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Total phenolic content and antioxidant potential of traditionally processed *Mucuna monosperma* seeds: An Indian under-utilized legume grain

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Introduction

Apart from common legume seeds, the earlier research works demonstrated the nutritive potential of certain promising under-utilized/wild legume grains, including the pulses of tribal utility. In this context, the seed materials of *Mucuna monosperma* DC ex Wight (common name: Negro bean, local name: periyattalargai), an Indian underutilized food legume received more attention. The boiled Negro bean seeds are eaten by tribes of Northeastern India, the Oceanic group of tribes, the Onges, Great Andamanese and Sompens. The biochemical composition and nutritional potential of Negro bean seeds are investigated by PUGALENTHI ET AL. (2003). Crude proteins, crude lipids, ash and nitrogen free extractives constituted 30.62%, 9.03%, 5.99% and 42.79%, respectively. The calorific value of 100 g dry matter of seed material is 374.91 kCal. The essential amino acids, leucine and isoleucine are present in relatively large quantities. The seeds are rich in minerals like potassium, Calcium, Magnesium and Iron (PUGALENTHI ET AL., 2003). Even though, the nutritional value of Negro bean seeds has reported earlier, there is no information regarding the antioxidant properties. Hence, the present study was carried out to analyze the total free phenolics content, antioxidant properties of methanolic extract of raw and traditionally processed Negro bean seeds with a view to promote them as a dietary ingredient in the supplementary therapeutic foods.

Material and Methods

The seed materials of Negro bean were collected from different locations of Tamil Nadu, India (Thudialur, Nambiyur, Othakuthirai, Pachamalai, and Panrutti). The first batch was stored without any treatment and considered as raw seeds. The whole seeds of second batch (25 g in each replicate) were soaked in distilled water in the ratio of 1:10 (w/v) for 8 h at 25°C and then cooked with fresh distilled water at 85-90°C (about 30 min). Third batch of samples were added into the red-soil suspension (1:5, W/V) and kept for 2 days in dark under moist cloth. Then the sprouts were separated, thoroughly washed and fried in sunflower oil at 185-190°C for 10 min. The fourth batch of seed materials roasted in a in an iron pot for 30 min at 120-130°C. One gram of powdered sample was treated with petroleum ether (1:10 w/v) for overnight on a magnetic stirrer, centrifuged at 2,800 x g for 10 min and the supernatant was discarded. Then the defatted residue was air-dried and sequentially extracted with 10 mL of 100%, 80%, 70%, and 50% methanol acidified with 1% conc. HCl in an ultra-sonic bath for 10 min followed by extraction in magnetic stirrer for 30 min. The extract was treated with 5 g of polyvinylpolypyrrolidone at 0°C for 30 min and purified by using a solid phase catridge and then the solvent was evaporated using

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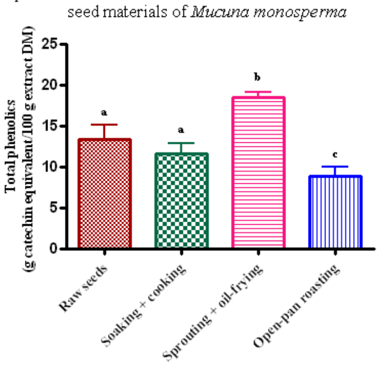
rotary vacuum evaporator and dried in lyophilizer and finally the residue was weighed and the total dry yield of extract was calculated. Then the extract was re-dissolved in water:methanol: formic acid (47.5:47.5:5%, v/v/v) solution in the ratio of 1 mg/mL of solvent and used for further analysis. The total free phenolic content of methanolic extract of each sample (raw and processed) with five replicate was estimated according to the method of SINGLETON ET AL. (1999). The ferric reducing/antioxidant power (FRAP) (PULIDO ET AL., 2000), inhibition of *β*-carotene bleaching (MILLER, 1971), DPPH (SANCHEZ-MORENO ET AL., 1998) and superoxide radical scavenging activities (ZHISHEN ET AL., 1999) of the methanolic extract were analyzed.

Results and Discussion

Total free phenolics

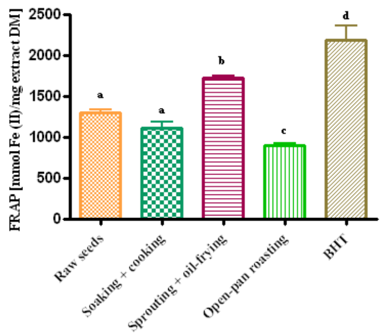
The total free phenolics content of methanolic extract of defatted raw seeds of Negro bean was found to be 14.80 g catechin equivalent/100 g extract DM (Figure 1). Such high yield of total free phenolics might be due to the repeated extraction of phenolic compounds using different concentrations of acidified methanol as a solvent. Because, recovery of phenolic compounds from food samples are mainly depend upon the type of solvent used and the type and duration of extraction. It is interesting to notice that the seed coat colour of Negro bean sample is dark brown. In addition to seed coat colour, the quantity of phenolic compounds in seed samples is influenced by soil, environmental conditions, genotype (cultivar/variety), agronomic practices (irrigation, fertilization and pest management), maturity level at harvest and post-harvest storage. Since, Negro bean grows wildly in adverse environmental conditions such as drought, poor soil *etc*., a high phenolic content in the seed materials contribute to the resistant function.

Fig. 1. Total free phenolic content in methanolic extract of raw and traditionally processed seed materials of *Mucuna monosperma*



Values are mean and ± standard deviation of five separate determinations.
Bars with different alphabet superscripts are significantly different ($p < 0.05$).

Fig. 2. Ferric reducing/antioxidant potential (FRAP) of methanolic extract of raw and differentially processed *Mucuna monosperma* seeds



Values are mean and ± standard deviation of five separate determinations.
Bars with different alphabet superscripts are significantly different ($p < 0.05$).

Reducing power

Ferric reducing/antioxidant power (FRAP) reflects total antioxidant power involving the single electron transfer reaction. Antioxidant potential of methanolic extracts of Negro bean seeds was estimated from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) complex. The reducing power of methanolic extract of raw seed materials of Negro bean was found to be 1,023 mmol Fe [II]/mg extract DM (Figure 2), which is also expected to demonstrate potential antioxidant activity in consumer's body.

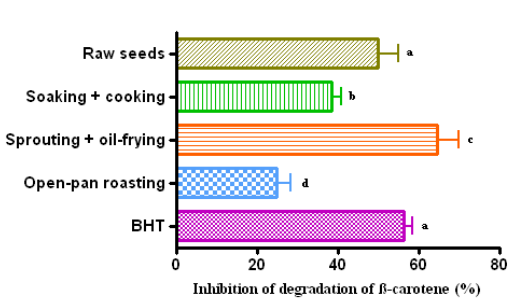
Inhibition of ß-carotene degradation

The *ß*-Carotene bleaching method is based on the loss of yellow colour of *ß*-carotene due to its reaction with radicals, which are formed by linoleic acid oxidation in an emulsion. The capacity of antioxidant compounds to prevent the discoloration/degradation of *ß*-carotene during the auto-oxidation of linoleic acid was measured. Antioxidant activity of 59.35% was demonstrated by the methanolic extract of raw Negro bean seeds in term of inhibition of *ß*-carotene degradation, which is more or less equal to that of positive control BHT (Figure 3). The results indicate that the presence of phenolic compounds in methanolic extract of Negro bean seeds can moderately prevent the degradation of ß-carotene caused by radical reactions.

DPPH radical scavenging activity

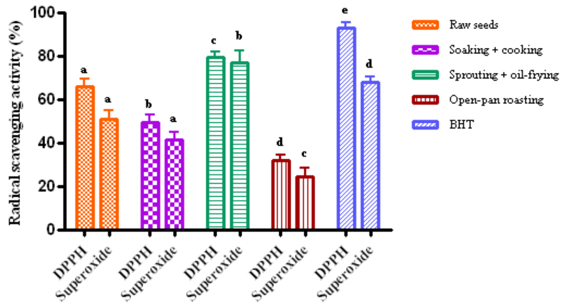
DPPH (2,2-Diphenyl-1-picrylhydrazyl), a stable organic free radical has a maximum absorption at 517 nm but upon reduction by an antioxidant, the absorption disappears. The DPPH radical scavenging activity of methanolic extract obtained from raw Negro bean seeds was found to be 72.12% (Figure 4). The interaction of phenolic compounds with DPPH was depending upon their structural confirmation; O-H bound dissociation energy, resonance delocalization of the antioxidant and steric-hindrance derived from bulky groups substituting hydrogen in the antioxidant compound. Potential radical scavenging activity revealed by the Negro bean seeds against a synthetic DPPH radical might confirm its hydrogen donating capacity.

Fig. 3. Inhibition of β-carotene degradation by methanolic extract of raw and differentially processed *Mucuna monosperma* seeds



Values are mean and ± standard deviation of five separate determinations. Bars with different alphabet superscripts are significantly different ($p < 0.05$

Fig. 4. DPPH and superoxide radical scavenging activities of methanolic extract of raw and differentially processed *Mucuna monosperma* seeds



Values are mean and ± standard deviation of five separate determinations. Bars with different pattern and different alphabet superscripts are significantly different ($p < 0.05$).

Superoxide radical scavenging activity

Superoxide radical is a biologically quite toxic oxygen molecule with one unpaired electron. Although it is a weak oxidant, it gives rise to the generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to the oxidative stress and lead to the genesis of several chronic diseases in human beings. The methanolic extract of Negro bean seeds deciphered a moderate scavenging activity (43.11%) against superoxide radicals (Figure 4). Thus, incorporation of such non-traditional legume grains with notable phenolic content in the regular diets of human population could play a preventive role against the superoxide radicals, and thus confers the alleviation of oxidative stress and ultimately disease protection in human body.

Effect of soaking + cooking

Significant level of reduction of total free phenolics was noticed during soaking + cooking treatment (20%) (Figure 1). Such significant loss during this treatment might be due to the leaching out of this compound into the soaking medium by increased permeability of the seed coat and also due to degradation of phenolics with the high temperature during subsequent cooking for a longer period (30 min). Soaking + cooking adversely affected the antioxidant activities (Figure 2-4), which might be due to the degradation of phenolic compounds under cooking at elevated temperature. Hence, such soaking + cooking treatment is not recommendable to use the Negro bean seeds as a natural source of antioxidants.

Effect of sprouting + oil-frying

It is important to recognize that, an appreciable level of increase of total free phenolics (21%) was observed during sprouting + oil-frying in Negro bean (Figure 1). This might be due to mobilization of stored phenolics by the activation of enzymes like polyphenol oxidase during sprouting and also due to the release of free phenolics from bounded form by the breakdown of cellular constituents and cell walls during subsequent thermal process (oil-frying). Sprouting + oil frying substantially increased the antioxidant activity (Figure 2-4), which was obviously attributed to elevation of its phenolic content. A significant level of positive correlation was noticed between the phenolic content and antioxidant properties of Negro bean seeds.

Effect of open-pan roasting

Open-pan roasting caused drastic losses of total free phenolics (30%) (Figure 1) as well as antioxidant properties (Figure 2–4) in the presently analyzed legume sample. This might be due to the disintegration of phenolic compounds by the direct action of high temperature during roasting. Therefore, open-pan roasting could be considered as a most aggressive practice and not a suitable method to preserve the phenolic compounds and their antioxidant properties in Negro bean seeds.

Conclusions and Outlook

Methanolic extract of Negro bean seed materials was found to contain appreciable levels total free phenolics with promising antioxidant properties. Considering the effect of different indigenous processing methods, sprouting + oil-frying extensively increased the total free phenolics content as well as antioxidant properties of the presently investigated wild type legume grain. Such viable processing technique could offer a good strategy to improve the phenolic content in Negro bean seeds for enhanced antioxidant activity. Therefore, such suitably processed Negro bean could be envisaged as a dietary ingredient in the formulation of supplementary foods with therapeutic value.

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