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Phytochemical and nutritional studies on pigeon pea and kidney bean cultivated in Egypt

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With 5 tables

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Some reports have been published in the literature concerning the nutritive value of leguminous seeds and their potential success as protein supplement in human nutrition (1, 2, 3, 4). The food balance sheet (1972–1973), given by the Ministry of Agriculture gives a figure of 31.2 g for legumes/Caput/day. The country at present grows a variety of legumes, most of which are usually eaten by those who cannot afford meat.

Although legumes have assumed an important role in the national dietary, they have at the same time been known for a long time to contain a wide variety of substances, which may be considered toxic to the animal body.

Two legumes, pigeon pea (*Cajanus indicus* spreng.) and kidney bean (*Phaseolus vulgaris* L.) variety Guiza III, were chosen. Phytochemical studies were carried out on the two seeds. Their nutritive value was also assessed and the toxic factor or factors were also estimated.

Experimental

Materials and methods

Preparation of the samples for analysis

The raw *Cajanus indicus* Spreng. and *Phaseolus vulgaris* L. (Guiza III) seeds used in these studies were brought from the Experimental station of the Ministry of Agriculture. 2 kg of each sample were divided into two portions, one half of it was cooked in boiling water for one hour, while the other half was kept raw. Both samples were air-dried and ground, then stored for analysis.

1. Phytochemical studies

The dried powdered seeds were separately subjected to the following tests:

a – Test for glycosides and/or carbohydrates according to *Gonzalez* and *Delgado* (5).

b – Test for cardenolides using *Keddé* reagent (6), *Legal's* colour test (7), *Baljet* colour reaction (8), Antimony trichloride (9), and *Raymond* colour reaction (10).

c – Test for tannis as described by *Ain Shoka* (11).

d – Test for flavonoids as described by *Ain Shoka* (11).

e – Test for saponins as described by *Ain Shoka* (11).
f – Test for unsaturated sterols and/or triterpens according to *Liebermann-Burchard* (12) and to *Salkowski* (13).
g – Test for alkaloids and bases as described by *Ain Shoka* (11).
h – Test for oxidase enzyme according to *Peach* and *Tracery* (14).
i – Estimation of trypsin inhibitor by the casein digestion method as described by *Laskowski* and *Laskowski* (15).
j – Determination of haemagglutinating activity of seed extracts according to the method of *Leiner* and *Hill* (16), which is based on the fact that the saline extract of the seeds agglutinate the washed rabbit red blood corpuscles.

2. *Analysis of the raw seeds*

Proximate analysis and determination of certain pharmacopoeial constants of the seeds for moisture, total nitrogen, ethereal extracts, fibre, ash (acid insoluble and water soluble ash), and minerals were carried out following the methods adopted by the A.O.A.C. (1965), and the procedure described in Egyptian Pharmacopoeia (1972).

3. *Extraction with successive organic solvents*

For the determination of percentage yield of extractives of each sample of dried powdered seeds of *Cajanus indicus* Spreng. and *Phaseolus vulgaris* L. Samples were successively extracted using solvents in the order of increasing polarity such as petroleum-ether (50–70° C), diethyl ether, chloroform and ethyl alcohol (95 %). For each organic solvent, the extraction was continued until no residue was obtained on evaporation of a small aliquot of the colourless percolate to dryness in a watch glass. Before using the next solvent, the marc was taken out of the extractor, carefully spread on a sheet of paper and the residual solvent allowed to evaporate at room temperature. This solvent-free powder was replaced in the same dry thimble and extracted with the following solvent in the order mentioned before.

4. *Amino acids estimation (for raw and cooked materials)*

The amino acid pattern and content of the seeds were determined after being hydrolysed with 6NHCl according to *Khans* and *Berker* (19). Measured amounts of the acid hydrolysates were spotted on Whatman No. 1 filter paper. Ascending paper chromatography technique was carried out using the buffered method of *Levy* and *Chung* (20). Cystine and methionine were oxidised with performic acid, then determined according to the method of *Jamalian* and *Pellet* (21). The colorimetric method of *Blauthi, Charezinski,* and *Berbec* (22) was followed for estimation of tryptophan in the alkali hydrolysates.

Results and discussion

Composition of seeds

At the outset, general analysis for the determination of moisture, fat, crude protein, carbohydrate, fibre, ash, calcium, phosphorus and iron content of the seeds were determined. Results are given in tab. 1. Both seeds contained good amounts of proteins, calcium, phosphorus and iron.

Extraction of protein

Extraction of seed protein with sodium hydroxide gave the highest yield of protein nitrogen as compared with other extractors (saline buffer pH 7 cold water and hot water as shown in tab. 2). These results agreed with those previously given by *Labarre* and *Delecourt* (23) and by

Tab. 1. Proximal analysis of seeds of *Cajanus indicus* Spreng. and *Phaseolus vulgaris* L. (Guiza III), calculated on dry-weight basis

Item	Moisture g/100 g	Protein g/100 g	Ether ext. g/100 g	Ash g/100 g	Acid-insoluble ash g/100 g	Water-soluble ash g/100 g	Fibre g/100 g	Carbohydrate g/100 g	Calcium mg/100 g	Phosphorus mg/100 g	Iron mg/100 g
Cajanus indicus Spreng.	10.55	25.2	1.85	4.12	0.15	4.23	9.59	68.78	170	272	8.9
Phaseolus vulgaris L. Guiza III	11.20	23.2	1.32	3.49	0.14	3.79	4.66	71.99	134	396	8.02

Tab. 2. Influence of solvent on yield and distribution of extractable nitrogen from dry defatted seed meal

Seed	Cold water	Hot water	Saline buffer pH 7	Sodium hydroxide pH 11
	g%	g%	g%	g%
Cajanus indicus Spreng.				
T.N.	1.770	1.121	3.012	3.125
N.P.N.	0.485	0.495	0.658	0.516
P.N.	1.485	0.676	2.354	2.609
Phaseolus vulgaris L. (Guiza III)				
T.N.	2.024	1.345	3.221	3.385
N.P.N.	0.513	0.506	0.704	0.685
P.N.	1.511	0.839	0.517	2.700

T.N. = total nitrogen
N. P.N. = non-protein nitrogen
P.N. = protein nitrogen

Kleminko (24), who found that dilute alkali solution is the best solvent to extract completely protein present in plant tissues.

Preliminary phytochemical screening of seeds

From tab. 3 it can be seen that the seeds of *Cajanus indicus* Spreng. and *Phaseolus vulgaris* L. contain carbohydrates and/or glycosides; flavonoids, saponins and unsaturated serols and/or triterpenes, but do not contain cardinolides, tannins, alkaloids, flavanols and flavonons, and oxidase enzyme. Extracts from the two seeds were also assayed for trypsin inhibitors and haemagglutinins activities. *Cajanus indicus* seeds contained 1724 trypsin inhibitor units, while *Phaseolus vulgaris* contained 2000 units. *Phaseolus vulgaris* L. contained 10 242 units haemagglutinin/g.

Tab. 3. Preliminary phytochemical screening of seeds of *Cajanus indicus* Spreng. and *Phaseolus vulgaris* L.

Test	*Cajanus indicus* Spreng.	*Phaseolus vulgaris* L. Guiza III
Carbohydrates and/or glycosides	+	+
Cardinolides	−	−
Tannins	−	−
Flavonoids	+	+
Flavanol + flavonone	−	−
Unsaturated sterols and/or triterpenes	+	+
Saponins	+	+
Alkaloids	−	−
Oxidaze enzyme	−	−
Trypsin inhibitors	+	+
Haemagglutinins	−	+

Percentage of extractives yielded to selective organic solvents and examination of the crude extracts

Tab. 4 shows that the total extractives yielded by successive extractions of powdered seeds of *Cajanus indicus* Spreng. are higher than those of *Phaseolus vulgaris* L.

(a) The examination of the crude extracts showed that the petroleum-ether (50–70) extracts of the two seeds consisted of dark brown soft masses, having a greasy touch. They had a characteristic odour and a characteristic taste. The two extracts gave positive results with *Liebermann-Burchard* test (*Lembowitch*, 12) and *Salkowski* test (*Robinson* 13), indicating the presence of unsaturated sterols and/or triterpenes. They gave negative test for carbohydrates, cardenolides, saponins and alkaloids.

(b) The ethereal extract of each seed was a yellowish brown soft mass. It has a characteristic odour and a bitter taste. The extract gave positive tests for reducing substances and negative tests for alkaloids, saponins and sterols and/or triterpenes.

(c) The chloroformic extract of each sample consisted of reddish brown, soft resinous mass having a characteristic odour and a slightly bitter taste. The extract gave positive tests for reducing substances and negative tests for alkaloids saponins and sterols and/or triterpenes.

Tab. 4. Percentage of extractives yielded to selective organic solvents

Solvents	Extractives	
	Cajanus indicus Spreng.	*Phaseolus vulgaris* L. Guiza III
Petroleum-ether (50–70)	0.95	1.07
Ether	0.46	0.61
Chloroform	0.31	0.13
Ethyl alcohol	12.10	5.20
Total	13.82	7.01

(d) The alcoholic extracts of the two seeds consist of dark brown semi-solid masses having a very characteristic odour and slightly bitter taste. They gave positive tests for reducing substances.

Chromatographic identification of the amino acids

Tab. 5 shows that 17 amino acids were identified on raw and cooked, *Cajanus indicus* Spreng. and *Phaseolus vulgaris* L. (Guiza III). Comparing our results on *Cajanus indicus* with the results given by other investigators for other varieties, it was seen that the amino acids, Leucine, isoleucine, cystine, and methionine were present in amounts lower than those given by *Orr* and *Watt* (25), and close to those obtained by *Cerrghelli, Busson, Toury,* and *Bergeret* (26). Phenyl alanine, valine and lysine are present in amounts similar to those reported by *Orr* and *Watt* (25) and by *Cerrghelli* et al. (26). In case of *Phaseolus vulgaris,* the tryptophan content was found to be lower than that obtained on other varieties by *Evans* and *Bandemer* (27) and by *Kakade* and *Evans* (28); at the same time it is higher than those values given by *Williams* (29). The threonine, methionine, phenyl alanine, arginine and histidine content were in agreement with the data reported by *Kakade* and *Evans* (28). The leucine + isoleucine, phenyl alanine and histidine content were also similar to the values given by *Evans* and *Bandemer* (27). Our values for lysine, cystine, phenyl alanine, valine, and histidine show that they are present in amounts lower than that given by *Kakade, Arnold, Liener,* and *Weibel* (30).

It was found that cooking the seeds destroyed the trypsin inhibitor and the haemagglutinin activity of seeds. *Liener* (31) in 1962 mentioned that trypsin inhibitor and haemagglutinins are heat-labile. Comparing the

Tab. 5. Amino acids milligram per gram of total nitrogen of raw and cooked seeds of *Cajanus indicus* Spreng. and *Phaseolus vulgaris* L.

Seeds	*Cajanus indicus* Spreng.		*Phaseolus vulgaris* L. Guiza III	
Amino acids	Raw mg/g N	Cooked mg/g N	Raw mg/g N	Cooked mg/g N
Leucine + isoleucine	743	763	792	813
Phenyl alanine	556	494	338	313
Valine	302	310	346	350
Methionine	62	44	62	44
Tyrosine	156	137	194	167
Threonine	206	239	264	306
Cystine	68	62	69	63
Lysine	436	297	459	313
Arginine	321	199	352	219
Histidine	272	261	163	156
Glycine	213	185	162	140
Glutamic acid	1165	1144	893	877
Aspartic acid	587	576	680	667
Tryptophan	22	23	77	88
Alanine	256	244	317	302
Serine	268	263	336	303

amino acid contents of the two cooked seeds with those of raw seeds (tab. 5), it was found that cooking the seeds resulted in slight losses of most of the amino acids studied, with the exception of leucine and isoleucine, valine, threonine, and tryptophan, which showed a slight increase after cooking. *Bandemer* and *Evans* (32) reported that heating the seeds causes small loss of most of its amino acids. *Myklestad, Bjoernstad,* and *Leif* (33) showed that most amino acid levels decreases as the temperature increases. *Taira Harue* (34), during the preparation of soybean-based foods, observed that lysine, cystine, arginine, tryptophan and serine were partially lost during heating. He stated that heating at high temperatures for a long time was most destructive, especially for lysine and cystine; he added that heating with water decreased the loss of lysine and arginine, but not other amino acids. Negative results were given by *Phadke* and *Sohonie* (35), who reported an increase in amounts of threonine, histidine, methionine and tryptophan for faba vulgaris autoclaved for five minutes. *Kakade* and *Evans* (28) reported also that certain amino acids, mainly leucine, isoleucine, threonine, tryphtophan, and valine, increased after autoclaving *Phaseolus vulgaris* for five minutes at 121° C, while arginine, lysine, and methionine were decreased after cooking the beans for 4 hours.

Lee and *Hannan* (36) attributed the destruction of amino acids on autoclaving for 4 hours, to the interaction between free amino groups of the basic amino acids and reducing carbohydrates.

Summary

1. The preliminary phytochemical screening of the two seeds established the presence of carbohydrates and/or glycosides, flavonoids, unsaturated sterols and/or triterpenes, saponins, trypsin inhibitors and haemagglutinins. In addition, it established the absence of cardenolides, tannins, alkaloids and oxidase enzyme.

2. Certain pharmacopoeial constants, including moisture, ash, acid-insoluble ash, water-soluble ash and crude fibre were determined.

3. The two seeds were subjected to successive extractions with different organic solvents such as petroleum ether (50–70° C), diethyl ether, chloroform and ethyl alcohol. The successive yields of extractives were determined. Examination of the crude extracts showed that petroleum ether extract contained sterols and/or triterpenes, while ether, chloroform, and ethyl alcohol extracts contained reducing substances.

4. General analysis of the two seeds for proteins, fats, carbohydrates, fibre and ash contents were carried out and the results were given in g/100 g dry seeds. Pigeon pea contained 25.2 g protein, 170 mg calcium and 8.9 mg iron. The protein content of kidney bean was 23 g, while calcium and iron contents were 134 mg and 8.02 mg respectively.

5. Extractions of the proteins using different solvents such as cold water, hot water, saline buffer pH 7 and sodium hydroxide pH 11 showed that sodium hydroxide was the best extractant.

6. The amino-acid content of the two seeds, whether raw or cooked, showed that they were deficient in methionine, cystine and tryptophan. Other essential amino acids were present in amounts higher than that given by the FAO provisional pattern.

7. Cooking the seeds by the popular methods used in the country resulted in an increase in the amounts of the amino acids, threonine, leucine and isoleucine,

while the other amino acids present remained unchanged or decreased. It was also observed that cooking the seeds destroyed the trypsin inhibitors and haemagglutinins found in the two seeds.

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