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Total phenolic content, *in vitro* antioxidant activity and type II diabetes relevant enzyme inhibition properties of methanolic extract of traditionally processed underutilized food legume, *Acacia nilotica* (L.) Willd ex. Delile

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Abstract: The methanolic extract of *Acacia nilotica* (L.) Willd ex. Delile (black thorn tree) seed materials, an underutilized food legume collected from India was found to contain total free phenolic content of 14.57 ± 1.69 g catechin equivalent/100 g extract DM. Encouraging levels of ferric reducing/antioxidant power (FRAP, 1840 mmol Fe[II]/mg extract), inhibition of *ß*-carotene degradation (53.26%) and radical scavenging activity against DPPH (64.91%) and superoxide (53.23%) radicals were noticed. Further, it also recorded 72.91% of α-amylase and 65.13% of α-glucosidase enzyme inhibition characteristics. Sprouting + oil-frying caused a apparent increase on the total free phenolic content and also significant improvement on the antioxidant and free radical scavenging capacity of methanolic extract of *A. nilotica* seeds, while soaking + cooking as well as open-pan roasting treatments showed diminishing effects. Moreover, inhibition of α-amylase and α-glucosidase enzyme activities was declined to 22.47 and 46.13%, respectively during sprouting + oil-frying treatment, which are more desirable for the dietary management of type II diabetes.

Keywords: *Acacia nilotica* seeds, total free phenolics, antioxidant activity, α-amylase inhibition, α-glucosidase inhibition, indigenous processing methods

Introduction

Legume grains are playing a pivotal role in the accustomed diets of human being throughout the world. 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 seeds of *Acacia nilotica* (L.) Willd ex. Delile (commonly known as black thorn tree), having admirable nutritional value received more attention. It is widely distributed throughout the tropical and subtropical regions of the world. The pods are non-splitting, scented and constricted between the seeds (Van Wyk and Van Wyk, 1997). Flower initiation is triggered by declining temperature and soil moisture (generally during autumn); green pods form during the dry season and ripen at the late dry/early wet season. Mature trees can produce up to 2 – 4 kg seed in a good fruiting season. Both young pods and mature seeds are edible. Pods are straight or slightly curved, 5 – 15 cm long on a pedicel, 0.5 – 1.2 cm wide with constrictions between the seeds giving the appearance of a string of pearls. Pods are fleshy when young, indehiscent, becoming black and hard at maturity. Seeds are deep blackish-brown in color, smooth, sub-circular, compressed, 6 – 7 mm long, 4.5 – 5 mm wide and the weight ranges from 5,000 – 16,000 seeds/kg.

In India, young pods and mature seeds of *A. nilotica* are known to be cooked and eaten by tribal people living in Western Rajasthan (Janardhanan *et al*., 2003). The mature seeds contained 234 g/kg of crude protein, 126 g/kg of crude fibre, 66.6 g/kg of crude fat, 39.7 g/kg of ash and 534 g/kg of carbohydrates on dry matter basis. Potassium, phosphorus, magnesium, iron and manganese occurred in high concentrations. The essential amino acid profile was found to meet the FAO/WHO recommended pattern except for cystine, methionine and threonine. Further, the *in vitro* protein digestibility of raw, dry heat-treated and autoclaved seeds were reported to be 61.2%, 77.4% and 80.2%, respectively. Biological value (60.4% vs. 54.2%), true digestibility (78.3% vs. 68.5%) and net protein utilization (47.3% vs. 37.1%) were significantly higher in autoclaved seeds than in raw seeds (Siddhuraju *et al*., 1996). Although the seeds possess certain antinutritional substances like hydrogen cyanide, phytate and trypsin inhibitor, both dry heating and autoclaving were reported as more effective detoxification methods (Siddhuraju *et al*., 1996). Further, supplementation of *A. nilotica* proanthocyanidins is reported to be a potentially powerful nutritional approach for management

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of cardiovascular disease risk in individuals with both metabolic syndrome and elevated low density lipoprotein cholesterol (Lerman *et al*., 2010).

Ayurvedic medicine practices have declared that green pods and seeds of *A. nilotica* can provide the nutrients and therapeutic ingredients to prevent, mitigate and treat various diseases (Singh *et al*., 2009a). It is used by traditional healers in Chattisgarh District of India for the treatment of various types of cancer. Different parts of *A. nilotica* are known to possess astringent, antibacterial, insect repellent, antioxidant, antidiabetic and antiviral properties (Bachaya *et al*., 2009). Traditionally the bark, leaves, pods and flowers are used against cancer, cold, congestion, cough, diarrhea, dysentery, fever, gall bladder, hemorrhoid, ophthalmia, sclerosis, small pox, tuberculosis, leprosy, bleeding piles, leucoderma and menstrual problems (Ambasta, 1994; Bhargava *et al*., 1998). In West Africa, the bark and gum of this plant are used against cancer, tumors and indurations of liver and spleen, the root for tuberculosis, the wood for smallpox and the leaves for ulcer (Kalaivani and Mathew, 2010). Bark decoctions of this tree are used in African traditional medicine for the treatment of diarrhea, dysentery, respiratory ailments, sore throats, dry coughs, children's fevers and toothache. Hutchings *et al*., (1996) also reported that the bark decoction can be used for eye complaints, as a nerve stimulant and aid for digestion.

Free radical scavenging potential of ethanolic extract of *A. nilotica* leaves was demonstrated by Kalaivani and Mathew (2010), whereas the chemopreventive mechanism of polyphenolics from bark against hepatocarcinoma was investigated by Singh *et al*. (2009b). Antioxidant and anti-quorum sensing activities of green pods of *A. nilotica* was analyzed by Singh *et al*. (2009a), while anthelmintic activity of fruits was proven by Bachaya *et al*. (2009). The antimutagenic, cytotoxic and antioxidant activities of tree bark have already been reported (Kaur *et al*., 2005). Antifungal activity of *A. nilotica* bark extract against *Candida albucans* was revealed by Pai *et al*. (2010). Further, *A. nilotica* bark was screened for antidiabetic effect in 3T3-L1 adipocytes and db/db mice (Babish *et al*., 2010). Role of *A. nilotica* against liver and kidney toxicity induced by cadmium was reported by Koriem *et al*. (2009). Immunomodulating property of *A. nilotica* fruits was investigated by Koko *et al*. (2008).

Even though, the nutritional value of seeds and medicinal properties of different parts of *A. nilotica* plant have reported earlier, the information regarding the antioxidant effects of seed materials are very meagre. In current situation, research in this direction is of prime importance to combat with free radical mediated chronic diseases. Hence, the present study was aimed to analyze the total phenolic content, antioxidant and type II diabetes related enzyme inhibition properties of methanolic extract of raw and traditionally processed *A. nilotica* seeds with a view to promote them as a nutraceutical/functional dietary ingredient.

Materials and Methods

Chemicals

(+)-Catechin hydrate, Polyvinylpolypyrrolidone (PVPP), Butylated Hydroxytoluene (BHT), 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, α-Glucosidase and *p*-Nitrophenyl-α-D-glucopyranoside were procured from Sigma-Aldrich Chemicals, USA, and all other chemicals were received from Merck, Darmstadt, Germany.

Seed samples

Seed materials of *A. nilotica* were collected from different locations of Tamil Nadu, India (Gobichettipalayam, Tirunelveli, Kanyakumari, Ramanathapuram and Dindigal). Seed samples from each location (about 500 g) were aggregated from 8 - 15 *A. nilotica* trees and mixed together to obtain a representative sample (2.5 kg). After acquisition, the seed samples were frozen immediately and stored at – 80°C until they reach laboratory. Then, the seed sample was randomly divided into four batches with five replicates (each consist of 25 g seeds) for implementing different processing methods and each experiment was repeated for three times. The first batch was stored without any treatment and considered as raw seeds and the remaining three batches were processed as described below.

Processing methods

Whole seeds of the second batch 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). The third batch of samples was added to a red-soil suspension (1:5, w/v), covered with moist cloth and kept in the dark for 2 days. The sprouts are washed with distilled water and then fried in sunflower oil at 185-190°C for 10 min. The fourth batch of seed materials was roasted in an iron pot for 30 min at 120-130°C and then the seeds were separated from the sand using a sieve and allowed to cool to room temperature.

Preparation of methanolic extract

All the processed as well as raw samples were frozen at -80°C and freeze-dried for 10 h. Then the samples were first cracked with the help of a wooden hammer into small pieces and subsequently powdered in a grinder (Siemens, Germany) to 1 mm particle size. One gram of seed flour was treated overnight with petroleum ether (1:10 w/v) on a magnetic stirrer and centrifuged at 2800 x g for 10 min; afterwards, the supernatant was discarded. The defatted residue was then 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 (Bandelin Sonorex, RK – 514 H, Berlin, Germany) for 10 min followed by extraction in magnetic stirrer for 30 min. After centrifugation, all the supernatants were pooled and made up to a known volume. The extract was treated with 5 g of PVPP at 0°C for 30 min, centrifuged and the supernatant was collected and purified using a Solid Phase Catridge (Strata-x-33 um polymeric sorbent, L100-1105, 200 mg/6 ml sample, 8B-S100-FCH-S from Phenomenex, USA). The phenolics were then eluted with 10 ml of 50% and 100% methanol and the solvent was evaporated using a rotary vacuum evaporator (Büchi Rotavapor – R, CH-9230, Switzerland) at 40°C and dried in lyophilizer (Virtis Freeze mobile 25 EL, New York). Finally the residue was weighed and the total dry yield of extract was calculated and then re-dissolved in water:methanol:formic acid (47.5:47.5:5%, v/v/v) solution in the ratio of 1 mg/ml for further analysis.

Analytical methods

The total free phenolic content of methanolic extract of raw and processed samples 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 degradation (Miller, 1971), scavenging activity against DPPH (Sanchez-Moreno *et al*., 1998) and superoxide radicals (Zhishen *et al*., 1999) as well as α-amylase and α-glucosidase inhibition activities (Worthington, 1993) were also analyzed.

Statistical analysis

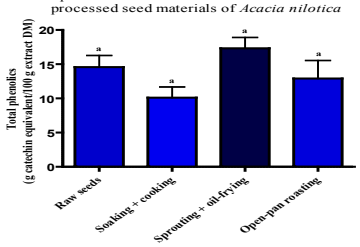
All the data were expressed as means ± standard deviation of three separate determinations with five replicates. One-way ANOVA with Dunnett's post hoc test to determine the significant differences between the control group (raw seeds) and experimental groups (processed seeds) as well as correlation analysis between phenolic content and medicinal properties were performed using GraphPad PRISM® version 5.00 for Windows, San Diego, California, USA.

Results and Discussion

Total free phenolics

The total free phenolics content of methanolic extract of defatted raw seeds of *A. nilotica* was found to be 14.57 g catechin equivalent/100 g extract DM (Figure 1). This value is higher when compared to the previous report on white bean (1.08 g catechin equivalent/100 g extract), pea (3.48 g catechin equivalent/100 g extract), faba bean (8.09 g catechin equivalent/100 g extract), lentil (6.01 g catechin equivalent/100 g extract) and broad bean (6.01 g catechin equivalent/100 g extract) (Amarowicz and Raab, 1997). Such high yield of total free phenolics might be due to the repeated extraction using different concentrations of acidified methanol. Because, recovery of phenolic compounds from food samples are mainly depend upon the type of solvent used and the duration of extraction. In addition, 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 conditions. For instance, low temperature during the onset and duration of seed fill were shown to increase the isoflavone content by several folds in soybean (Kim *et al*., 2006). Since, *A. nilotica* grows wildly in adverse environmental conditions such as drought, poor soil etc., a high phenolic content in the seed materials may contribute to the resistant function.

Figure 1. Total free phenolic content in methanolic extract of raw and traditionally processed seed materials of *Acacia nilotica*

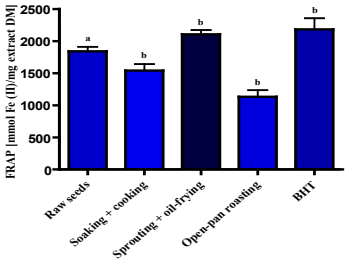


Values are mean and ± standard deviation of three separate determinations with five replicates.
Bars with same alphabet superscripts are significantly not different ($p < 0.05$) according to one-way ANOVA with Dunnett's post hoc test.

Reducing power

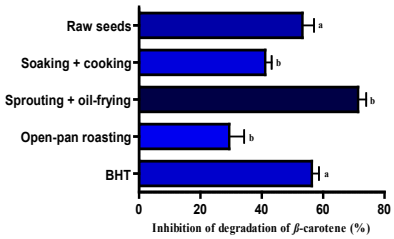
The FRAP assay measures the antioxidant effect of any substance in the reaction medium in term of its reducing ability and it reflects total antioxidant power involving the single electron transfer reaction. Antioxidant potential of methanolic extracts of *A. nilotica* seeds was estimated from their ability to reduce TPTZ-Fe(III) complex to TPTZ-Fe(II) complex. The reducing power of methanolic extract of raw seed materials of *A. nilotica* (1840 mmol Fe [II]/mg extract DM, Figure 2) was found to be higher when reviewed earlier report on seed samples of *Tamarindus indica* (517 mmol Fe [II]/mg extract

Figure 2. Ferric reducing/antioxidant potential (FRAP) of methanolic extract of raw and differentially processed *Acacia nilotica* seeds



Values are mean and ± standard deviation of three separate determinations with five replicates.
Bars with different alphabet superscripts are significantly different ($p < 0.05$) according to one-way ANOVA with Dunnett's post hoc test.

Figure 3. Inhibition of *β*-carotene degradation by the methanolic extract of raw and differentially processed *Acacia nilotica* seeds



Values are mean and ± standard deviation of three separate determinations with five replicates.
Bars with different alphabet superscripts are significantly different ($p < 0.05$) according to one-way ANOVA with Dunnett's post hoc test.

DM) (Siddhuraju, 2007); moth bean (618 mmol Fe [II]/mg extract DM) (Siddhuraju, 2006); light brown (545 mmol Fe [II]/mg extract DM) and dark brown (487 mmol Fe [II]/mg extract DM) varieties of cowpea (Siddhuraju and Becker, 2007). However, this value was lower in comparison to the positive control, BHT (2182 mmol Fe[II]/mg extract DM, Figure 2) and comparable with other underutilized legume grains such as *Vigna vexillata* (1967 mmol Fe[II]/mg extract DM) (Sowndhararahan *et al*., 2011) and brown variety seeds of horse gram (1724 mmol Fe [II]/mg extract DM) (Siddhuraju and Manian, 2007).

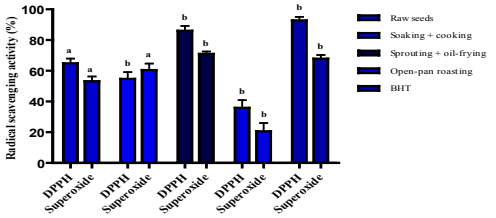
Inhibition of ß-carotene degradation

Methanolic extract of raw *A. nilotica* seeds demonstrated 53.26% of inhibition of *ß*-carotene degradation, which is equal to that of positive control BHT (Figure 3). This value is higher than that of former reports on *Lathyrus filiformis* (28%) (Pastor-Cavada *et al*., 2009) and large black soybean (25%) (Shon *et al*., 2007), but lower when compared to pseudo-cereals including *Amaranthus cruentus* var. R-104 (73.5%) and *Chenopodium quinoa* var. JQ (70.4%) (Nsimba *et al*., 2008). The *ß*-carotene bleaching method was 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. In this assay, the oxidation of linoleic acid generates peroxyl free radicals due to the abstraction of hydrogen atom from di-allylic methylene groups of linoleic acid. The free radicals oxidized the highly unsaturated *ß*-carotene and consequently the orange coloured chromophore of *ß*-carotene was degraded and the results were monitored spectrophotometrically. The capacity of antioxidant compounds to prevent the discoloration/degradation of *ß*-carotene during the auto-oxidation of linoleic acid was measured. The results indicate that the presence of phenolic compounds in methanolic extract of *A. nilotica* 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. This method is based on the reduction of alcoholic DPPH solution in the presence of hydrogen donating antioxidant compound due to the formation of a non-radical form (DPPH-H). The DPPH radical scavenging activity of methanolic extract obtained from raw *A. nilotica* seeds was found to be 64.91% (Figure 4). This is higher than the previous reports on an under-utilized legume, *Mucuna pruriens* (50%) (Randhir *et al*., 2009) and certain common legumes like *Phaseolus vulgaris* var. Bayo Victoria (40%) (Rocha-Guzman *et al*., 2007); *Vigna radiata* (25%) (Randhir *et al*., 2004); soybean (44%) (Boateng *et al*., 2008); Navy bean (14%) and Pinto bean (43%) (Anton *et al*., 2008) and comparable to *Vicia faba* (60%) (Randhir and Shetty, 2004); kidney bean (62%) (Boateng *et al*., 2008) and *Caesalpinia bonducella* (69%) (Shukla *et al*., 2009). However, the radical scavenging activity revealed by *A. nilotica* methanolic extract was significantly lower than that of BHT. The free radical scavenging activity of the phenolic compounds was depends on number of available hydroxyl groups and different structural features such as O-H bound dissociation energy, resonance delocalization of the antioxidant and steric-hindrance derived from bulky groups substituting hydrogen in the antioxidant compound. Potential DPPH radical scavenging activity revealed by methanolic extract of *A. nilotica* seeds might confirm its hydrogen donating capacity and also its proposed ability to protect the consumers' health from various free-radical related diseases.

Figure 4. DPPH and superoxide radical scavenging activities of methanolic extract of raw and differentially processed *Acacia nilotica* seeds



Values are mean and ± standard deviation of three separate determinations with five replicates.
Bars with different alphabet superscripts are significantly different ($p < 0.05$) according to one-way ANOVA with Dunnett's post hoc test.

Superoxide radical scavenging activity

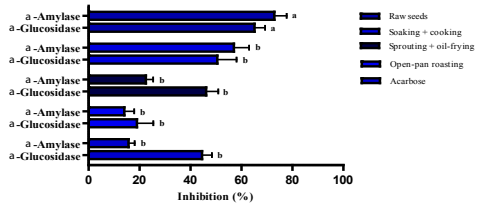
Superoxide radicals, a biologically quite toxic oxygen molecule with one unpaired electron and deployed by the immune system to kill the invading microorganisms, but also deleterious to cellular macromolecules on the other hand. Although superoxide anion was a weak oxidant, it gives rise to the generation 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. Presently investigated methanolic extract of *A. nilotica* raw seeds exhibited 53.23% of superoxide radical scavenging activity, which is parallel to that of BHT (Figure 4). This is higher than that of brown (37.23%) and black (23.43%) varieties of horse gram (Siddhuraju and Manian, 2007); moth bean (19.73%) (Siddhuraju, 2006); light brown (32%) and dark brown (32.8%) varieties of cowpea (Siddhuraju and Becker, 2007) and comparable to *Pisum sativum* (55%) (Troszynska *et al*., 2002). However, it is lower when matched to the seeds of *Bauhinia vahlii* (82.6%) (Sowndhararajan *et al*., 2010) and *Caesalpinia bonducella* (74.71%) (Shukla *et al*., 2009). The methanolic extract of *A. nilotica* seeds deciphered a moderate scavenging activity against superoxide radicals and thus, its incorporation in the regular diets of human population could play a preventive role against the dangerous superoxide radical , which is produced continuously during body metabolism.

α-Amylase inhibition activity

Research priorities on type II diabetes are now-a-days becoming more prevalent with increased emphasis on its management through dietary practice. Considering the diet-linked challenge of type II diabetes, consumption of foods rich in α-amylase and α-glucosidase inhibitors - so called hypoglycemic foods - are receiving more attention and being investigated extensively. α-Amylase and α-glucosidase are well-known key enzymes, playing a vital role in the management of hyperglycemia linked type II diabetes. Acarbose, miglitol and metformin are certain examples of commercially available enzyme inhibitors for the clinical treatment of type II diabetes. However, these drugs are reported to cause various side effects such as abdominal distention, flatulence and possibly diarrhea due to the excessive inhibition of pancreatic α-amylase, which resulted in the abnormal bacterial fermentation of undigested carbohydrates in the colon (Ranilla *et al*., 2008). Hence, at present there is an increasing interest among the food scientists to find out an alternative natural source of α-amylase inhibitor with potential antioxidant activity without any side effects for the dietary management of type II diabetic patients (Kwon *et al*., 2006; Cheplick *et al*., 2007; Ranilla *et al*., 2008).

α-Amylase catalyzes the hydrolysis of glycosidic linkages in the starch and release the hydrolyzed products, which constitute the first step in enzymatic degradation of this polymer. α-Amylase inhibitors are starch blockers, which can binds with the reactive sites of amylase enzyme and alter its catalytic activity and thus reducing the blood sugar level. The methanolic extract of raw seed materials of *A. nilotica* showed 72.91% of α-amylase inhibition (Figure 5) under *in vitro* assay conditions. This value is lower than Mucuna pruriens seeds (87%) (Randhir *et al*., 2009), but comparable with that of mung bean (65%) and higher than the cereal grains such as wheat, buckwheat, corn and oats (38 – 55%) (Randhir *et al*., 2008); Foxtail millet (32%), Proso millet (55%) and finger millet (55%) (Shobana *et al.*, 2009; Kim *et al.*, 2011). Since high amylase inhibition results in many harmful side effects in human beings, low level of α-amylase inhibitors from natural fruits, vegetables and legume grains are reported to offer a good strategy to control postprandial hyperglycemia (McDougall *et al.*, 2005; Kwon *et al*., 2006),. In this connection, the α-amylase inhibition activity observed in raw wild type legume grain of the present study seems to be not suitable to implement in the dietary practice of type II diabetes.

Figure 5. α-Amylase and α-glucosidase inhibition activities of methanolic extract of raw and differentially processed *Acacia nilotica* seeds



Values are mean and ± standard deviation of three separate determinations with five replicates.
Bars with different alphabet superscripts are significantly different ($p < 0.05$) according to one-way ANOVA with Dunnett's post hoc test.

α-Glucosidase inhibition activity

The cells lining the small intestine release the α-glucosidase that results in the cleavage of di- and oligosaccharides into glucose and its absorption in the intestine. The delay of glucose absorption could have a beneficial effect in controlling the postprandial blood sugar level. α-Glucosidase inhibitor can retard rate of glucose absorption in the intestine, by inhibiting the cleavage of di- and oligosaccharides through competitive and reversible inhibition of intestinal α-glucosidase enzyme. A moderate level of α-glucosidase inhibition (65.13%) was observed in the methanolic extract of *A. nilotica* seeds, which is higher to that of acarbose (Figure 5). This value is higher than that of earlier results given for wheat, buckwheat, corn and oats (18 – 31%) (Randhir *et*

al., 2008), but lower when look at Foxtail millet (82.5%), Proso millet (77%), sorghum (95%) and finger millet (78%) (Shobana *et al*., 2009; Kim *et al*., 2011); *Mucuna pruriens* seeds (79%) (Randhir *et al*., 2009) and *Psoralea corylifolia* seeds (77.5%) (Oh *et al*., 2010). However, it should be noted that these results are based on *in vitro* biochemical tests and are indicative of anti-glycemic effects in the prevention/ management of type II diabetes and have limited implications to what happens under *in vivo*.

Effect of soaking + cooking

Substantial level of reduction of total free phenolics was noticed during soaking + cooking treatment (30%) (Figure 1). Similarly, 60 and 28% of losses of phenolics were observed during soaking + autoclaving in light brown and dark brown coloured seeds of cowpea, respectively (Siddhuraju and Becker, 2007); 82% loss in *Vigna vexillata* (Sowndhararajan *et al*., 2011) and 48% in *Bauhinia vahlii* seeds (Sowndhararajan *et al*., 2010). Such non-significant loss of total free phenolics 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 significantly affected the antioxidant activity in terms of reducing power, inhibition of *ß*-carotene degradation, and DPPH radical scavenging activities (except superoxide radical inhibition) as well as α-amylase and α-glucosidase inhibition properties of presently investigated seed sample (Figure 2 - 5). Similarly, Xu and Chang (2008) reported the loss of antioxidant activity during soaking (6 – 34%) as well as cooking (33 – 82%) in food legumes such as green pea, yellow pea, chickpea and lentil. Further, cooking alone was also recognized to reduce the antioxidant activity in *Lathyrus sativus* (Starzynska-Janiszewska *et al*., 2008); *Phaseolus vulgaris* (Granito *et al*., 2008) and *Chenopodium quinoa* (Dini *et al*., 2010). Such a decreased antioxidant and enzyme inhibition properties of *A. nilotica* might be due to the degradation of phenolic compounds under cooking at elevated temperature. Hence, such soaking + cooking treatment is not recommendable to use *A. nilotica* seeds as a natural source of antioxidants and type II diabetes related enzyme inhibitors.

Effect of sprouting + oil-frying

An appreciable level of increase of total free phenolics (16%) was observed during sprouting + oil-frying in *A. nilotica* grains (Figure 1). Similarly, sprouting for 2 days + autoclaving was reported to increase the total free phenolics by 9%, 20%, 27% and 50% in wheat, buckwheat, corn and oats, respectively (Randhir *et al*., 2008). A very high increase on the level of total free phenolics was also noticed in *Vigna radiata* (217%) after 7 days of germination (Fernandez-Orozco *et al*., 2008). Further, Zielinski (2003) reported that germination of *Glycine max* caused an increase of total free phenolics from 2.6 to 3.1 mg/g extract DM. A major portion of phenolic compounds was stored in seeds as soluble conjugate or insoluble forms. Hence, significant level of increase exhibited by the *A. nilotica* seeds under sprouting + oil-frying treatment 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 bound-phenolics by the breakdown of cellular constituents and cell walls during subsequent thermal process (oil-frying).

Sprouting + oil frying was found to increase the antioxidant activity at significant level (Figure 2- 5). In agreement, germination was reported to increase the antioxidant activity of *Mucuna pruriens* seeds, lupin seeds, mung bean seeds, faba bean, peas and common beans (Shetty *et al*., 2002; Randhir *et al*., 2004, 2009; Lopez-Amoros *et al*., 2006; Duenas *et al*., 2009). Similarly, sprouting + autoclaving was also reported to significantly increase the antioxidant activity in wheat, buckwheat, corn and oats (Randhir *et al*., 2008). Such significant increase of antioxidant properties of *A. nilotica* seeds was obviously attributed to the elevation of their phenolic content during sprouting + oil-frying treatment. A significant level of positive correlation was noticed between the phenolic content and antioxidant properties (except superoxide inhibition effect) of *A. nilotica* seeds (Table 1).

Table 1. Correlation analysis between phenolic content and antioxidant and functional properties of raw and differentially processed *Acacia nilotica* seeds

Compound	Pearson coefficient (r)					
	FRAP	BCB	DPPH	SO	AIA	GIA
Total phenolics	0.6307*	0.6390*	0.6277*	0.2936	-0.2388	0.1017

* Correlation is significant at 0.05 (p two-tailed).
FRAP - Ferric reducing/antioxidant potential; BCB - *β*-Carotene bleaching assay; DPPH - DPPH free radical scavenging activity; SO - Superoxide radical scavenging activity; AIA - α-Amylase inhibition activity; GIA - α-Glucosidase inhibition activity.

Nonetheless, significant level of loss of α-amylase and α-glucosidase inhibition activities was observed in *A. nilotica* grain after sprouting + oil-frying treatment (Figure 5). This is in agreement with that of earlier observation on *Mucuna pruriens* seeds during germination (Randhir *et al*., 2009). The α-amylase and α-glucosidase inhibition levels recorded in sprouting + oil-fried seeds were similar to that of synthetic antidiabetic agent (acarbose) and

hence, more desirable for the dietary maintenance of type II diabetes. Such low level of α-amylase and moderate inhibition of α-glucosidase can regulate the blood sugar level of the diabetic patients without any adverse side effects. Thus, sprouting + oil frying could be considered as a mild and favourable treatment to exploit *A. nilotica* seeds as a therapeutic food source.

Effect of open-pan roasting

Open-pan roasting caused only a little level of loss of total free phenolics (11%) in *A. nilotica* sample (Figure 1). Similar level of removal of phenolic content was recorded in moth bean (11%) (Siddhuraju, 2006); dark brown seed coated cowpea (16%) (Siddhuraju and Becker, 2007) and *Bauhinia vahlii* (20%) (Sowndhararajan *et al*., 2010), but drastic losses of 48 and 64% were also reported in light brown coloured cowpea and *Vigna vexillata* seeds, respectively (Siddhuraju and Becker, 2007; Sowndhararajan *et al*., 2011).

Drastic losses of antioxidant as well as enzyme inhibition properties were caused by open-pan roasting (Figure 2 – 5). Similarly, decrease of antioxidant activity during roasting was reported in black-eyed peas, kidney beans and pinto beans (Boateng *et al*., 2008) and amaranth, quinoa, wheat and buckwheat (Alvarez-Jubete *et al*., 2010) Although the open-pan roasting method did not affect the phenolic content at significant level, it exhibited diminishing effect on functionality properties of *A. nilotica* samples. This might be due to the disintegration of phenolic compounds by the direct action of high temperature during roasting. There is no correlation between the enzyme inhibition properties and phenolic content of *A. nilotica* seeds (Table 1). 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 and health relevant functionalities in *A. nilotica* seeds.

Conclusion

Methanolic extract of *A. nilotica* seeds was found to contain appreciable levels total free phenolics with promising antioxidant and type II diabetes related enzyme inhibition properties. A significant correlation was recognized between the phenolic content and antioxidant properties (except superoxide inhibition activity), while it was lacking in the case of enzyme inhibition characteristics. Considering the effect of different indigenous processing methods, soaking + cooking exhibited an ample loss of antioxidant and enzyme inhibition properties. Open-pan roasting did not show any significant level of reduction of phenolic content, but drastically affected the antioxidant and starch digestive enzyme inhibition characteristics, and thus considered as the most aggressive practice. Alternatively sprouting + oil-frying was noticed to extensively increase the total free phenolics content as well as antioxidant properties of *A. nilotica* grain. Such viable processing technique could offers a good strategy to improve the phenolic content in *A. nilotica* seeds for enhanced antioxidant activity and functionality towards inhibition of starch digestive enzymes relevant to potential type II diabetes management. Therefore, such suitably processed *A. nilotica* grain could be envisaged as a nutraceutical/ functional dietary ingredient, after conducting *in vivo* experiments. Further, identification of phenolic constituents of *A. nilotica* seeds is in progress.

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References

Alvarez-Jubete, L., Wijngaard, H., Arendt, E. K. and Gallagher, E. 2010. Polyphenol composition and *in vitro* antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. Food Chemistry 119: 770 – 778.

Amarowicz, R. and Raab, B. 1997. Antioxidative activity of leguminous seed extracts evaluated by chemiluminescence methods. Zeitschrift für Naturforschung 52: 709 –712

Ambasta, S. P. 1994. The useful plants of India. Publication and Information Directorate, CSIR, New Delhi (pp. 4).

Anton, A. A., Ross, K. A., Beta, T., Fulcher, R. G. and Arntfield, S. D. 2008. Effect of pre-dehulling treatments on some nutritional and physical properties of navy and pinto beans (*Phaseolus vulgaris* L.). LWT Food Science and Technology 41: 771 – 778.

Babish, J.G., Pacioretty, L.M., Bland, J.S., Minich, D.M., Hu, J. and Tripp, M.L. 2010. Antidiabetic screening of commercial botanical products in 3T3-L1 adipocytes and db/db mice. Journal of Medical Foods 13: 535 - 547.

Bachayaa, H. A., Iqbala, Z., Khana, M. N., Sindhua, Z. D. and Jabbara, A. 2009. Anthelmintic activity of *Ziziphus nummularia* (bark) and *Acacia nilotica* (fruit) against *Trichostrongylid nematodes* of sheep. Journal of Ethnopharmacology 123: 325 – 329.

Bhargava, A., Srivastava, A. and Kumbhare, V. C. 1998. Antifungal activity of polyphenolic complex of *Acacia nilotica* bark. Indian Forestry 124: 292 – 298.

Boateng, J., Verghese, M., Walker, L. T. and Ogutu, S.

2008. Effect of processing on antioxidant contents in selected dry beans (*Phaseolus* spp. L.). LWT Food Science and Technology 41: 1541 – 1547.

Cheplick, S., Kwon, Y., Bhowmik, P. and Shetty, K. 2007. Clonal variation in raspberry fruit phenolics and relevance for diabetes and hypertension management. Journal of Food Biochemistry 31: 656 – 679.

Dini, I., Tenore, G. C. and Dini, A. 2010. Antioxidant compound contents and antioxidant activity before and after cooking in sweet and bitter *Chenopodium quinoa* seeds. LWT Food Science and Technology 43: 447 – 451.

Duenas, M., Hernandez, T., Estrella, I. and Fernandez, D. 2009. Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (*Lupinus angustifolius* L.). Food Chemistry 117: 599 – 607.

Fernandez-Orozco, R., Frias, J., Zielinski, H., Piskula, M. K., Kozlowska, H. and Vidal-Valverde, C. 2008. Kinetic study of the antioxidant compounds and antioxidant capacity during germination of *Vigna radiata* cv. emerald, *Glycine max* cv. jutro and *Glycine max* cv. merit. Food Chemistry 111: 622 – 630.

Granito, M., Paolini, M. and Perez, S. 2008. Polyphenols and antioxidant capacity of *Phaseolus vulgaris* stored under extreme conditions and processed. LWT Food Science and Technology 41: 994 – 999.

Hutchings, A., Scott, A. H., Lewis, G. and Cunningham, A. B. 1996. Zulu Medicinal Plants. An Inventory. University of Natal Press, Pietermaritzburg.

Janardhanan, K., Vadivel, V. and Pugalenthi, M. 2003. Biodiversity in Indian under-exploited/tribal pulses. In: Jaiwal, P. K. and Singh, R. P. (Eds), Improvement strategies for Leguminosae Biotechnology, pp. 353 – 405. The Netherlands: Kluwer Academic Publishers.

Kalaivani, T. and Mathew, L. 2010. Free radical scavenging activity from leaves of *Acacia nilotica* (L.) Wild. ex Delile, an Indian medicinal tree. Food and Chemical Toxicology 48: 298 – 305.

Kaur, K., Michael, H., Arora, S., Harkonen, P. and Kumar, S. 2005. *In vitro* bioactivity-guided fractionation and characterization of polyphenolic inhibitory fractions from *Acacia nilotica* (L.) Willd. ex Del. Journal of Ethnopharmacology 99: 353 – 360.

Kim, J. A., Jung, W. S., Chun, S. C., Yu, C. Y., Ma, K. H. and Gwag, J. G. 2006. A correlation between the level of phenolic compounds and the antioxidant capacity in cooked-with-rice and vegetable soybean (*Glycine max* L.) varieties. European Journal of Food Research and Technology 224: 259 – 270.

Kim, J. S., Hyun, T. K. and Kim, M. J. 2011. The inhibitory effects of ethanol extracts from sorghum, foxtail millet and proso millet on α-glucosidase and α-amylase activities. Food Chemistry 124: 1647 – 1651.

Koko, W.S., Mesaik, M.A., Yousaf, S., Galal, M. and Choudhary, M.I. 2008. *In vitro* immunomodulating properties of selected Sudanese medicinal plants. Journal of Ethnopharmacology 19: 26 – 34.

Koriem, K.M., Farrag, A.R., Badawy, M.A. and El-Toumy, S.A. 2009. Role of some Egyptian medicinal plants against liver and kidney toxicity induced by cadmium chloride. Toxicology Mechanisms and Methods 19: 524 – 534.

Kwon, Y. I., Vattem, D. V. and Shetty, K. 2006. Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension. Asia Pacific Journal of Clinical Nutrition 15: 107 – 118.

Lerman, R. H., Minich, D. M., Darland, G., Lamb, J. J., Chang, J. L., Hsi, A., Bland, J. S. and Tripp, M. L. 2010. Subjects with elevated LDL cholesterol and metabolic syndrome benefit from supplementation with soy protein, phytosterols, hops rho iso-alpha acids, and *Acacia nilotica* proanthocyanidins. Journal of Clinical Lipidology 4: 59 – 68.

Lopez-Amoros, M. L., Hernandez, T. and Estrella, I. 2006. Effect of germination on legume phenolic compounds and their antioxidant activity. Journal of Food Composition and Analysis 19: 277 – 283.

McDougall, G. J., Shpiro, F., Doboson, P., Smith, P., Black, A. and Stewart, D. 2005. Different polyphenolic compounds of soft fruits inhibit α-amylase and α-glucosidase. Journal of Agricultural Food Chemistry 53: 2760 – 2766.

Miller, H. E. 1971. A simplified method for the evaluation of antioxidants. Journal of American Oil Chemical Society 48: 91 – 92.

Nsimba, R. Y., Kikuzaki, H. and Konishi, Y. 2008. Antioxidant activity of various extracts and fractions of *Chenopodium quinoa* and *Amaranthus* spp. seeds. Food Chemistry 106: 760 – 766.

Oh, K. Y., Lee, J. H., Curtis-Long, M. J., Cho, J. K., Kim, J. Y., Lee, W. S. and Park, K. H. 2010. Glycosidase inhibitory phenolic compounds from the seed of *Psoralea corylifolia*. Food Chemistry 121: 940 – 945.

Pai, M.B., Prashant, G.M., Murlikrishna, K.S., Shivakumar, K.M. and Chandu, G.N. 2010. Antifungal efficacy of *Punica granatum*, *Acacia nilotica*, *Cuminum cyminum* and *Foeniculum vulgare* on *Candida albicans*: an *in vitro* study. Indian Journal of Dental Research 21: 334 – 336.

Pastor-Cavada, E., Juan, R., Pastor, J. E., Alaiz, M. and Vioque, J. 2009. Antioxidant activity of seed polyphenols in fifteen wild *Lathyrus* species from South Spain. LWT Food Science and Technology 42: 705 – 709.

Pulido, R., Bravo, L. and Saura-Calixto, F. 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. Journal of Agricultural Food Chemistry 48: 3396 – 3402.

Randhir, R. and Shetty, K. 2004. Microwave-induced stimulation of L-Dopa, phenolics and antioxidant activity in fava bean (*Vicia faba*) for Parkinson's diet. Process Biochemistry 39: 1775 – 1784.

Randhir, R., Kwon, Y. I. and Shetty, K. 2008. Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain sprouts and seedlings. Innovative Food Science and Emerging Technologies 9: 355 – 364.

Randhir, R., Kwon, Y. I. and Shetty, K. 2009. Improved

health-relevant functionality in dark germinated *Mucuna pruriens* sprouts by elicitation with peptide and phytochemical elicitors. Bioresource Technology 100: 4507 – 4514.

Randhir, R., Lin, Y. T. and Shetty, K. 2004. Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. Process Biochemistry 39: 637 – 646.

Ranilla, L. G., Kwon, Y. I., Genovese, M. I., Lajolo, F. M. and Shetty, K. 2008. Antidiabetes and antihypertension potential of commonly consumed carbohydrate sweeteners using *in vitro* models. Journal of Medicinal Foods 11: 337 – 348.

Rocha-Guzman, N. E., Gonzalez-Laredo, R. F., Ibarra-Perez, F. J., Nava-Berumen, C. A. and Gallegos-Infante, J. A. 2007. Effect of pressure cooking on the antioxidant activity of extracts from three common bean (*Phaseolus vulgaris* L.) cultivars. Food Chemistry 100: 31 – 35.

Sanchez-Moreno, C., Larrauri, J. A. and Saura-Calixto, F. A. 1998. A procedure to measure the antiradical efficiency of polyphenols. Journal of the Science of Food and Agriculture 76: 270 – 276.

Shetty, P., Atallah, M. T. and Shetty, K. 2002. Effects of UV treatment on the proline-linked pentose phosphate pathway for phenolics and L-Dopa synthesis in dark germinated *Vicia faba*. Process Biochemistry 37: 1285 – 1295.

Shobana, S., Sreerama, Y. N. and Malleshi, N. G. 2009. Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: Mode of inhibition of α-glucosidase and pancreatic amylase. Food Chemistry 115: 1268 – 1273.

Shon, M. Y., Kim, T. H. and Sung, N. J. 2007. Antioxidants and free radical scavenging activity of *Phellinus baumii (Phellinus of Hymenochaetaceae)* extracts. Food Chemistry 82: 593 – 597.

Shukla, S., Metha, A., John, J., Singh, S., Mehta, P. and Vyas, S. P. 2009. Antioxidant activity and total phenolic content of ethanolic extract of *Caesalpinia bonducella* seeds. Food and Chemical Toxicology 47: 1848 – 1851.

Siddhuraju, P. and Becker, K. 2007. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. Food Chemistry 101: 10 – 19.

Siddhuraju, P. and Manian, S. 2007. The antioxidant activity and free radical-scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. Food Chemistry 105: 950 – 958.

Siddhuraju, P. 2006. The antioxidant activity and free radical-scavenging capacity of phenolics of raw and dry heated moth bean (*Vigna aconitifolia*) (Jacq.) Marechal seed extracts. Food Chemistry 99: 149 – 157.

Siddhuraju, P. 2007. Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated *Tamarindus indica* seed coat. LWT Food Science and Technology 40: 982 – 990.

Siddhuraju, P., Vijayakumari, K. and Janardhanan, K. 1996. Chemical composition and nutritional evaluation of an underexploited legume, *Acacia nilotica* (L.) Del. Food Chemistry 57: 385 – 391.

Singh, B. N., Singh, B. R., Sharma, B. K. and Singh, H. B. 2009b. Potential chemoprevention of *N*-nitrosodiethylamine-induced hepatocarcinogenesis by polyphenolics from *Acacia nilotica* bark. Chemical-Biology Interactions 181: 20 – 28.

Singh, B. N., Singh, B. R., Singh, R. L., Prakash, D., Sarma, B. K. and Singh, H. B. 2009a. Antioxidant and anti-quorum sensing activities of green pod of *Acacia nilotica* L. Food and Chemical Toxicology 47: 778 – 786.

Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology 299: 152 – 178.

Sowndhararajan, K., Siddhuraju, P. and Manian, S. 2011. Antioxidant and free radical scavenging capacity of the underutilized legume, *Vigna vexillata* (L.) A. Rich. Journal of Food Composition and Analysis 24: 160 – 165.

Sowndhararajan, K., Siddhuraju, P. and Manian, S. 2010. *In vitro* Evaluation of the antioxidant activities in the differentially processed seeds from underutilized legume, *Bauhinia vahlii* Wight & Arn. Food Science and Biotechnology 19: 503 – 509.

Starzynska-Janiszewska, A., Stodolak, B. and Jamroz, M. 2008. Antioxidant properties of extracts from fermented and cooked seeds of Polish cultivars of *Lathyrus sativus*. Food Chemistry 109: 285 – 292.

Troszynska, A., Estrella, I., Lopez-Amores, M. L. and Hernandez, T. 2002. Antioxidant activity of pea (*Pisum sativum* L.) seed coat acetone extract. LWT Food Science and Technology 35: 158 – 164.

Van Wyk, B. and Van Wyk, P. 1997. Field guide to trees of Southern Africa, p. 496, Cape Town, South Africa: Struik Publishers (A division of New Holland publishing South Africa).

Worthington, V. 1993 Worthington Enzyme Manual. Worthington Biochemical Corporation (pp. 36 – 261), Freehold, NJ.

Xu, B. and Chang, S. K. C. 2008. Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. Food Chemistry 110: 1 – 13.

Zhishen, J., Mengcheng, T. and Jianming, W. 1999. The determination of flavonoid contents on mulberry and their scavenging effects on superoxide radical. Food Chemistry 64: 555 – 559.

Zielinski, H. 2003. Contribution of low molecular weight antioxidants to the antioxidant screen of germinated soybean seeds. Plant Foods for Human Nutrition 58: 1 – 20.