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ORIGINAL ARTICLE

Effect of certain indigenous processing methods on the bioactive compounds of ten different wild type legume grains

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Abstract In recent years, research efforts are under-way on the possibilities of utilization of natural source of bioactive compounds for the dietary management of certain chronic diseases such as diabetes, obesity, cardiovascular diseases, cancer etc. In this connection, seed materials of promising wild type under-utilized food legume grains such as *Acacia nilotica* (L.) Willd. Ex Delile, *Bauhinia purpurea* L., *Canavalia ensiformis* (L.) DC., *Cassia hirsuta* L., *Caesalpinia bonducella* F., *Erythrina indica* L., *Mucuna gigantea* (Willd.) DC., *Pongamia pinnata* (L.) Pierre, *Sebania sesban* (L.) Merr. and *Xylia xylocarpa* Roxb. Taub., collected from South India, were investigated for certain bioactive compounds. All the samples were found to constitute a viable source of total free phenolics (3.12–6.69 g/100 g DM), tannins (1.10–4.41 g/100 g DM), L-Dopa (1.34–5.45 g/100 g DM) and phytic acid (0.98–3.14 g/100 g DM). In general, the seed materials of *X. xylocarpa* recorded high levels of total free phenolics and tannins, whereas the maximum levels of L-Dopa and phytic acid were noticed in *M. gigantea* and *S. sesban*, respectively. Further, presently investigated all the bioactive compounds were drastically reduced during soaking in tamarind solution + cooking as well as soaking in alkaline solution + cooking, and thus these treatments were considered to be more aggressive practices. Open-pan roasting also demonstrated a significant reduction of total free phenolics, tannins and moderate loss of L-Dopa and phytic acid. Alternatively, sprouting + oil-frying showed significant level of increase of total free phenolics (9–27%) and tannins (12–28%), but diminishing effect on phytic acid and L-Dopa. Hence, among the presently employed treatments, sprouting + oil-frying could be recommended as a suitable treatment for the versatile utilization of these wild under-utilized legume grains for the dietary management of certain chronic diseases.

Keywords Wild type legume grains · Bioactive compounds · Total free phenolics · Tannins · L-Dopa · Phytic acid · Processing methods

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Introduction

Legume grains have been playing a key role in the traditional diets of human beings throughout the world and they are excellent source of protein, dietary fibre, starch, micronutrients and bioactive compounds with low level of fat (Messina 1999). The total per capita consumption of legume grains has been increased markedly over the past two decades in US, due to increased attention to beans being classified as functional foods (Luthria and Pastor-Corrales 2006). Accumulation of chemical, biochemical, clinical and epidemiological evidences indicating a positive correlation between the consumption of legume seeds and decreasing incidence of several chronic diseases such as cancer, cardiovascular diseases, obesity and diabetes (Troszynska et al. 2002). Such obvious health benefits of legume seeds are attributed to presence of certain bioactive compounds such as phenolic acids, flavonoids and tannins (Siddhuraju and Manian 2007). Therefore, at present the studies on bioactive compounds, which are responsible for health promoting/disease preventing effect,

are being increased in addition to the evaluation of nutritive profiles of legume grains.

Actually in olden days, the bioactive compounds like total free phenolics, tannins, L-Dopa and phytic acid were considered as antinutritional substances and their presence in food/feed-stuffs was reported to be undesirable from the nutritional point of view (Vadivel and Pugalenthi 2008). But, now-a-days, the health beneficial role of such bioactive compounds has been explored by a large number of research studies. Particularly, these bioactive compounds were demonstrated to possess many favourable medicinal properties, including potential antioxidant activity (Siddhuraju and Manian 2007; Herken et al. 2007; Randhir et al. 2008). As a consequence of health beneficial effects, presence of such bioactive compounds in the diet has been viewed in a positive light in recent years by both scientists and consumers and resulted in a push to procure foods with specific health benefits such as functional foods.

Apart from common legume seeds, the earlier research efforts revealed the nutritive potential of certain promising under-utilized/wild legume seeds, including the pulses of tribal utility. Among the various under-utilized legumes, seed materials of *Acacia nilotica* (L.) Willd. Ex Delile, *Bauhinia purpurea* L., *Canavalia ensiformis* (L.) DC., *Cassia hirsuta* L., *Caesalpinia bonducella* F., *Erythrina indica* L., *Mucuna gigantea* (Willd.) DC., *Pongamia pinnata* (L.) Pierre, *Sebania sesban* (L.) Merr. and *Xylia xylocarpa* Roxb. Taub., merit a wider use as a food legume. Their distribution, agronomic traits, nutritional value, mode of consumption was described in detail by Janardhanan et al. (2003). In South India, these wild type legume grains are being traditionally consumed by certain ethnic groups, particularly the Kanikkar, Lambadi, Uraali and Dravidian tribes living in Tamilnadu, Kerala, Karnataka and Andhrapradesh States. Beside good nutritional profiles, these wild legume grains also reported to contain health beneficial bioactive compounds like total free phenolics, tannins, L-Dopa and phytic acid (Janardhanan et al. 2003). Hence in recent years, research efforts are under-way to incorporate these wild type legume grains in the dietary management of various chronic diseases, including diabetes, obesity and cardiovascular diseases.

Generally before consumption, all the legume grains are subjected to appropriate processing method in order to improve their nutritive quality. But, most of the common processing methods have been shown to reduce the levels of bioactive compounds such as polyphenolics in commercial legume grains (Rocha-Guzman et al. 2007), while increase on the levels of tannins, catechins and polyphenols was reported during cooking by Vidal-Valverde et al. (1993). The information on effect of processing methods on the bioactive components of wild type legume grains is very meager. So, it is very important to identify a suitable processing method(s), which will cause minimum level of loss of bioactive compounds in wild type under-utilized legume seeds. In this connection, in the present study, an attempt has been made to analyze the effect of certain indigenous processing techniques; particularly those are used by Indian tribal groups, on the levels of bioactive compounds of ten different under-utilized legume seeds collected from South India.

Materials and methods

Chemicals Poly-vinyl-polypyrrolidone, (+)-catechin hydrate, vanillin, tannic acid, L-Dopa, phytic acid were procured from Sigma-Aldrich Chemicals, USA; Sephadex LH-20 was obtained from Pharmacia Fine Chemicals, Sweden; Anion exchange resine was purchased from Bio-Rad, USA, and all other chemicals were received from Merck, Darmstadt, Germany.

Collection of seed samples The seed materials of *Acacia nilotica* (Kottayam, Kerala), *Bauhinia purpurea* (Visakapatnam, Andhrapradesh), *Canavalia ensiformis* (Tirunelveli, Tamilnadu), *Cassia hirsuta* (Gundalpet, Karnataka), *Caesalpinia bonducella* (Sikkumagalur, Karnataka), *Erythrina indica* (Kadambur, Tamilnadu), *Mucuna gigantea* (Trivendrum, Kerala), *Pongamia pinnata* (Coimbatore, Tamilnadu), *Sebania sesban* (Nanjankoodu, Karnataka) and *Xylia xylocarpa* (Sreesailam, Andhrapradesh) were collected during 2008–2009. After removing the immature and damaged seeds, the mature seeds were dried under shaded condition for 2 days. Then the seed materials were randomly divided into five batches and the first batch was stored without any treatment, which is considered as raw seeds and the remaining four batches were processed as described below.

Soaking in tamarind solution + cooking The tamarind solution was prepared by dissolving 10 g of tamarind pulp in 500 mL of distilled water (pH 2.75). The whole seeds of wild legumes (50 g) were soaked in tamarind solution in the ratio of 1:10 (*w*/*v*) and kept in dark for 8 h at 25°C. The soaked samples were rinsed and then cooked with distilled water (in the ratio of seed to water, 1:10 *w*/*v*) at 85–90°C on a hot plate until they became soft when felt between the fingers (about 45 min). This treatment was adapted based on the fact that the Kanikkar tribe living in Kerala state, India has been following this method for the consumption of wild legume seeds.

Soaking in alkaline solution + cooking The whole seeds (50 g) were soaked in 500 mL of 0.2% $NaHCO_3$ solution (pH 8.6) for 8 h in dark at room temperature (25°C) in the

bean to alkaline solution ratio of 1:10 (*w*/*v*). After soaking, the alkaline solution was drained off and the seeds were cooked with distilled water at 85–90°C for about 45 min. This method of processing is common among the Dravidian tribal sect living in Tirunelveli District, Tamilnadu state, India.

Sprouting + oil-frying Clean red-soil (100 g) was taken in a tray and made into a paste with distilled water in the ratio of 1:5 (*w*/*v*). Then, 50 g of each seed sample was added into the red-soil suspension and mixed well. The tray was covered with a moist cloth and kept for 7 days in dark at 25°C. Then the sprouts were separated and thoroughly washed with tap water. The sprouts, thus obtained, were fried with Biskin oil (100% sunflower oil) at 85–90°C on a hot plate for about 15 min. This treatment was based on the practice of Lambadi ethnic group of Karnataka and Andhrapradesh states, India.

Open-pan roasting The seed materials (50 g) were taken in an iron pot along with acid-treated clean and fine sand to prevent the burning of seed coat and also to ensure the uniform distribution of heat. The seed materials were roasted for 30 min at 120–130°C. Then the seeds were separated by using a sieve, and allowed to cool to room temperature. In general, the Uraali tribal group lives near the Kadambur hills of Sathyamangalam, Erode District, Tamilnadu state, India has been using open-pan roasting for the consumption of under-utilized legume seeds in their regular diet.

Preparation of seed flour All the processed as well as raw samples were freezed at −80°C and freeze-dried for 48 h. Then the samples were first cracked with the help of a wooden hammer into small pieces and subsequently powdered in a seed mill (Siemens, Germany) to 1 mm particle size, freeze-dried for 24 h and stored at 9°C until further use.

Total free phenolics The total free phenolics were extracted from seed samples by taking 1 g of defatted seed flour sequentially with 10 mL of 100%, 80% and 50% methanol and 70% acetone acidified with 1% conc. HCl in an ultra-sonic bath (Bandelin Sonorex, RK – 514 H, Berlin, Germany) for 30 min. After centrifugation, all the super-natants were pooled and made up to a known volume. The extract was treated with 1 g of poly-vinyl-polypyrrolidone at 0°C for 30 min. Then the contents were purified by using a Solid Phase Catridge (SPC) (Strata-x-33 um polymeric sorbent, L100-1105, 200 mg/6 mL sample, 8B-S100-FCH-S from Phenomenex, USA). The total free phenolics were eluted with 10 mL of 50% and 100% methanol and used for estimation according to the method of Singleton et al. (1999). Based on the standard curve prepared with (+)-Catechin hydrate (20–100 μg), the amount of total free phenolics in the extract was calculated and expressed in g/100 g seed flour on dry matter basis.

Tannins The tannins were extracted from seed materials by taking 1 g of defatted seed flour sequentially with 100%, 90%, 80% and 70% acetone solutions acidified with 1% conc. HCl. After centrifugation, all the supernatants were pooled together and made up to a known volume with acetone. Then the extract was purified by using Sephadex LH-20 column chromatography (96×1.6 cm) with acetone: water (50:50, *v*/*v*) as a solvent (Troszynska et al. 2002). After collecting 20 fractions (5 mL each), the active fractions were identified and pooled together and used for quantification by using vanillin reagent method (Price et al. 1978). The aliquot (500 μl) was taken in a test tube and treated with 5 mL of 0.5% vanillin and 5 mL of 4% HCl. After standing for 30 min at room temperature, the contents were mixed well and the absorbance was measured at 500 nm in a UV–Visible Spectrophotometer (Perkin-Elmer, Lambda 35). The standard curve was prepared by taking different concentrations of tannic acid and the level of tannins was calculated.

L-Dopa Finely ground seed flour (1 g) was treated with 10 mL of petroleum ether and kept in an ultra-sonic bath for 30 min. Then the defatted pellet was extracted with 10 mL of 0.1 N HCl. The contents were vortexed for 10 min at room temperature (25°C) and kept in an ultra-sonic bath for 30 min under ice bath condition and subsequently it was kept on a magnetic stirrer for 1 h at room temperature. The supernatant was collected by centrifugation (13,000 × g, 15 min) and the extraction procedure was repeated twice and all the supernatants were pooled and diluted to a known final volume and used for further analysis. The L-Dopa content was quantified by measuring the ultra-violet light absorption at 282 nm in a UV–Visible Spectrophotometer after correction for background absorption according to Brain (1976) method. The L-Dopa content of seed samples was calculated by using the standard curve prepared with synthetic L-Dopa and expressed in g/100 g seed flour on dry matter basis.

Phytic acid The phytic acid was extracted from raw and differentially processed seed samples by taking 1 g of defatted seed flour with 10 mL of 2.4% HCl and incubated for 10 min in ultra-sonic bath. Then the contents were centrifuged at 13,000 × g for 5 min and the supernatant was collected. Similarly, the residue was re-extracted twice and all the supernatants were pooled together and made up to a known volume with distilled water. The extract was purified by using an anionic-exchange column chromatog-

raphy (0.7 cm × 15 cm) containing 0.5 g of anion-exchange resin (100–200 mesh, chloride form; AG1-X4, Bio-Rad Co., CA, USA). The phytic acid was eluted with 2 M HCl and used for quantification according to Latta and Eskin (1980) method. The purified extract (100 µl) was diluted to 3 mL with distilled water and 1 mL of Wade reagent (0.03% $FeCl_3.6H_2O$ and 0.3% sulfosalicylic acid) was added. The contents were vortexed and centrifuged at 3500 × g for 5 min. Then the absorbance of the supernatant was measured at 500 nm. The phytic acid content was calculated by using the standard curve prepared with synthetic phytic acid.

Statistical analysis All the data were analyzed and expressed as means ± standard deviation of five separate determinations (n=5). The statistical analysis was carried out by using SPSS for Windows (SPSS Inc., Chicago, IL, version 11.0). Values of analyzed compounds were found to be normal distributed by using Kolmogorov-Smirnov-test. Means of the groups regarding different processing methods were compared by one-way ANOVA and Dunnett post-hoc test using the raw seeds as a control. Two-tailed P values<0.05 were considered statistically significant.

Results and discussion

Total free phenolics The phenolic compounds constitute one of the most numerous and ubiquitously distributed group of plant secondary metabolites, which are ranged from simple molecules (eg. phenolic acids, phenylpropanoids and flavonoids) to highly polymerized compounds (eg. Lignins and melanins). Now-a-days, the phenolic compounds are demonstrated to prevent the development of many chronic diseases such as atherosclerosis, diabetes, cancer etc. Such protective effect of phenolics might be associated with their powerful antioxidant and free radical scavenging properties (Siddhuraju 2007). The seed coat of legume grains are reported to contain numerous types of phenolics, which playing an important protective role against oxidative damage in consumer's body (Troszynska et al. 2002).

The total free phenolics content of raw seed materials of different wild legume grains were found to range between 3.12 and 6.69 g/100 g seed flour DM (Table 1). These values are higher when compared to previous reports on broad bean (2.39 g/100 g DM); pea (2.26–3.48 g/100 g DM); white bean (1.08 g/100 g DM); black bean (4.40 g/100 g DM) and common bean (1.88–2.53 g/100 g DM), but comparable with that of faba bean (5.59 g/100 g DM); Adzuki bean (8.97 g/100 g DM); red bean (5.54–9.36 g/100 g DM); red lentil (5.80 g/100 g DM); green lentil (6.76 g/100 g DM) and brown bean (9.14 g/100 g DM) (Amarowicz and Pegg 2008).

In general, the total free phenolics content of presently investigated under-utilized legume grains was appears to be higher when compared to the literature (Janardhanan et al. 2003). This might be due to the repeated extraction of phenolic compounds by using both methanol and acetone as solvents. Because, recovery of phenolic compounds from legume grains is mainly depends upon the type of solvent used and the duration of extraction. Acetone and methanol extracts of seed samples exhibited higher phenolic yield when compared to either methanol or acetone used alone (Agboola et al. 2009).

The seed samples of *Xylia xylocarpa* (6.69 g/100 g DM) registered significantly (p<0.05) higher level of total free phenolics, which is followed by *Mucuna gigantea* (6.47 g/100 g DM) and *Acacia nilotica* (6.24 g/100 g DM) (Table 1). It is interesting to notice that the seed coat colour of these seed materials is dark brown/black. Relationships between seed coat colour and phenolics level are still controversial. While Barampama and Simard (1993) found a positive relationship between the seed coat colour and phenolic content, Guzman-Maldonado et al. (1996) did not find any relationship. However, there are some reports available with high correlation between cultivar lines and phenolic content (De Mejia et al. 2003). In addition to seed coat colour, it is well documented that 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. 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 these under-utilized legumes grow wildly in adverse environmental conditions such as drought, poor soil etc., a high phenolic content contributes to the resistant function.

Although the dietary intake of phenolics varies considerably among the geographical regions, it is estimated that the daily intake of total free phenolics was ranged from 20 mg to 1 g, which is higher than the intake of vitamin E. Hence, in recent years, food technologists are keen to harness the nutritional benefits of phenolics, namely its antioxidant or free radical scavenging, food preservative, antimicrobial, anti-mutagenic, therapeutic and pharmaceutical properties.

Tannins Beside simple phenolics mainly found in cellular vacuoles, some polymerized form of phenolics with varying degree of solubility such as tannins are also noticed in legume seeds. Tannins are defined as a unique group of phenolic metabolites of relatively high molecular weight.

Table 1 Effect of certain indigenous processing methods on the total free phenolics content of wild type legume grains[a,b,c]

Name of the wild type legume grain	Raw seeds	Processed seeds			
		Soaking in tamarind solution + cooking	Soaking in alkaline solution + cooking	Sprouting + oil-frying	Open-pan roasting
Acacia nilotica	6.2^{a}±0.39	3.7^{b}±0.05 (−40)	2.0^{c}±0.03 (−67)	7.6^{d}±0.14 (+18)	4.6^{e}±0.12 (−26)
Bauhinia purpurea	5.8^{a}±0.14	3.8^{b}±0.14 (−34)	2.9^{c}±0.21 (−50)	6.3^{d}±0.23 (+9)	4.1^{e}±0.13 (−28)
Canavalia ensiformis	4.6^{a}±0.26	3.2^{b}±0.12 (−30)	2.4^{c}±0.17 (−49)	5.4^{d}±0.14 (+14)	2.7^{e}±0.09 (−41)
Cassia hirsuta	5.2^{a}±0.18	3.8^{b}±0.25 (−26)	2.8^{c}±0.23 (−44)	6.6^{d}±0.17 (+21)	2.5^{e}±0.27 (−52)
Caesalpinia bonducella	3.7^{a}±0.21	2.3^{b}±0.18 (−38)	2.0^{c}±0.16 (−45)	4.8^{d}±0.10 (+23)	1.9^{e}±0.03 (−47)
Erythrina indica	3.1^{a}±0.22	2.1^{b}±0.15 (−31)	1.4^{c}±0.04 (−56)	4.3^{d}±0.25 (+27)	2.3^{e}±0.17 (−26)
Mucuna gigantea	6.5^{a}±0.25	4.5^{b}±0.13 (−31)	3.3^{c}±0.13 (−49)	7.8^{d}±0.05 (+17)	3.3^{e}±0.14 (−48)
Pongamia pinnata	4.8^{a}±0.32	3.6^{b}±0.24 (−26)	1.7^{c}±0.05 (−65)	6.5^{d}±0.17 (+25)	2.1^{e}±0.17 (−36)
Sebania sesban	3.5^{a}±0.18	2.1^{b}±0.19 (−39)	1.9^{c}±0.08 (−45)	4.8^{d}±0.19 (+26)	2.3^{e}±0.19 (−36)
Xylia xylocarpa	6.7^{a}±0.20	4.2^{b}±0.16 (−36)	3.7^{c}±0.02 (−44)	7.8^{d}±0.21 (+15)	3.3^{e}±0.21 (−50)

[a] Values are mean ± standard deviation of five separate determinations ($n=5$)

[b] Values given within the parenthesis with negative/positive sign indicate the percentage of reduction/improvement

[c] Values in the same row with different alphabet superscripts are significantly different ($p<0.05$)

Concerning chemical structure, they can be divided into four groups: condensed tannins, hydrolyzable tannins, phlorotannins and complex tannins (Serrano et al. 2009). Tannins possess ideal structural chemistry for better free radical scavenging activity and hence, they exhibit more effective antioxidant activity under in vitro conditions than tocopherols and ascorbic acid (Shukla et al. 2009). The free radical scavenging power of tannins is closely connected with their spatial confirmation and degree of polymerization. Further, both the hydrolysable and condensed tannins are demonstrated to possess more effective and greater antioxidant activity than simple phenolics.

The tannins content of raw under-utilized legume grains were found to falls between 1.10 and 4.41 g/100 g DM (Table 2). These values are found to be higher when compared to previous reports on green pea (0.003–0.17 g/100 g DM); yellow pea (0.15 g/100 g DM); chickpea (0.18 g/100 g DM); lentil (0.012–0.88 g/100 g DM); red kidney bean (0.012–0.55 g/100 g DM); black bean (0.04–0.67 g/100 g DM) and common bean (0.02–0.13 g/100 g DM) (Amarowicz and Pegg 2008). Such a high level of tannins in wild legume seeds when compared to the literature (Janardhanan et al. 2003) might be due to the type of solvent used for extraction in the present analysis (acidified acetone). Similarly, Chavan et al. (2001) and Troszynska et al. (2002) reported the maximization of extraction of tannins from beach pea and yellow pea seed coats, respectively, when acetone was used as a solvent compared to methanol.

It is noticeable that, the seed samples with dark brown coloured seed coat like *Xylia xylocarpa* (4.41 g/100 g DM) and *Mucuna gigantea* (3.28 g/100 g DM) as well as black coloured seed coat *Acacia nilotica* (3.04 g/100 g DM) were registered significantly ($p<0.05$) higher level of tannins than the other seeds. It is postulated that high level of condensed tannins or proanthocyanidin are seen in dark coloured beans than in yellow or white coloured beans. Since, the level of phenolics was relatively low in pale coloured seeds; it is possible to assume that the major phenolics in dark coloured coated seeds could be proanthocyanidins. Recent studies have demonstrated a quantitative pattern of heredity for tannins content and that tannins level is also associated with seed coat colour inheritance. Several factors, such as plant type, cultivar, age of the plant

Table 2 Effect of certain indigenous processing methods on the tannins content of wild type legume grains[a,b,c]

Name of the wild type legume grain	Raw seeds	Processed seeds			
		Soaking in tamarind solution + cooking	Soaking in alkaline solution + cooking	Sprouting + oil-frying	Open-pan roasting
Acacia nilotica	3.0^{a}±0.12	2.2^{b}±0.11 (−28)	1.6^{c}±0.05 (−46)	3.8^{d}±0.28 (+21)	1.5^{e}±0.14 (−49)
Bauhinia purpurea	2.3^{a}±0.18	1.4^{b}±0.14 (−37)	1.1^{c}±0.14 (−51)	3.2^{d}±0.21 (+28)	1.1^{e}±0.23 (−52)
Canavalia ensiformis	1.6^{a}±0.06	1.1^{b}±0.06 (−35)	0.96^{c}±0.12 (−42)	1.9^{d}±0.04 (+12)	0.85^{e}±0.12 (−48)
Cassia hirsuta	2.3^{a}±0.18	1.5^{b}±0.04 (−36)	1.1^{c}±0.25 (−51)	3.2^{d}±0.22 (+27)	1.3^{e}±0.17 (−45)
Caesalpinia bonducella	1.7^{a}±0.24	1.1^{b}±0.12 (−35)	0.91^{c}±0.18 (−48)	2.4^{d}±0.19 (+27)	0.98^{e}±0.10 (−44)
Erythrina indica	1.1^{a}±0.11	0.72^{a}±0.15 (−34)	0.68^{a}±0.15 (−38)	1.5^{a}±0.24 (+25)	0.59^{b}±0.25 (−46)
Mucuna gigantea	3.3^{a}±0.12	2.4^{b}±0.08 (−25)	1.9^{c}±0.13 (−39)	4.5^{d}±0.05 (+27)	1.9^{e}±0.03 (−42)
Pongamia pinnata	2.8^{a}±0.15	1.8^{b}±0.16 (−37)	1.6^{c}±0.24 (−43)	3.2^{a}±0.12 (+12)	1.5^{d}±0.07 (−45)
Sebania sesban	1.8^{a}±0.08	1.2^{b}±0.24 (−35)	0.96^{c}±0.04 (−47)	2.4^{d}±0.18 (+26)	0.88^{e}±0.09 (−51)
Xylia xylocarpa	4.4^{a}±0.14	3.3^{b}±0.08 (−26)	2.9^{c}±0.16 (−33)	5.5^{d}±0.17 (+20)	2.7^{e}±0.21 (−39)

[a] Values are mean ± standard deviation of five separate determinations ($n=5$)

[b] Values given within the parenthesis with negative/positive sign indicate the percentage of reduction/improvement

[c] Values in the same row with different alphabet superscripts are significantly different ($p<0.05$)

or plant parts, stage of development and environmental conditions were reported to govern the tannins content in legume grains. Presence of high content of tannins in the presently studied wild legume seeds might be due to the metabolism of polyphenolic compounds or polymerization of existing phenolic compounds during development or maturation (Chavan et al. 2001).

According to Serrano et al. (2009), the mean daily intake of condensed tannins among US population (>2 year old) was estimated to be 53.6 mg/person/day, whereas 450 mg/person/day in the Spanish diet. There are a lot of epidemiological data, which suggested that tannins intake may prevent the onset of many chronic diseases. The positive biological effects including antioxidant, anticarcinogenic, anti-mutagenic, antimicrobial, antiviral and anti-diabetic properties of tannins have been extensively studied.

L-Dopa L-Dopa (L-3,4-Dihxdroxyphenylalanine) is a non-protein phenolic amino acid, mainly used in the treatment of Parkinson's disease, since it is the precursor of dopamine (Pugalenthi et al. 2005). L-Dopa has also been investigated as a dietary supplement to manage hypertension, renal failure and liver cirrhosis. Further, the protective effects of L-Dopa on small bowel injury, ulcer, gastro-intestinal diseases, diabetes as well as antioxidant stress were scientifically proved by earlier studies (Pugalenthi et al. 2005). The seed materials of wild legume grains, especially the *Mucuna* species is reported to contain appreciable level of L-Dopa (Pugalenthi and Vadivel 2007).

The raw seed materials of different wild legume grains of the present study recorded the L-Dopa content of 1.34–5.45 g/100 g DM (Table 3). These values are found to be comparable with that of certain under-utilized legumes such as *Cassia floribunda* (1.57 g/100 g DM); *C. obtusifolia* (1.34 g/100 g DM); *Canavalia ensiformis* (2.64 g/100 g DM) and *C. gladiata* (2.83 g/100 g DM) (Vadivel and Janardhanan 2005), but lower than that of *Mucuna cochichinensis* (6.11 g/100 g DM) and *M. veracruz* (7.12 g/100 g DM) (Adebowale et al. 2005).

The L-Dopa content varies considerably at significant level ($p<0.05$) among the wild legumes of the present investigation. The seed samples of *Mucuna gigantea* recorded the maximum level of L-Dopa content (5.45 g/100 g DM), while the low level was observed in *Erythrina*

Table 3 Effect of certain indigenous processing methods on the L-Dopa content of wild type legume grains[a,b,c]

Name of the wild type legume grain	Raw seeds	Processed seeds			
		Soaking in tamarind solution + cooking	Soaking in alkaline solution + cooking	Sprouting + oil-frying	Open-pan roasting
Acacia nilotica	2.6^{a}±0.20	2.1^{b}±0.25 (−18)	1.9^{c}±0.06 (−29)	1.7^{d}±0.17 (−34)	2.3^{a}±0.13 (−13)
Bauhinia purpurea	3.8^{a}±0.24	2.9^{b}±0.24 (−26)	2.5^{c}±0.24 (−34)	2.2^{d}±0.22 (−42)	3.2^{e}±0.18 (−17)
Canavalia ensiformis	2.4^{a}±0.14	1.6^{b}±0.05 (−31)	1.3^{c}±0.04 (−46)	1.2^{d}±0.05 (−48)	2.0^{e}±0.08 (−14)
Cassia hirsuta	2.9^{a}±0.06	2.1^{b}±0.14 (−27)	1.9^{c}±0.08 (−37)	1.6^{d}±0.13 (−45)	2.5^{e}±0.06 (−16)
Caesalpinia bonducella	3.7^{a}±0.08	2.7^{b}±0.13 (−27)	2.3^{c}±0.16 (−38)	2.1^{d}±0.06 (−41)	3.2^{e}±0.12 (−13)
Erythrina indica	1.3^{a}±0.17	0.96^{b}±0.03 (−28)	0.88^{c}±0.09 (−34)	0.7^{d}±0.05 (−47)	1.1^{a}±0.13 (−16)
Mucuna gigantea	5.4^{a}±0.12	4.2^{b}±0.18 (−22)	3.8^{c}±0.17 (−31)	3.3^{d}±0.08 (−39)	4.9^{e}±0.15 (−10)
Pongamia pinnata	1.9^{a}±0.28	1.4^{b}±0.11 (−27)	1.3^{c}±0.25 (−33)	1.2^{d}±0.09 (−40)	1.6^{a}±0.22 (−16)
Sebania sesban	3.5^{a}±0.16	2.4^{b}±0.14 (−30)	2.2^{c}±0.18 (−38)	2.0^{d}±0.14 (−42)	2.9^{e}±0.17 (−17)
Xylia xylocarpa	2.3^{a}±0.14	1.7^{b}±0.10 (−24)	1.6^{c}±0.15 (−30)	1.5^{d}±0.16 (−35)	1.9^{e}±0.11 (−15)

[a] Values are mean ± standard deviation of five separate determinations (n=5)

[b] Values given within the parenthesis with negative sign indicate the percentage of reduction

[c] Values in the same row with different alphabet superscripts are significantly different (p<0.05)

indica. In general, the *Mucuna* species is naturally a potential source of L-Dopa and commercially used for the extraction of this compound for the treatment of Parkinsonism. Such a wide variability in L-Dopa content among wild legumes could be cause by both environmental effect and genetic nature. For instance, presence of more L-Dopa was noticed in the *Mucuna* plants growing near the equator (within 10°) than the plants cultivated far away from equatorial regions in earlier investigations. Further, the L-Dopa synthesis is reported to be high in plants growing at low latitudes, near the equator (Pugalenthi and Vadivel 2007). It was also hypothesized that variation in the intensity of light and backscattered ultraviolet radiation, both generally more near the equator, may be among the factors explaining why the L-Dopa content was found to be high in plants growing at low latitudes.

Phytic acid Phytic acid (myo-inositol hexaphosphate) is widely found in cereals, nuts, legumes, oil seeds, pollen and spores, constituting about 1–5% and generally the legume seeds are regarded as the major source of dietary phytate (Herken et al. 2007). In recent years, the phytic acid is considered as an antioxidant, anti-carcinogenic, hypoglycemic and hypolipidemic agent, in addition to the fact that a high phytate diet can be effectively used in the treatment of hyper-calciuria and kidney stones in human beings (Schlemmer et al. 2009). The wild type legume seeds were found to contain 0.98–3.14 g/100 g DM of phytic acid (Table 4). These values are comparable with that of an earlier report on *Phaseolus vulgaris* (0.61–2.38 g/100 g DM); *Vicia faba* (0.51–1.77 g/100 g DM); *Pisum sativum* (0.22–1.22 g/100 g DM); *Vigna unguiculata* (0.37–2.90 g/100 g DM); *Cicer arietinum* (0.28–1.60 g/100 g DM) and *Lens culinaris* (0.27–1.51 g/100 g DM) (Schlemmer et al. 2009).

Considerable level of variation on the phytic acid content of presently investigated under-utilized legume grains might be attributed to both genetic and environmental conditions. In general, the cultivar, which contains appreciably high amount of protein is observed to be associated with high phytic acid content. Hence, as the protein content increases, the phytate level is also found to increase in the seed samples. Al-Numair et al. (2009) reported that the amount of phytic acid is always exceeds

Table 4 Effect of certain indigenous processing methods on the phytic acid content of wild type legume grains[a,b,c]

Name of the wild type legume grain	Raw seeds	Processed seeds			
		Soaking in tamarind solution + cooking	Soaking in alkaline solution + cooking	Sprouting + oil-frying	Open-pan roasting
Acacia nilotica	2.5^{a}±0.05	1.9^{b}±0.08 (−24)	1.6^{c}±0.06 (−35)	1.1^{d}±0.12 (−53)	2.1^{e}±0.16 (−14)
Bauhinia purpurea	1.7^{a}±0.16	1.2^{b}±0.14 (−26)	1.2^{c}±0.14 (−31)	0.9^{d}±0.14 (−46)	1.4^{e}±0.08 (−18)
Canavalia ensiformis	2.2^{a}±0.06	1.7^{b}±0.15 (−23)	1.4^{c}±0.17 (−34)	1.0^{d}±0.08 (−51)	1.9^{a}±0.14 (−13)
Cassia hirsuta	1.5^{a}±0.14	1.0^{b}±0.16 (−31)	0.96^{c}±0.13 (−35)	0.79^{d}±0.06 (−47)	1.2^{a}±0.12 (−18)
Caesalpinia bonducella	0.98^{a}±0.13	0.7^{b}±0.08 (−23)	0.68^{c}±0.05 (−30)	0.54^{d}±0.12 (−45)	0.8^{a}±0.04 (−15)
Erythrina indica	1.7^{a}±0.09	1.2^{b}±0.11 (−28)	1.2^{c}±0.08 (−33)	0.96^{d}±0.08 (−44)	1.4^{e}±0.05 (−20)
Mucuna gigantea	2.4^{a}±0.12	1.9^{b}±0.06 (−22)	1.6^{c}±0.04 (−32)	1.5^{d}±0.10 (−40)	2.0^{e}±0.08 (−16)
Pongamia pinnata	1.8^{a}±0.14	1.3^{b}±0.13 (−31)	1.1^{c}±0.06 (−41)	0.9^{d}±0.04 (−49)	1.6^{e}±0.09 (−15)
Sebania sesban	3.1^{a}±0.08	2.4^{b}±0.07 (−25)	2.0^{c}±0.15 (−35)	1.9^{d}±0.05 (−40)	2.7^{e}±0.14 (−12)
Xylia xylocarpa	1.8^{a}±0.06	1.3^{b}±0.04 (−29)	1.1^{c}±0.13 (−40)	0.8^{d}±0.14 (−54)	1.6^{e}±0.16 (−12)

[a] Values are mean ± standard deviation of five separate determinations (n=5)

[b] Values given within the parenthesis with negative sign indicate the percentage of reduction

[c] Values in the same row with different alphabet superscripts are significantly different (p<0.05)

than that of phosphorus for all the legume cultivars, which indicates that the ratio would be more than 100%. Generally, in legume seeds, the phytic acid level is positively correlated with total phosphorous, correlation coefficients being greater than 0.90. Factors that affect the total phosphorous content, such as soil, available phosphorous and fertilizer can also influence the phytic acid concentration.

The estimated daily phytic acid intake of human population was about 750 mg in U.S.A.; 600–800 mg/day in U.K.; 393 mg/day among Canadian children; 2,000–2,200 mg/day in Nigeria; 1890 and 569 mg/day in Malawi and New Guinea, respectively and 1487 mg/day in East India (Plaami 1997). But, historically it has been considered as an antinutrient and postulated to impede the bioavailability of minerals. Nevertheless, the research studies conducted by Grases et al. (2004) showed that there is no negative effect on the mineral balance and element bioavailability due to the oral administration of phytic acid, even in the second generation rats.

Effect of treatments on total free phenolics Reduction of significant level (p<0.05) of total free phenolics was noticed during soaking in tamarind solution + cooking treatment (26–40%) (Table 1). But, in contrast increase in flavonoid content of pearl millet during cooking process was reported by Gupta and Nagar (2010). Soaking in tamarind solution may leads to softening of cell wall tissues under acidic environment, which is usually accompanied by release of bounded phenolic compounds, and hence may be leached or diffused into the soaking/cooking medium. Ranilla et al. (2009) reported that treatments with a previous soaking and/or draining step after cooking process resulted in significant loss of phenolic constituents. Likely, the previous soaking process in this experiment may lead to significant reduction of phenolic compounds in wild legume grains.

Soaking in alkaline solution + cooking treatment resulted in significant loss of total free phenolics (44–67%) (Table 1). This is in agreement with that of previous reports on *Vigna radiata* (32%) (Grewal and Jood 2006); *Cajanus cajan* (50%) and *Vigna unguiculata* (37%) (Onwuka 2006). Such significant level of reduction of total free phenolics during this treatment might be because of the leaching out of this compound into the soaking medium due to increased

permeability of the seed coat under alkaline environment or due to solubilisation of this compound in alkaline solution under the influence of concentration gradient and also due to degradation of phenolics with the high temperature during subsequent cooking. Such loss of phenolics can also be explained by a lixiviation phenomenon that drives phenolics into the cooking water. This process is a function of temperature and will promote diffusion of phenolics into cotyledons also (Barroga et al. 1985; Rocha-Guzman et al. 2007).

It is important to recognize that, significant level of increase of total free phenolics (9–27%) was observed during sprouting + oil-frying in the under-utilized legume grains. 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 level of elevation on the level of total free phenolics was 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 DM. Earlier research studies indicated that, a major portion of total free phenolics was stored in seeds as soluble conjugate or insoluble forms. Hence, the little level of increase noticed in wild legume 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 release of free phenolics from bounded form by the breakdown of cellular constituents and cell walls during subsequent thermal process (oil-frying). Further, thermal treatment (oil-frying) likely induce the hydrolysis of conjugated phenolics and resulted in the release of free phenolic compounds (Ranilla et al. 2009).

Significant reduction of total free phenolics was noticed during open-pan roasting (26–52%) (Table 1). Similarly, dry-heat treatment was reported to decrease the total free phenolics content in cowpea seeds (48–60%) (Siddhuraju and Becker 2007). Degradation of total free phenolics as a result of direct heat exposure could be a reason for this reduction observed in under-utilized legume seeds under open-pan roasting.

Effect of treatments on tannins Soaking in tamarind solution + cooking caused significant level of reduction of tannins in wild type legume grains (25–37%) (Table 2). Such loss of tannins during this treatment could be attributed to leaching out of this compound into the acidic soaking medium and chemical transformation or decomposition of tannins and also the formation of tannins-protein complex under acidic soaking medium as well as by thermal conditions during subsequent cooking.

Soaking in alkaline solution + cooking resulted in significant level of removal of tannins (33–51%), but these values are found to be lower when compared to an earlier report on *Bauhinia purpurea* (69–78%) (Vijayakumari et al. 2007) and *Phaseolus vulgaris* (68%) (Shimelis and Rakshit 2007). Such losses may be a function of increased permeability of the seed coat, which leads to leaching out of this compound, due to the alkaline environment and subsequent degradation during cooking.

Alternatively, substantial level of increase of tannins was recorded (12–28%) during sprouting + oil-frying treatment (Table 2). These results are in agreement with those reported by Fernandez-Orozco et al. (2008), who found a high level of increase of tannins (53%) in lupin sprouts. Khattak et al. (2007) reported a small rise on the tannins content of chickpea, while several authors observed large level of increase of tannins in soybean and other beans during germination. Similarly, Duenas et al. (2009) have also noticed a significant level of increase on both flavonoid and non-flavonoid polyphenolic compounds during germination of lupin seeds. Such significant increase of tannins in the presently investigated seeds might be probably due to the polymerization of existing phenolic compounds into insoluble and high molecular weight polymers like tannins. According to Bunea et al. (2008), the increase in concentration of certain phenolic compounds after thermal treatment may be explained either by their better release from the food matrix as a result of breakdown of supramolecular structures containing phenolic groups or because of their thermal stability.

Open-pan roasting caused a drastic level of loss of tannins (39–52%) in the presently studied legume grains (Table 2). This is in agreement with that of an earlier report on loss of tannins (54–72%) in light brown colour seed-coated *Vigna unguiculata* (Siddhuraju and Becker 2007). Such a substantial reduction of tannins in the wild seeds might be due to the fact that some of the polyphenolic compounds, like tannins are known to accumulate in the cellular vacuoles and the direct heat may denature them. Other reasons could be Maillard reaction, caramelization, chemical oxidation of tannins and maderisation.

Effect of treatments on L-Dopa Soaking in tamarind solution + cooking resulted in a significant level of removal of a non-protein amino acid, the L-Dopa (18–31%) in all the investigated legume samples (Table 3). Similarly, Srivastava and Khokhar (1996) also observed the significant effect of tamarind solution on another non-protein amino acid, ß-ODAP in *Lathyrus sativus* seeds. However, the level of L-Dopa loss is appears to be lower in relation to reduction of phenolics and tannins in respective seed samples under this treatment, which could be explained by two factors: 1. Permeability of the seed coat along with

the diffusion rate of L-Dopa and 2. Presence of L-Dopa in the intact cell compartments of cotyledons rather than the seed coat.

A drastic level of reduction of L-Dopa was observed in the seed materials of wild legumes (29–46%) during soaking in alkaline solution + cooking treatment, which is in consonance with that of an earlier report on velvet bean (81%) (Vadivel and Pugalenthi 2010). Furthermore, D'Mello and Walker (1991) also reported that the treatment of *Canavalia ensiformis* seeds with alkaline solution (potassium-bi-carbonate) at 80 °C resulted in decline of another non-protein amino acid, L-Canavanine to a negligible level. Such obvious reduction of L-Dopa in underutilized legumes under this treatment is most likely due to enhanced leaching out of this compound by the increased seed coat permeability caused by the alkaline soaking medium and also due to the chemical conversion of L-Dopa into melanin pigment under alkaline conditions in addition to partial denaturation of L-Dopa by heat under cooking.

Sprouting + oil-frying treatment was found to cause significant level of loss of L-Dopa (34–48%) in the presently analyzed seed materials (Table 3). These results suggested that the L-Dopa degrading enzymes, such as polyphenol oxidase could be synthesized upon germination of seeds to metabolize the L-Dopa. From the earlier research works, it was reported that, only a short-term germination of legume grains is sufficient to capture a good amount of L-Dopa, after which it may be mobilized to some other products, which are not yet identified (Randhir et al. 2008). Hence, the raise noticed in total free phenolics and tannins after sprouting + oil-frying treatment of the present study (Tables 1 & 2) could be mobilized from L-Dopa. Because, it is postulated that, during initial stages of germination, most of the phenolics may have been diverted towards antioxidant function and L-Dopa production, when the need for lignifications was minimal. But, as germination proceed, the L-Dopa content has reduced markedly, which indicating that the precursor metabolites are potentially diverted from L-Dopa production towards the synthesis of phenolics and tannins, which are required for lignifications process associated with growth. This may be one of the reasons explaining why the L-Dopa content was reduced after sprouting + oil-frying treatment in wild legume grains.

Open-pan roasting resulted in moderate level of reduction of L-Dopa (10–17%) in wild legume seeds. This is in consonance with that of a previous report on the reduction of L-Dopa content in *Entada scandens* (27%) (Vadivel et al. 2008). Such loss of L-Dopa under this treatment might be due to either partial oxidation of this compound or racemisation under high temperature. Another reason could be that there may be a chance of modification of chemical properties of L-Dopa, since the seed samples were subjected to a very high temperature (120–130°C).

Effect of treatments on phytic acid Significant reduction of phytic acid was observed during soaking in tamarind solution + cooking treatment (22–31%) in wild type legume seeds (Table 4). These results are in agreement with the previous findings of Srivastava and Khokhar (1996) in different lines of *Lathyrus sativus* seeds. These losses are mainly due to leaching out of this compound into the acidic soaking medium and the leaching is particularly favoured when the compound possesses low molecular weight and ionic character. Deshpande and Cheryan (1983) also reported that the loss of phytic acid can be enhanced by increasing the ionic concentration of the soaking medium.

Soaking in alkaline solution + cooking treatment resulted in 30–41% of loss of phytic acid in the seed samples. Similarly, 27% of reduction of phytic acid was noticed in *Bauhinia purpurea* seeds (Vijayakumari et al. 2007). Nonetheless, high level of removal of phytic acid was also reported in *Phaseolus vulgaris* during this treatment (Shimelis and Rakshit 2007). Such significant loss of phytic acid is likely due to either leaching out of this compound into the alkaline soaking medium and/or formation of insoluble complexes between phytic acid and other nutrients such as phytate-protein and phytate-protein-mineral complexes under alkaline soaking environment and/or hydrothermal processing.

Sprouting + oil-frying exhibited a significant level of removal of phytic acid in the presently analyzed underutilized legume grains (40–54%) (Table 4). Such a drastic level of reduction were higher in relation to earlier reports on *Cajanus cajan* (17%) (Oloyo 2004) and *Vigna radiata* (13%) (Grewal and Jood 2006). Similarly, Al-Numair et al. (2009) reported that germination reduces the phytic acid content by 18–34% in *Vicia faba* and 23–36% in *Phaseolus vulgaris*. Recently, germination and cooking treatments are reported to reduce 13 and 15% of phytic acid, respectively in cowpea seed flour (Herken et al. 2007). But, on the other hand, high level of reduction of phytic acid is also observed in certain common legume grains during germination (Khattak et al. 2007). The removal of phytic acid during germination in legume grains is attributed to the enzymatic (phytase) hydrolysis of phytic acid followed by diffusion. Phytase activity is detected in all the sprouts of cereal and legume grains. Since, the seeds need a lot of energy for their sprouting process, the naturally occurring phytase enzyme becomes active upon sprouting. Consequently, the phytase hydrolyzes the phytic acid into phosphate and myo-inositol phosphate, which represent an important primary source of energy for seed sprouting.

Moderate level of reduction of phytic acid (12–20%) was noticed in wild legume seeds upon open-pan roasting (Table 4). These values are found to be lower when compared to an earlier report on *Entada scandens* (49%) (Vadivel et al. 2008). Such minimal loss of phytic acid

during this treatment might be partially due to degradation of myo-inositol hexa-phosphate (phytic acid) into penta- and tetra- phosphates under high temperature (120–130°C). Prolonged input of energy is necessary to denature this heat-stable compound.

Conclusion

Ten different wild type unconventional legume seeds collected from various agro-ecological regions of South India were found to contain appreciable levels of bioactive compounds such as total free phenolics, tannins, L-Dopa and phytic acid. Among the under-utilized legume grains, significantly higher level of total free phenolics and tannins were noticed in *Xylia xylocarpa* seeds, while *Mucuna gigantea* and *Sesbania sesban* registered maximum levels of L-Dopa and phytic acid, respectively. Considering the effect of different indigenous processing methods, soaking in tamarind solution + cooking as well as soaking in alkaline solution + cooking treatments exhibited drastic level of loss of all the investigated compounds. Further, open-pan roasting also demonstrated a significant level of reduction of total free phenolics, tannins, and moderate loss of L-Dopa and phytic acid. Nonetheless, sprouting + oil-frying was found to increase the content of total free phenolics and tannins, but showing diminishing effect on L-Dopa and phytic acid. Hence, among the presently employed processing methods, sprouting + oil-frying could be considered as a suitable processing method and can be recommended for the consumption of these wild type legume seeds in order to increase the dietary intake of health beneficial bioactive compounds. Implementation of such suitable processing technique will increase opportunities for the versatile utilization of under-utilized legume seeds with high levels of bioactive compounds in the dietary management of certain chronic diseases such as diabetes, cancer, cardiovascular diseases, etc.

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