



Antioxidant and type II diabetes related enzyme inhibition properties of methanolic extract of an underutilized food legume, *Canavalia ensiformis* (L.) DC: Effect of traditional processing methods

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ABSTRACT

The methanolic extract of *Canavalia ensiformis* (L.) DC (Jack bean) seed, an underutilized food legume collected from India was analyzed for antioxidant and health relevant functional properties. The raw seeds contained total free phenolic content of 12.98 ± 1.63 g catechin equivalent/100 g extract DM. The raw seed extract exhibited ferric reducing/antioxidant power (FRAP, 1218 mmol Fe[II]/mg extract), inhibition of β -carotene degradation (49.81%), radical scavenging activity against DPPH (56.78%) and superoxide (35.89%). In addition, 77.56% of α -amylase and 75.45% of α -glucosidase enzyme inhibition characteristics were found under *in vitro* starch digestion bioassay. Sprouting + oil-frying caused an apparent increase on the total free phenolic content with significant improvement on antioxidant and free-radical scavenging capacity, while soaking + cooking as well as open-pan roasting treatments showed diminishing effects. Inhibition of α -amylase and α -glucosidase enzyme activities were declined to 22.69 and 42.69%, respectively during sprouting + oil-frying treatment is more desirable for the dietary management of type II diabetic patients.

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1. Introduction

Natural antioxidants from plant-based sources offer an alternative source of dietary ingredients to promote healthy life. For example, α -amylase and α -glucosidase inhibitors are known to regulate/prevent hyperglycemia linked type II diabetes. In this research health relevant functionality of seeds from *Canavalia ensiformis* (L.) DC (commonly known as jack bean) is explored. The *C. ensiformis* originated from South America and grown in the tropics and subtropics produces high yields especially in regions of low altitude, high temperature and relative humidity (Molina, Argueta, & Bressani, 1974). Although some may be cultivated in drought-prone areas of Arizona and Mexico, *C. ensiformis* is considered one of the few pulses that grow well on highly leached, nutrient-depleted low land tropical soils. *C. ensiformis* is often grown on walls as an ornamental plant in the North Coast of Columbia and Nigeria (Udedibie, 1990) and as cover crop in western countries. Total yield of forage and dry seeds of *C. ensiformis* range from 1 to 10 t/ha/yr (Kessler, 1990).

The plant is often used as high protein food and forage crop in Southwestern United States, Mexico, Central American countries,

Brazil, Peru, Ecuador and West Indies (Sauer & Kaplan, 1969). *C. ensiformis* seed comprises of 84–89% cotyledon and 11–15% of seed coat (Sridhar & Seena, 2006) and seed coat colour range from maroon to red (Janardhanan, Vadivel, & Pugalethi, 2003). Indian tribal sects, Kurumba, Malayali, Irula and other Dravidian groups cook the mature seed as food (Janardhanan et al., 2003), the westerns consume immature pods of *C. ensiformis* as vegetables, and the seeds are roasted and grounded to make coffee-like drink (Bressani, Brenes, Gracia, & Elias, 1987). The proximate composition of the jack bean seeds are 24–32% crude protein, 1.8–9.6% crude lipid, 4.65–10% crude fibre, 2–4.6% ash and 43–60% carbohydrates (Janardhanan et al., 2003). It is known to have anti-nutritional compounds such as concanavalin A, L-canavanine, canatoxin, polyamines, protease inhibitors, flatulence factors, cyanides, saponins, urease and L-Dopa.

The seed decoction or powdered seeds from *C. ensiformis* are used as an antibiotic and antiseptic (Gill & Nyawuame, 1994). Proteins with complete sequence homology to bovine insulin present in *C. ensiformis* seed coat are used as antihuman insulin antibodies (Oliveira, Sales, Machado, Fernandes, & Xavier, 1999). It can be used as treatment for diabetes as it has been shown to significantly lower the blood glucose level in alloxanized mice. Seed proteins of *C. ensiformis* have considerably decrease cholesterol level in hypercholesterolemia rats (Marfo, Wallace, Timpo, &

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Simpson, 1990) and Marchetti, Mastromarino, Rieti, Seganti, and Orsi (1995) found inhibition of herpes simplex, rabies and rubella viruses by *C. ensiformis* lectins. In addition, Swaffar and Ang (1999) have also demonstrated the inhibitory effect of L-canavanine isolated from *C. ensiformis* against MIA PaCa-2 pancreatic cancer cells.

There can be no doubt that nutritional value of seeds and medicinal properties of different parts of *C. ensiformis* plant are greatly reported, but there has been very little information on antioxidant and medicinal properties of seed materials. Hence, the present research attempts to analyze the total free phenolics content, antioxidant and type II diabetes related enzyme inhibition properties of methanolic extract of raw and traditionally processed *C. ensiformis* seeds.

2. Materials and methods

2.1. Chemicals

(+)-Catechin hydrate, polyvinylpyrrolidone, butylated hydroxytoluene, 2,4,6-tris-(2-pyridyl)-s-triazine, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), β -carotene, linoleic acid, tween-40, riboflavin, methionine, nitro-blue tetrazolium, starch, α -amylase, and α -glucosidase and *p*-nitrophenyl- α -D-glucopyranoside were from Sigma–Aldrich Chemicals, USA, and all other chemicals were from Merck, Darmstadt, Germany.

2.2. Seed samples

The seed of *C. ensiformis* (red colour seed coat) were collected from different locations of South India such as Kempnaickenpalayam (Erode District, Tamilnadu), Vazhikkadavu (Malapuram District, Kerala), Gundalpet (Mysore District, Karnataka) and Neyar Dam (Trivandrum District, Kerala). Seed sample from each location (about 500 g) was aggregated and mixed from 8 to 15 plants in order to obtain a representative sample. After acquisition, the seed samples were frozen immediately and stored at -80°C prior to experiments. Seed samples were randomly divided into four batches of five replicates (each consist of 25 g seeds) for different processing methods. The first batch was stored without any treatment and considered as raw seeds and the remaining three batches were treated differently (as described below).

2.3. Soaking + cooking

The seeds from second batch were soaked in distilled water in ratio of 1:10 (w/v) and kept in darkness for 8 h at 25°C . Then the seeds were removed and cooked with fresh distilled water (in a ratio of seed to water, 1:5 w/v) at $85\text{--}90^{\circ}\text{C}$ on a hot plate until the seeds become soft when felt between fingers (about 30 min). This treatment method was adapted from Kanikkar tribe living in Kerala state, India.

2.4. Sprouting + oil-frying

Clean red-soil (100 g) was made into paste with distilled water in a ratio of 1:5 (w/v) and place in tray (five replications). Then, 25 g from the third batch of samples were mixed into the red-soil suspension. The trays were covered with a moist cloth and kept for 2 days in dark at 25°C . Then grown sprouts were separated and washed thoroughly with tap water before frying in sunflower oil at $185\text{--}190^{\circ}\text{C}$ on a hot plate for about 15 min. This treatment is based on the practice of Lambadi ethnic group of Karnataka and Andhra Pradesh states, India.

2.5. Open-pan roasting

The fourth batch of seed was roasted for 30 min at $120\text{--}130^{\circ}\text{C}$ on an iron pot containing clean and fine acid-treated sand. The seeds were then separated using a sieve and allowed to cool to room temperature. This treatment method was adapted from the Uraali tribal group living near the Kadambur hills of Sathya-mangalam, Erode District, Tamilnadu state, India.

2.6. Preparation of methanolic extract

After each individual treatment, all the raw and processed samples (except roasted samples) were frozen at -80°C and freeze-dried for 10 h. Firstly, the samples were cracked into small pieces with a wooden hammer, grinded (Siemens, Germany) into powder form (1 mm particle size), and freeze-dried for 8 h before storing at -20°C . Secondly, one gram of each sample was treated with petroleum ether (1:10 w/v) overnight on a magnetic stirrer, centrifuged at 3000 rpm for 10 min and the supernatant was discarded. Then the defatted residue was air-dried, extracted with 10 ml of 100%, 80%, 70% and 50% methanol acidified with 1% conc. of HCl in an ultra-sonic bath (Bandelin Sonorex, RK – 514 H, Berlin, Germany) for 10 min and stirred magnetically for 30 min. After centrifugation, the supernatants were pooled and made up to a known volume. The extract was treated with 5 g of polyvinylpyrrolidone at 0°C for 30 min and the supernatant was collected and purified with Solid Phase Cartridge (Strata-x-33 μm polymeric sorbent, L100-1105, 200 mg/6 ml sample, 8B-S100-FCH-S, Phenomenex, USA). The purified phenolics were then eluted with 10 ml of 50% and 100% methanol, evaporated using rotary vacuum evaporator (Büchi Rotavapor – R, CH-9230, Switzerland) at 40°C and dried in lyophilizer (Virtis Freeze mobile 25 EL, New York) for 1 h. Finally the residue was weighed to calculate the total dry yield of extract. The extract was then re-dissolved in water:-methanol:formic acid (47.5:47.5:5%, v/v/v) solution in the ratio of 1 mg/ml of solvent to be used for further analysis.

2.7. Analytical methods

The total free phenolic content of methanolic extract from raw and processed samples were estimated according to method adapted from Singleton, Orthofer, and Lamuela-Raventos (1999). The ferric reducing/antioxidant power (FRAP) (Pulido, Bravo, & Saura-Calixto, 2000), inhibition of β -carotene bleaching (Miller, 1971), radical scavenging activity against DPPH (Sanchez-Moreno, Larrauri, & Saura-Calixto, 1998) and superoxide (Zhishen, Mengcheng, & Jianming, 1999) as well as α -amylase and α -glucosidase inhibition activities (Worthington, 1993) of the methanolic extract were analyzed.

2.8. Statistical analysis

All data were analyzed and expressed as means $\pm$ standard deviation (in five replications, $n = 5$). One-way ANOVA with Dunnett's post test were used to determine the significant differences between the experimental batches as well as correlation analysis were performed using GraphPad PRISM[®] version 5.00 for Windows, San Diego, California, USA.

3. Results and discussion

3.1. Total free phenolics

The total free phenolics content of methanolic extract of defatted raw seeds of *C. ensiformis* was found to be 12.98 g catechin equivalent/100 g extract DM (Table 1). This value was higher as

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