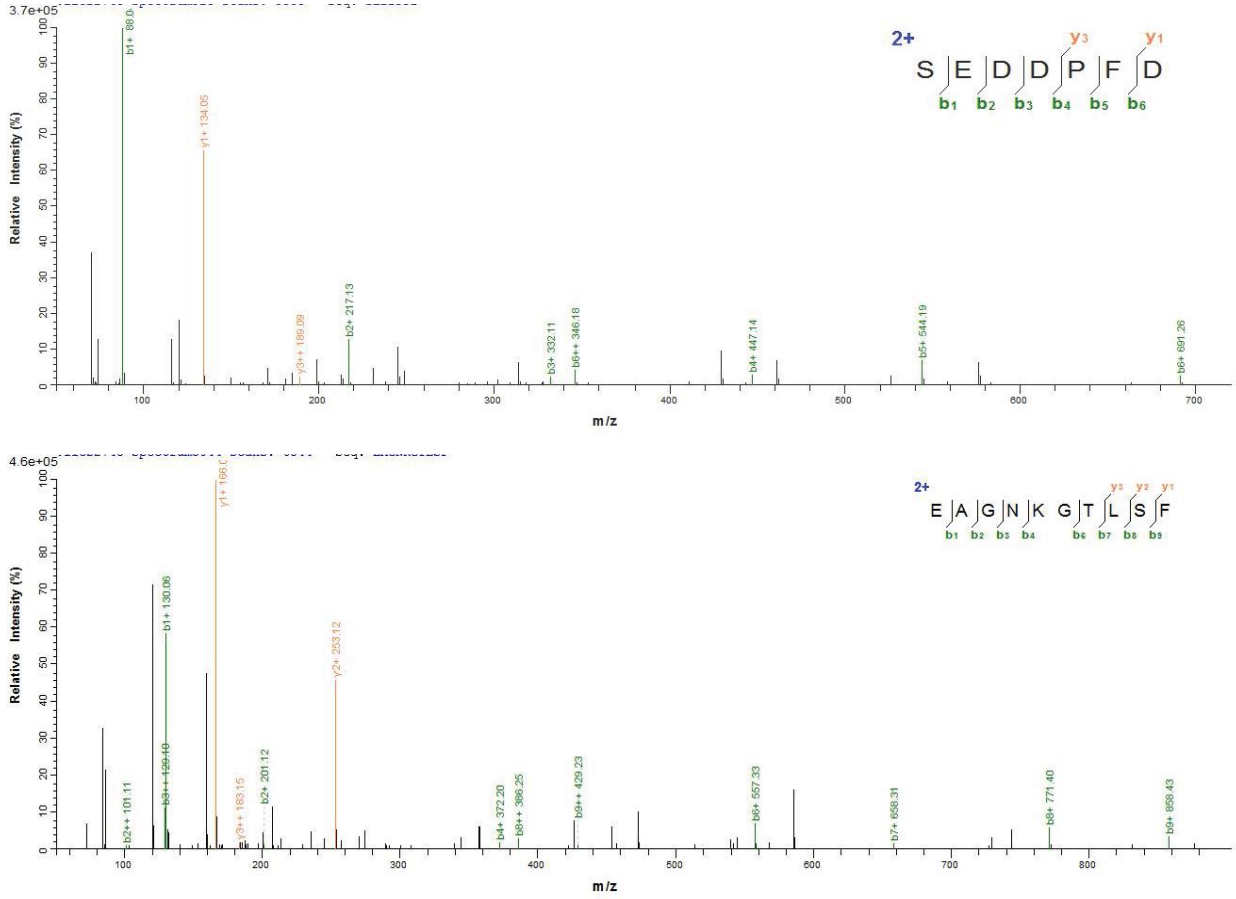

Fig. S2. The MS/MS spectra of the identified peptides (A) SEDDPFD, (B) EAGNKGTLSF

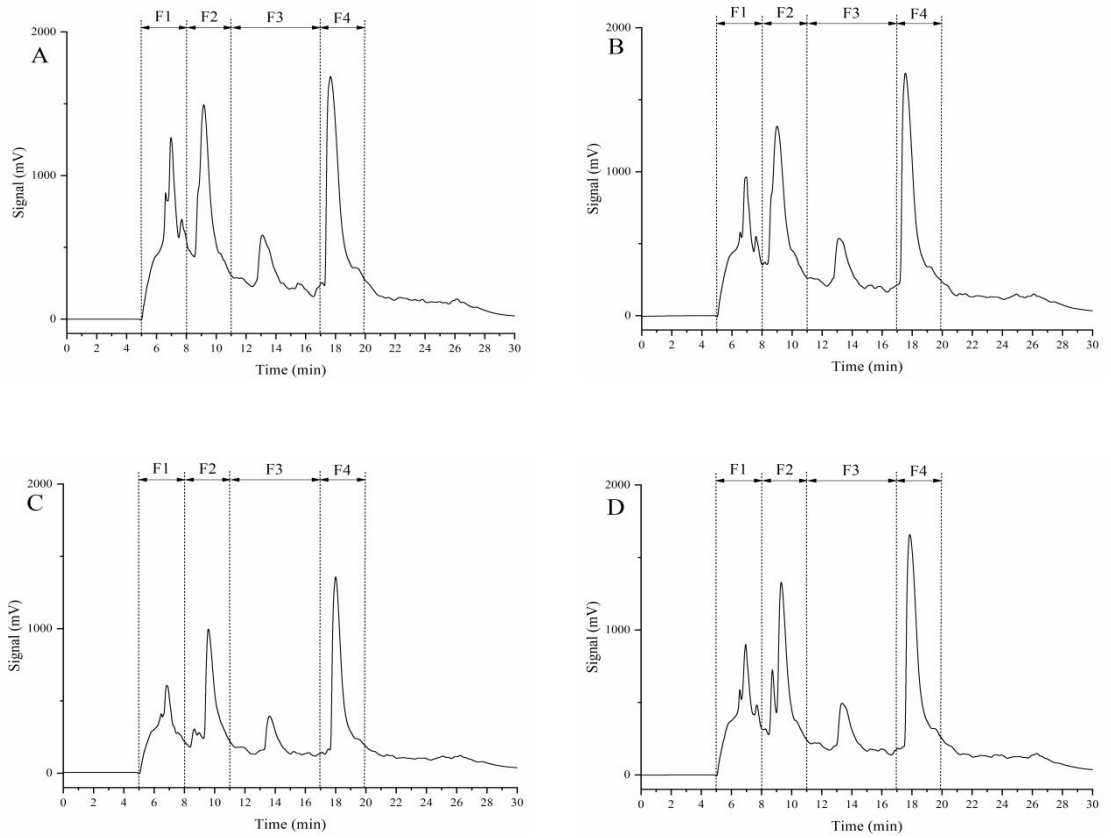

Fig. S3. The chromatograms of < 3kDa peptide fraction of (A) RAW, (B) GR, (C) GRB and (D) GRM *in vitro* gastrointestinal digest obtained by semi-preparative RP-HPLC, collected fractions were coded as F1–F4