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# Original Article

# Preliminary assessment of the nutritional composition of underexploited wild legumes from semi-arid *Caatinga* and moist forest environments of northeastern Brazil

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#### ABSTRACT

The seeds of 14 wild legume species from Brazil were analyzed to assess their potential as food. The seeds showed high levels of crude protein (10.9–50.0%), dietary fiber (0.8–52.3%) and energy (905–1804 kJ 100 g $^{-1}$ ), and the amino acid composition (mg g $^{-1}$  N) was comparable to that of soybeans, but with higher amounts of lysine (456–10,884) and histidine (199–918). The seeds showed lectin activity (20–2560 and 60–2560 HU g $^{-1}$  flour for untreated or treated erythrocytes, respectively) as well as trypsin inhibitory activity (4.06–27.35  $\mu g$  TI mg $^{-1}$  flour), urease (225–23,895 U kg $^{-1}$  flour) and toxic activities (LD $_{50}$ 0.22–0.12 g kg $^{-1}$  body weight). Most of the minerals, as with edible legume seeds, were present in high levels when considering reference daily intake (RDI) and the nutrient content claims of the United States Food and Drug Administration (FDA). Thus, these wild legume seeds can be promising alternatives sources of food to overcome malnutrition problems.

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# 1. Introduction

The northeastern region of Brazil possesses an area of 1,539,000 km², where 45 million people live. Approximately 50% of this population lives in poverty, hence they do not have an adequate food supply. This situation results in a high index of malnutrition and other nutritional disabilities. On the other hand, 60% of this region of semi-arid climate is covered by xerophytic *Caatinga* vegetation (dry land vegetation), with small groves of

tropical moist forest in the mountains. In *Caatinga* vegetation, there are almost a thousand vascular plant species. Among these, over 200 species are legumes (Sampaio et al., 2000).

A possible measure to overcome the malnutrition problem faced by the northeastern Brazilian population would be the cultivation of these lesser known wild plants, which have already increased food resources for human and/or animal nutrition. Certainly many of these underexploited legumes contain proteins and other nutritionally important components that could be used as alternative food sources to complement conventional crops. The search for alternative food ingredients, especially in developing countries, is of utmost importance, particularly because of the high cost of animal protein sources (Arun et al., 2003).

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Besides their high protein content and low cost, legume seeds have other desirable features, such as abundance of carbohydrates and fiber, low fat content (except oilseeds), and B complex vitamins and minerals (Sridhar and Seena, 2006). In addition, legume seeds display nutraceutical properties against cardiovascular disease, diabetes, digestive tract diseases, overweight and obesity (Duranti, 2006).

Little-known legumes can play an important role in agriculture since they have potential to contribute to the world food production because of their adaptation to adverse environmental conditions and high resistance to diseases and pests (Sridhar and Seena, 2006). However, most of the Brazilian wild legumes have not been biochemically and nutritionally investigated; thus lack of nutritional information continues to limit their use. Thus, considering the great Brazilian biodiversity, it is reasonable to carry out an evaluation of the potential of its wild plants for exploitation.

In view of this, the aim of the present work was to investigate the food potential of wild legumes from northeastern Brazil. For this, the proximate composition, amino acid profile, and determination of antinutrients and/or toxic substances and mineral composition were determined in 14 wild legume seeds from semi-arid Caatinga vegetation and moist forest in northeastern Brazil. These seeds (Table 1) belong to Leguminosae family and are underexploited by local population, being barely used in a nonsustainable way in folk medicine and large-scale extraction of timber for construction and charcoal. Moreover, these species are poorly studied for their potential use in the food industry and medicine, making it difficult to take conservation actions. In light of this, the population of the semi-arid region still faces serious public health problems that could be mitigated by the sustainable use of biodiversity. Therefore, the knowledge of the chemical composition and nutritional value of these species may contribute to public action and thus to the conservation of these species.

**Table 1**Compiled information on the studied wild legumes from Northeastern Brazil.

Family – Subfamily Botanical name [type of environment/vegetation: harvesting date] voucher number	Common name (in Portuguese)	Traditional use by local population	Information on nutritional and antinutritional properties		
Fabaceae – Caesalpinoideae Caesalpinia bracteosa Tul. [Semi-arid Caatinga: January/2005] EAC 39616	"Catingueira"	Used in civil construction, as charcoal, as vermifuge, treatment of respiratory infections, dysentery, hepatitis and anemia (Maia, 2004)	Not available		
Caesalpinia ferrea Mart. [Araripe National Forest, moist forest: January/2005] EAC 39616	"Jucá", "pau-ferro"	Treatment of diabetes and bronchial and lung diseases (Maia, 2004)	Not available		
Dimorphandra gardneriana Tul. [Araripe National Forest, moist forest: March/2007] EAC 39617	"Fava d'anta"	Treatment of hemorrhoids, varicose veins, haematome and other vascular diseases (Agra et al., 2007)	Not available		
Hymenaea courbaril L. [Araripe National Forest, moist forest: January/2005] EAC 38108	"Jatobá"	Human food	Not available		
Pterogyne nitens Tul. [Araripe National Forest, moist forest: March/2007] EAC 39793	"Madeira-nova"	Not described	Not available		
Senna obtusifolia (L.) H.S. Irwin & Barneby [Semi-arid Caatinga: July/2005] EAC 39320	"Mata pasto"	Used as abortive, laxative, sedative and in the treatment of amenorrhea and rheumatism (Agra et al., 2007)	Not available		
Senna rugosa (G. Don) H.S. Irwin & Barneby [Araripe National Forest, moist forest: September/2005] EAC 38112	"Lagarteiro"	Not described	Not available		
Fabaceae – Faboideae Dioclea megacarpa Rolfe [Araripe National Forest, rainforest: January/2005]	"Mucunã", "olho-de-boi"	Not described	Lectin activity (Moreira et al., 1997)		
EAC 38110 Erythrina velutina Willd. [Semi-arid Caatinga: September/2005]	"Mulungu"	Used in the treatment of insomnia, coughs and as	Lectin activity (Moraes et al., 1996)		
EAC 35979  Lonchocarpus sericeus (Poiret) Kunth [Semi-arid coast zone: October/2006]  EAC 39615	"Ingá"	vermifuge (Agra et al., 2007) Not described	Proximate composition, energy, antinutrients, amino acid composition and feeding trials with rats (Proll et al., 1998)		
Fabaceae – Mimosoideae Albizia lebbeck (L.) Benth.	"Timbaúba"	Used in civil construction and	Chemical composition and		
[Semi-arid Caatinga: November/2006] EAC 39608 Enterolobium contortisiliquum (Vell.) Morong	"Orelha-de-macaco",	as charcoal (data non-published)  Used as anti-inflammatory	secondary metabolites (Babayemi et al., 2004) Kunitz-type trypsin		
[Semi-arid Caatinga: September/2005] EAC 38115	"orelha-de-negro"	(Agra et al., 2007)	inhibitory activity (Batista et al., 1996)		
Parkia platycephala Benth. [Araripe National Forest, moist forest: March/2007] EAC 38109	"Visgueiro"	Used as animal feed (data non-published)	Lectin activity (Mann et al., 2001)		
Piptadenia moniliformis Benth. [Semi-arid Caatinga: March/2007] EAC 35974	"Catanduva"	Used in civil construction and as charcoal (Maia, 2004)	Not available		

#### 2. Materials and methods

#### 2.1. Biological and chemical reagents

Soybean trypsin inhibitor (Sigma T9128), trypsin from human pancreas (Sigma T6424), protease from *Bacillus licheniformis* (Sigma P3910), L-BAPNA (Sigma B3133), urease from *Canavalia ensiformes* (Sigma U0251), total dietary fiber assay kit (Sigma TDF100A), were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All other chemical reagents used in the experiments were of analytical grade. Rabbit blood was obtained from animals of colonies maintained at the Federal University of Ceará (Fortaleza, Brazil). Female mice of Swiss strain were obtained from an outbred colony maintained at the Federal University of Ceará (Fortaleza, Brazil).

#### 2.2. Seed samples and processing

Harvesting of plant material was carried out in *Caatinga* forest, in one rainforest (Araripe National Forest) and in the coastal zone, all in Ceará State, northeastern Brazil. Mature wild seeds (100–1000 g) were collected during the dry period (from January 2005 to March 2007), with help of natives. Only one harvest was performed due to difficulties pertinent to the semi-arid region that undergoes severe droughts and to the limitations established by Brazilian environmental protection agencies. Plants were identified and voucher specimens were deposited in the Herbarium Prisco Bezerra–EAC, Universidade Federal do Ceará (Fortaleza, Ceará, Brazil). Table 1 lists the studied species with their botanical classification, locality and harvesting date, voucher number, common name, traditional use by local population and the status of knowledge of their nutritional and antinutritional properties in the literature

The seeds were separated from pods, sun-dried and ground to obtain fine flour (mesh size 1.0 mm) by using a blender and a coffee mill and stored in air-tight containers at  $4\,^{\circ}\text{C}$  until analysis.

#### 2.3. Proximate composition and energy

Moisture content was determined at 110 °C using an oven until constant weight was obtained, at least for 72 h (AOAC, 1998). Nitrogen was determined by the standard macro-Kjeldahl method (AOAC, 1998) using a digestion apparatus combined with the photocolorimetric method described by Baethgen and Alley (1989) to obtain total crude protein content ( $N \times 6.25$ ). Crude lipid content was determined by n-hexane extraction (AOAC, 1998) and ash content by using a muffle furnace at 550 °C (AOAC, 1998). Total dietary fiber was determined by Prosky-AOAC method (AOAC, 1998) using the total dietary fiber assay kit (Sigma-Aldrich Co., St. Louis, MO, USA). All determinations were run in triplicates (three analyses of the same sample). The digestible carbohydrate content was determined by calculating the percentile difference from all the other constituents according to the formula: [100 g dry weight – (g crude protein + g crude lipid + g ash + g dietary fiber)]/ 100 g. The energy content was estimated by multiplying the percentages of crude protein, crude fat and digestible carbohydrates by their respective modified Atwater factors, which are 17, 37 and 17, respectively (FAO, 2003).

## 2.4. Amino acid analysis

Defatted seed flours were hydrolyzed with 6 M HCl containing  $10~{\rm g~L^{-1}}$  phenol at  $110~{\rm ^{\circ}C}$  for 22 h, in sealed glass tubes under  $N_2$  atmosphere. HCl and phenol were removed by evaporation and the amino acid compositions were established after chromatography on a Biochrom 20 system (Pharmacia). Tryptophan was deter-

mined according to the method described by Pintér-Szakács and Molnár-Perl (1990). All determinations were run in triplicates.

# 2.5. Mineral composition

The determination of minerals (K, Na, Ca, Mg, Fe, Zn, Cu, Mn, Cr and Mo) was performed by atomic emission spectroscopy (ICP-OES). For each test 200 mg of seed meal were treated with 3 mL concentrated HNO $_3$  (Merck, Uppsala, Sweden) and 2 mL H $_2$ O $_2$  30% (v/v) (Merck). This mixture was heated in microwave oven (Multiwave, Anton Par) under pressure, with heating program set to 20 min and cooling to 15 min. After decomposition the suspension was diluted to 30 mL with deionized water (Milli-Q) and a calibration curve was prepared using multielementar solution for quantitation of minerals (Gouveia et al., 2002). All determinations were run in triplicates.

# 2.6. Antinutritional and/or toxic factors

Crude extracts were prepared according to Vasconcelos et al. (1997) and used for detection of lectin and toxic activities. Lectin activity was assessed by serial twofold dilution of samples (Vasconcelos et al., 1997). The extracts were diluted with 0.15 M NaCl and mixed with rabbit erythrocytes (20 mg mL $^{-1}$ suspension prepared in 0.15 M NaCl), untreated or treated with proteases from B. licheniformes in a concentration of 1 mg  $mL^{-1}$ . The degree of agglutination was visually monitored after the tubes had been left to stand at 37 °C for 30 min, at room temperature  $(22 \pm 3 \, ^{\circ}\text{C})$  and after additional 30 min. The lectin activity was expressed as haemagglutination titre (HU g<sup>-1</sup> flour), which is the reciprocal of the highest dilution giving visible agglutination of rabbit erythrocytes untreated and treated with proteases from B. licheniformis. Trypsin inhibitor activity was determined according to the method described by Kakade et al. (1969), with a slight modification (Hamerstrand et al., 1981), using trypsin enzyme and L-BAPNA as substrate. The activity was expressed as the amount of trypsin inhibited, calculated from a calibration curve using soybean trypsin inhibitor. Urease assay was carried according to Kaplan (1969), with minor modifications of the procedure well described by Vasconcelos et al. (1997). Acute toxicity to mice (n = 6) was verified by intraperitonial injection (30  $\mathrm{mL}\,\mathrm{kg}^{-1}$  body weight) of diluted and crude water extract of each seed according to Vasconcelos et al. (1997). This procedure was approved by the Animal Experimentation Ethics Committee of Universidade Federal do Ceará (CEPA), who adopts the guidelines of Brazilian College of Animal Experimentation (COBEA). All determinations were run in triplicates.

# 2.7. Statistical analysis

The data of proximate composition and of antinutritional and/ or toxic components were presented as mean  $\pm$  standard deviations of triplicates. For other analyses, the data were shown as mean of three determinations and the standard deviation values were omitted since they were less than 5% of mean.

# 3. Results and discussion

In the present study only one sample was collected because we are dealing with wild plants of the semi-arid region of Brazil. This region undergoes severe periods of droughts making it difficult the harvesting of samples in more than one period along the year. The amount of seeds available for harvesting is limited, since these plant species are not cultivated and often their natural environments have been destroyed by local population through unsustainable activities. Besides, there are also the difficulties imposed by the strict regulation of environmental protection Brazilian

**Table 2**Proximate composition and energy value of 14 wild legumes seeds from Northeastern Brazil.

Legume seeds	Energy <sup>a</sup> (kJ/100 g)	Moisture (%)	% Dry weight basis							
			Protein <sup>b</sup>	Protein <sup>b</sup> Lipid		Dietary fiber	Digestible carbohydrate			
Albizia lebbeck	1240	8.9 ± 0.1	$42.9 \pm 2.8$	$4.3 \pm 0.1$	$3.5 \pm 0.1$	$28.6 \pm 0.8$	20.7			
Caesalpinia bracteosa	1159	$15.9 \pm 0.5$	$29.3 \pm 1.5$	$10.9 \pm 0.2$	$3.7 \pm 0.1$	$40.9 \pm 0.7$	15.2			
Caesalpinia ferrea	1000	$10.3 \pm 0.6$	$42.7 \pm 1.6$	$1.8 \pm 0.2$	$5.6 \pm 0.2$	$37.7 \pm 0.9$	12.2			
Dimorphandra gardneriana	972	$10.7 \pm 0.1$	$\textbf{32.3} \pm \textbf{1.2}$	$2.7 \pm 0.0$	$2.0 \pm 0.1$	$44.0 \pm 3.3$	19.0			
Dioclea megacarpa	1618	$12.8 \pm 0.5$	$44.8 \pm 1.9$	$2.5 \pm 0.0$	$2.5 \pm 0.0$	$5.3 \pm 0.0$	44.9			
Enterolobium contortisiliquum	1291	$10.3 \pm 0.5$	$\textbf{50.0} \pm \textbf{3.4}$	$3.4 \pm 0.0$	$3.5 \pm 0.4$	$24.6 \pm 0.3$	18.5			
Erythrina velutina	1299	$10.3\pm1.0$	$39.0 \pm 3.6$	$12.4 \pm 0.1$	$\boldsymbol{5.0\pm0.2}$	$\textbf{33.2} \pm \textbf{1.4}$	10.4			
Hymenaea courbaril	1804	$11.4\pm0.2$	$10.9 \pm 0.4$	$8.0 \pm 0.4$	$2.5 \pm 0.4$	$\boldsymbol{0.8 \pm 0.0}$	77.8			
Lonchocarpus sericeus	1709	$6.7 \pm 0.6$	$\textbf{35.7} \pm \textbf{1.6}$	$29.6 \pm 0.1$	$3.5 \pm 0.1$	$30.8 \pm 0.3$	0.4			
Parkia platycephala	1048	$10.0 \pm 0.1$	$\textbf{32.2} \pm \textbf{1.9}$	$13.4 \pm 0.7$	$1.8 \pm 0.1$	$52.3 \pm 1.0$	0.3			
Piptadenia moniliformis	1390	$11.4\pm1.1$	$44.7 \pm 2.0$	$\textbf{7.2} \pm \textbf{0.1}$	$3.2 \pm 0.1$	$23.5 \pm 0.2$	21.4			
Pterogyne nitens	1275	$\textbf{8.2} \pm \textbf{0.1}$	$28.3 \pm 1.6$	$11.8 \pm 0.3$	$5.0 \pm 0.0$	$33.9 \pm 0.8$	21.0			
Senna obtusifolia	905	$11.5 \pm 0.1$	$15.8 \pm 1.8$	$6.3 \pm 0.2$	$\textbf{6.8} \pm \textbf{0.1}$	$47.4 \pm 0.6$	23.7			
Senna rugosa	1048	$\textbf{8.2} \pm \textbf{0.1}$	$22.3 \pm 1.3$	$\boldsymbol{0.7 \pm 0.0}$	$1.9 \pm 0.0$	$37.3 \pm 0.4$	37.8			
<sup>d</sup> RDI	$8368  (kJ  day^{-1})$	_	$50 \ (g  day^{-1})$	$65 (g  day^{-1})$	-	$25 \; (g  day^{-1})$	$300  (\mathrm{g}  \mathrm{day}^{-1})$			

All values are means ± standard deviation of three analyses of the same sample.

- <sup>a</sup> Estimated by multiplying the percentage of total protein, total fat and digestible carbohydrates by the respective modified at water factors 17, 37 and 17, respectively. <sup>b</sup> N × 6.25.
- <sup>c</sup> The available carbohydrate content was determined by calculating the percentile difference from all the other constituents according to the formula: [100 g dry weight (g crude protein+g crude lipid+g ash+g dietary fiber)]/100 g.
- d Reference daily intake in 101.9 (c) (8) iv. FDA U.S. Food and Drug Administration (2010).

agencies, which establish limits to the amount of seeds to be collected and the frequency of collections.

The results of the proximate composition and energy value analysis of the 14 northeastern Brazil wild legume seeds are in Table 2. In the present study the results on nutrient contents were expressed per 100 g dry weight to be possible to compare the nutrient composition considering that the study is dealing with wild plants, that the different species were gathered in different stages of maturation and that they have different characteristics (e.g., some seeds are juicier than others, some seeds have thicker tegument, etc.). Moisture values ranged from  $6.7 \pm 0.6\%$  in Lonchocarpus sericeus to  $15.9 \pm 0.5\%$  in Caesalpinia bracteosa. Five species showed moisture values comparable to those reported for different cultivars of cowpea ranging from 6.1 to 8.9% (Onwuliri and Obu, 2002), which is an important protein source for northeastern Brazil population, and to values described for Indian wild legume seeds ranging from 5.7 to 8.5% (Vadivel and Janardhanan, 2005). However, the other nine species showed high moisture values (>10%). According to Puzzi (2000), soybean should be stored with moisture ranging from 11 to 14% in order to prevent proliferation of microorganisms and the occurrence of hydrolytic reactions that may lead to loss of seeds nutritional value. Therefore, the high moisture values do not compromise the nutritional quality of the seeds. Protein values ranged from 10.9  $\pm$  0.4% in Hymeneaea courbaril to 50.0  $\pm$  3.4% in Enterolobium contortisiliquum. Only H. courbaril and Senna obtusifolia showed protein values below 20%. Therefore, twelve legume species showed protein values comparable or much higher than those reported for some cultivated edible legume seeds in Brazil, like six cowpea cultivars ranging from 19.5 to 26.1% (Maia et al., 2000), five soybean cultivars ranging from 36.1 to 48.5% (Vasconcelos et al., 1997) and black beans with 23.2% (Trugo et al., 2000). In addition, these protein values are similar or higher than those described for Indian wild legumes ranging from 20.3 to 35.0% (Vadivel and Janardhanan, 2005) and for Mexican wild lupins which vary from 37.20 to 45.41% (Ruiz and Sotelo, 2001) and represent 22-100% of reference daily intakes (RDIs) established for adults and children four or more years of age based on a 2000 kcal (8368 kJ) intake (FDA, 2010). In recent years, the occurrence of malnutrition in children and adults decreased at an accelerated pace, but increased the prevalence of overweight and obesity in the population of northeastern Brazil. Thus, it has been established an antagonism of temporal trends between malnutrition and obesity, defining a common feature of the

process of nutritional transition in Brazil (Batista Filho and Rissin, 2003). This results from the fact that northeastern Brazilian population food is characterized by being richer in energy than in protein, causing a nutritional imbalance. Poor populations in northeastern Brazil, both rural and urban, depend on legumes. especially beans for protein intake, since the animal protein, that is considered the best in terms of amino acid balance and digestibility, is more expensive. Therefore, the study of unconventional proteins is very timely to analyze the feasibility of their incorporation into the diet as a way to combat the nutritional deficiencies of the poorest populations, resulting from the intake of foods that do not reach nutritional requirements. In the present study total lipids was expected to be lower than the actual amount since analysis of crude lipid did not include step of acid digestion prior to solvent extraction. This treatment is required for polar and complex lipids which occur as part of the membranes in plant foods, which often associated with protein and carbohydrates. However, the estimated crude fat done may include true fats and oils, fatty acid esters, compound lipids and fat-soluble vitamins or provitamins such as the carotenoids, all of which of nutritional value. The ether extract may also contain significant concentrations of indigestible waxes, resins and essential oils, which are not as important in the nutritional point of view.

The lipid content ranged from 0.7% in Senna rugosa to 29.6% in L. sericeus. The lipid content in L. sericeus is higher than those described for soybean seeds, an important oilseed, with values ranging from 18.3 to 21.5% (Vasconcelos et al., 1997). Analysis of fatty acid composition of seeds with lipid content over 20% could reveal new sources of edible legume oils with composition at least similar to that presented by soybean oil, rich in unsaturated fatty acids. The ash values ranged from 1.8  $\pm$  0.1% in *Parkia platycephala* to 6.8  $\pm$  0.1% in *S. obtusifolia*, being these values comparable to those reported for lupin (3.2%), soybean (5.3%) and black bean (4.6%) by Trugo et al. (2000). Dietary fiber values ranged from 0.8% in H. courbaril to 52.3% in P. platycephala. Seventy five percent of the analyzed species showed dietary fiber values over 30%, being very close to that described for the main fiber source of Brazilian population, the black beans. The extremely high values of 30% dietary fiber represent 120% of the RDI (FDA, 2010). The consumption of dietary fiber has been related to several health promoting effects, such as prevention of cardiovascular disease, diabetes, digestive tract diseases, overweight and obesity (Duranti, 2006). The digestible carbohydrates values ranged between 0.3% in P. platycephala and 77.8% in H. courbaril. Due to their balanced composition, rich in protein, oil and digestible carbohydrate, seeds showed high energy values, which varied from 905 kJ  $100 \, \mathrm{g}^{-1}$  in S. obtusifolia to  $1804 \, \mathrm{kJ} \, 100 \, \mathrm{g}^{-1}$  in H. courbaril. These energy values are comparable or much higher than those reported for black beans ( $1413 \, \mathrm{kJ} \, 100 \, \mathrm{g}^{-1}$ ) and based upon FDA regulations these values may be considered as good sources to high sources of energy, representing 10-22% of RDI (FDA, 2010).

The amino acid composition of the wild seeds from northeastern Brazil is shown in Table 3. The wild seeds showed essential amino acid profile similar to that described for soybean (Trugo et al., 2000; Vasconcelos et al., 2001), but with higher amounts of lysine  $(456-1088 \text{ mg g}^{-1} \text{ N})$  and histidine  $(199-918 \text{ mg g}^{-1} \text{ N})$ . Excepting for histidine content, most of the wild seeds showed lower amounts of all essential amino acids if compared to egg white profile. This fact was also demonstrated for soybean seeds (Trugo et al., 2000; Vasconcelos et al., 2001). According to the requirements of essential amino acids established by FAO/WHO/ UNU (1985) for children in different age groups, wild seeds were highly deficient in tryptophan (0-65 mg g<sup>-1</sup> N) and methionine + cystine  $(0-380 \text{ mg g}^{-1} \text{ N})$  and, in a lower proportion, in leucine  $(311-585 \text{ mg g}^{-1} \text{ N})$  and isoleucine  $(23-375 \text{ mg g}^{-1} \text{ N})$ . This essential amino acids profile is characteristic of leguminous seeds with Met + Cys as the primarily limiting amino acids (Trugo et al., 2000; Ruiz and Sotelo, 2001). Therefore, according to these results Erythrina velutina, P. platycephala, Piptadenia moniliformis, Pterogyne nitens, S. obtusifolia and S. rugosa present an adequate amino acid profile when compared to soybean, one of the most important plant protein sources.

The presence of antinutritional and/or toxic components is one of the major limiting factors for use of the nutritional qualities of the legumes (Liener, 1994). The lectin, trypsin inhibitory, urease and toxic activities are shown in Table 4. In the studied legumes, lectin activity against treated or untreated rabbit erythrocytes was detected only in six species. *P. platycephala* showed the strongest lectin activity, 2560 HU g $^{-1}$  for both untreated or treated rabbit erythrocytes. These values are higher than those described for

cowpea cultivars (99.6-540.3  $HU g^{-1}$  for treated rabbit erythrocytes) by Vasconcelos et al. (2010). The seed aqueous extracts of Dioclea megacarpa, E. velutina, L. sericeus, S. obtusifolia and S. rugosa also showed haemagglutinating activity. As shown in Table 1, the lectins from D. megacarpa, E. velutina and L. sericeus had been previously purified. Haemagglutinins (lectins) combine with cells lining the intestinal mucosa and interfere with nutrient absorption. The vast majority of known plant lectins are inactivated by cooking or roasting (Liener, 1994), and therefore the presence of these antinutritional factors in the same studied seeds constitute a transposable barrier to their use. The trypsin inhibitory activity ranged from 4.1  $\pm$  0.4 in S. rugosa to 27.4  $\pm$  0.2  $\mu$ g TI mg<sup>-1</sup> flour in C. ferrea. The highest values are close to those described for some V. unguiculata cultivars (12.00  $\pm$  0.56 to 30.56  $\pm$  0.54  $\mu$ g TI mg<sup>-1</sup> flour) by Maia et al. (2000) and are less than half the value described for the Bays soybean cultivar ( $62.5 \pm 2.6 \,\mu g \, Tl \, mg^{-1}$  flour) studied by Vasconcelos et al. (2001). Dietary trypsin inhibitors are blamed to be responsible for the poor digestibility of dietary protein by interference with the proper function of trypsin leading to growth inhibition and pancreatic hypertrophy (Liener, 1994). In spite of their antinutritional effects, trypsin inhibitors are often inactivated by appropriate heat treatment (Maia et al., 2000), as shown for lupins, soybeans and black beans (Trugo et al., 2000). Regarding the urease activity, seeds presented values ranging from  $225 \pm 7$  in S. obtusifolia to  $23,895 \pm 3388 \text{ U kg}^{-1}$  flour in *L. sericeus*, about 10 times lower than those of Bays soybean cultivar and two times lower than those of Rio Balsas soybean cultivar (Vasconcelos et al., 2001). Toxic activity was present in five studied seeds, and the most potent was that shown by Albizia lebbeck, with a  $LD_{50}$  0.22  $\pm$  0.01 g kg $^{-1}$  body weight, being about two times less toxic than that of Bays sovbean cultivar (Vasconcelos et al., 2001). The concern with urease and toxins in this work is due to results by Vasconcelos et al. (2001) which have described deleterious effects of these components in soybean seeds included in diets for growing rats. Therefore, the wild seeds studied in this work present similar or even lower content of antinutrients and/or toxic factors in comparison with common legumes widely used in human food. In

**Table 3**Amino acid composition of 14 wild legume seeds from Northeastern Brazil compared with amino acid composition of plant and animal standard proteins, soybeans and egg white, and with patterns of amino acid requirement for different children age groups.

Legume seeds	Amino acids (mg g <sup>-1</sup> N)																	
	Essential										Non-essential							
	Thr	Val	Ile	Leu	Lys	Phe	Tyr	Met	Cys	Trp	His	Asx	Glx	Ser	Gly	Ala	Arg	Pro
Albizia lebbeck	278	223	375	585	872	271	581	24	32	41	806	500	522	398	273	106	872	182
Caesalpinia bracteosa	358	249	23	342	862	346	262	49	45	23	344	394	587	398	432	6	1202	148
Caesalpinia ferrea	318	154	154	311	789	281	222	44	101	21	419	471	757	369	378	8	1190	184
Dimorphandra gardneriana	256	317	308	506	693	474	340	67	56	22	234	369	581	371	247	307	984	203
Dioclea megacarpa	292	154	188	356	1088	304	116	0	0	11	737	494	348	203	262	33	1490	180
Enterolobium contortisiliquum	239	213	188	509	931	326	234	64	52	12	379	577	884	356	314	311	509	164
Erythrina. velutina	273	346	332	457	789	524	501	132	52	131	199	341	489	292	256	302	758	209
Hymenaea courbaril	211	261	276	390	861	254	353	0	0	0	918	408	375	257	501	175	922	131
Lonchocarpus sericeus	153	191	140	358	618	211	173	16	101	29	736	568	594	274	258	262	1500	96
Parkia platycephala	242	304	236	426	970	284	218	67	211	23	258	531	818	344	296	341	574	132
Piptadenia moniliformis	255	278	229	417	939	360	224	303	73	10	296	373	490	293	326	53	1120	215
Pterogyne nitens	299	250	178	418	576	363	137	29	146	32	278	462	656	381	299	356	1160	112
Senna obtusifolia	321	241	210	498	576	294	239	89	89	54	263	542	818	356	328	341	529	519
Senna rugosa	299	288	168	421	456	265	182	0	151	65	374	789	819	434	385	386	597	238
Standards																		
Soybeans <sup>a</sup>	238	291	237	480	409	378	314	86	102	34	190	696	1140	264	242	266	532	333
Ovalbumin <sup>b</sup>	308	346	361	482	689	559	275	327	149	91	146	382	538	339	204	363	650	182
Child requirements <sup>c</sup>																		
2-5 Years	212	219	175	412	362		394		156	69	119							
10-12 Years	175	156	175	275	275		138		138	6	119							

Values are means of triplicates of the same sample. The standard deviation values were all less than 5% of means.

<sup>&</sup>lt;sup>a</sup> Vasconcelos et al. (2001).

<sup>&</sup>lt;sup>b</sup> Purchased from Sigma-Aldrich Co. (St. Louis, USA).

<sup>&</sup>lt;sup>c</sup> FAO/WHO/UNU (1985).

**Table 4**Toxic and/or antinutritional factors (lectin, trypsin inhibition, urease and toxic activities) of 14 wild legume seeds from Northeastern Brazil.

Legume seeds	Lectin <sup>a</sup>		Trypsin inhibitory <sup>b</sup>	Urease <sup>c</sup>	Toxic <sup>d</sup>
	Untreated	Treated			
Albizia lebbeck	NDe	ND	$20.8 \pm 0.8$	$5613\pm197$	$0.22 \pm 0.01$
Caesalpinia bracteosa	ND	ND	$16.2\pm0.8$	$11,\!278 \pm 1307$	$NL^f$
Caesalpinia ferrea	ND	ND	$27.4 \pm 0.2$	$822\pm50$	NL
Dimorphandra gardneriana	ND	ND	$25.8 \pm 0.2$	$1240\pm62$	NL
Dioclea megacarpa	1280	2560	$10.8 \pm 0.1$	$47,178 \pm 3351$	$\boldsymbol{0.72 \pm 0.03}$
Enterolobium contortisiliquum	ND	ND	$26.2\pm0.1$	$3684\pm173$	$1.12 \pm 0.04$
Erythrina velutina	1280	1280	$24.0 \pm 0.8$	$3645\pm171$	$1.01 \pm 0.02$
Hymenaea courbaril	ND	ND	$4.5\pm0.2$	$620\pm28$	NL
Lonchocarpus sericeus	1280	320	$\textbf{8.3} \pm \textbf{0.3}$	$23,895 \pm 3388$	NL
Parkia platycephala	2560	2560	$ND^g$	$5907 \pm 967$	NL
Piptadenia moniliformis	ND	ND	$\textbf{8.9} \pm \textbf{0.5}$	$1899\pm228$	NL
Pterogyne nitens	ND	ND	$\textbf{7.0} \pm \textbf{0.2}$	$1470\pm10$	$0.80 \pm 0.03$
Senna obtusifolia	20	80	ND	$225\pm7$	NL
Senna rugosa	80	160	$4.1 \pm 0.4$	$465\pm13$	NL

All values are means  $\pm$  standard deviation of triplicates the same sample except for lectin value that was expressed only as mean.

**Table 5**Mineral composition of 14 legume seeds from Brazil compared with patterns of mineral requirement for adults and children four or more years of age based on a 2000 kcal (8368 kJ) intake.

Legume seeds	Minerals (m	$ m g100g^{-1}$ seed		Minerals ( $\mu g 100  g^{-1}$ seed flour)						
	K	Na	Ca	Mg	Fe	Zn	Mn	Cu	Cr	Мо
Albizia lebbeck	604	7.5	253	110	6.0	3.3	3.0	690	130	600
Caesalpinia bracteosa	890	14.1	249	142	11.4	3.9	5.2	1500	20	30
Caesalpinia ferrea	1351	9.2	268	160	5.7	5.6	1.5	2200	200	340
Dimorphandra gardneriana	813	11.8	97	213	4.6	3.0	3.6	890	290	40
Dioclea megacarpa	738	7.8	31	111	5.2	1.8	1.6	620	280	190
Enterolobium contortisiliquum	844	9.2	41	244	20.2	2.0	2.3	890	280	20
Erythrina. velutina	1581	8.8	113	157	6.1	3.8	3.2	1600	150	410
Hymenaea courbaril	366	10.2	58	117	3.8	1.5	2.5	1040	280	70
Lonchocarpus sericeus	1137	10.6	224	102	5.9	5.7	3.7	1500	160	320
Parkia platycephala	826	8.1	116	229	5.9	2.3	1.7	1080	110	90
Piptadenia moniliformis	888	9.0	103	135	4.4	3.0	2.2	800	230	290
Pterogyne nitens	896	1.4	61	102	1.5	3.5	1.7	700	150	$ND^a$
Senna obtusifolia	1219	7.9	504	102	0.7	3.2	1.1	610	160	380
Senna rugosa	813	5.5	116	124	5.5	1.3	2.7	360	90	50
<sup>b</sup> RDI	3500	2400	1000	400	18	15	2.0	2000	120	75
	$(mg  day^{-1})$	$(mgday^{-1})$	$(mg  day^{-1})$	$(mg  day^{-1})$	$({\rm mgday}^{-1})$	$(mg  day^{-1})$	$(mgday^{-1})$	$(\mu gday^{-1})$	$(\mu g  day^{-1})$	$(\mu g  day^{-1})$

Values are means of three analyses of the same sample. The standard deviation values were all less than 5% of means.

addition, these protein compounds are easily eliminated by moist heat treatment or cooking, since they are heat-labile.

The data on mineral composition (Table 5) showed that most of the studied minerals showed high levels when considering RDI and the nutrient content claims of FDA (2010). The seeds showed high contents (mg  $100~\rm g^{-1}$  seed flour) of potassium (366–1581), 12–45% RDI; magnesium (102–244), 26–61% RDI; iron (1.5–20.2), 8.3–112% RDI; zinc (1.3–5.6), 10–37% RDI and manganese (1.1–5.2), 55–528% RDI. Similarly, they showed high amounts ( $\mu$ g  $100~\rm g^{-1}$  seed) of copper (360–2200), 36–110% RDI; chromium (20–290), 17–242% RDI and molybdenum (0–600), 0–800% RDI. The seeds are also good sources of calcium (31–504 mg  $100~\rm g^{-1}$ ), 3–50% RDI and show low amounts of sodium (1.4–14.1 mg  $100~\rm g^{-1}$  seed flour), 0.06–0.6% RDI. The mineral profile shown in this work is close to those described for wild legumes of the genus *Canavalia*, *Cassia* and *Mucuna* from south India studied by Vadivel and Janardhanan (2005). Besides, this

mineral profile showing major quantities of K, Mg, Fe, Zn, Mn, Cu, Cr and Mo is similar to those reported by Onwuliri and Obu (2002) for different varieties of cowpea and black beans, important mineral sources in Brazilian people diet, especially of zinc and iron. Therefore, these seeds are good sources of minerals, since their levels are similar to those of beans, satisfying partially or completely the mineral requirements established by the reference daily intakes shown in Table 5 (FDA, 2010).

## 4. Conclusions

The wild legume seeds collected in the semi-arid and moist tropical forest environments from northeastern Brazil show chemical composition and energy values comparable to or much higher than those reported for wild legumes from other regions of the world, or for cultivated legumes such as beans and soybeans.

<sup>&</sup>lt;sup>a</sup> The lectin activity is expressed as haemagglutination titre (HU g<sup>-1</sup>), which is the reciprocal of the highest dilution giving visible agglutination in rabbit erythrocytes untreated and treated with proteases from *Bacillus licheniformis* (Sigma–Aldrich Co., St. Louis, USA);.

<sup>&</sup>lt;sup>b</sup> Trypsin inhibition activity is expressed as μg of trypsin inhibited per mg of flour (μgTI mg<sup>-1</sup> flour);.

<sup>&</sup>lt;sup>c</sup> The urease activity is expressed in units of enzyme per kg of flour (U kg<sup>-1</sup> flour). The units were calculated using information from Sigma–Aldrich Co. that 1 g of pure enzyme contains 870,000 units.

 $<sup>^{</sup>m d}$  Toxic activity is represented as LD<sub>50</sub>, 50% lethal dose. One LD<sub>50</sub> designates the amount of protein in g kg $^{-1}$  mouse body weight producing convulsion and death of 50% of tested animals injected by intraperitoneal route.

<sup>&</sup>lt;sup>e</sup> Not detected by used methodology; the method detects haemagglutination from 5.2 μg protein mL<sup>-1</sup>.

f Not lethal at a dose of 1 g per kg mice body weight.

g Not detected by used methodology; the method detects inhibition from 1 ng soybean trypsin inhibitor.

<sup>&</sup>lt;sup>a</sup> Not detected by used methodology.

<sup>&</sup>lt;sup>b</sup> Reference daily intake in 101.9 (c) (8) iv. FDA U.S. Food and Drug Administration (2010).

They contain significantly high levels of proteins and dietary fiber. The antinutritional and/or toxic factors detected in the seeds should not pose a significant problem to human health if the seeds are properly processed, since it is known that important food legumes show high levels of these compounds before heat processing. In addition, they contain higher levels of some essential amino acids compared to recommended levels, as well as amino acid profile similar to that of sovbean seeds. Likewise, the seeds mineral composition is close to those of beans, which are relevant sources of minerals for Brazilian people. The contents of potassium, magnesium, iron, zinc, manganese, copper, chromium and molybdenum meet the RDI for adults and children four or more years of age based on a 2000 kcal (8368 kJ) intake (FDA, 2010) Thus, these wild legume seeds can be promising alternatives sources of food to overcome the malnutrition problem faced by Brazilian northeastern people, as well as to create basic sustainability elements to prevent extinction of these species. Another perspective is the utilization of these species as tool in agricultural biotechnology for production of new, more nutritious, transgenic crops.

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