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Research Article

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Isolation and determination of nutritional and antinutritional compounds from the seeds of selected plant species

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ABSTRACT

The aim of the present study was to analyze and quantify the presence of nutritional compounds like soluble proteins, carbohydrates and antinutritional compounds such as lectins, protease inhibitors, polyphenols and tannins in the seeds of Artocarpus heterophyllus (jack fruit), *Canavalia ensiformis (*jack bean), *Lens culinaris* (lentil) *and Pisum sativum* (pea). *Of the four plant species, the soluble protein content was high in the seeds of pea and least in jack bean thus indicating their positive nutritional role in the diet. High content of lectins was detected in all the four seed extracts showing their potent biotechnological applications. The seed extracts were tested for proteolytic and protease inhibitor activity to determine the enzymatic characteristics. Jackfruit showed comparatively high proteolytic activity indicating the probable role of plant proteases in defence responses that are triggered by pathogens or pests.The comparative studies on trypsin inhibition in the four seed extracts revealed no significant inhibitory activity, indicating their non toxic nature and possible use in nutrition. The above results suggested that seeds of these plant species have nutritive value as they are sources of valuable proteins and carbohydrates and besides they also contain a many bioactive compounds that cannot be considered as nutrients but exert physiological effects on humans.*

Key words: *Artocarpus heterophyllus, Canavalia ensiformis*, *Lens culinaris*, *Pisum sativum*, nutritional and antinutritional compounds.

INTRODUCTION

The increase in number of chronic diseases and expanding costs of health care have gained the interest of researchers to refine the existing regimes concerning nutrition and to demonstrate the positive and adverse effects of compounds present in plants on human and animal health [7]. Nutritional compounds from plant sources serve as a cheap source of valuable proteins, saccharides, and several micronutrients including minerals, vitamins and dietary fibre. The contribution of such nutritional compounds in the daily diet has many beneficial physiological effects as it allows preventing common metabolic diseases, such as diabetes mellitus, coronary heart disease, cancer and lowering of the plasma cholesterol level [6].

They also contain a wide range of bioactive microconstituents that cannot be considered as nutrients, however possess many beneficial properties, like antioxidant, anti-inflammatory, antimicrobial, which may be useful in disease prevention. Of particular interest are carbohydrates, enzyme inhibitors, lectins and polyphenols, tannins etc. for their role as disease preventive agents in diets [9].

Anti-nutritional factors are naturally occurring compounds that classified under a broad group of secondary metabolites. They can be the compounds that are present in human or animal foods which cause anti-nutritional effects and anti-physiological effects such as impaired reproductive function or reduced immunocompetence or substances which reduce feed intake in animals [12]. It has been revealed that anti-nutritional factors are also known as anti-nutrients which are toxic substances that can be found in most food and are able to restrict the nutrient availability to the body [4].

Nutritionists believe that anti-nutritional factors responsible to influence physiological characteristic and retard growth of animals [17]. ANCs include proteinase inhibitors, lectins, phytates, polyphenols etc [1]. Most of food antinutrients have an impact on the digestive system, like the inhibition of digestive enzymes (*e.g.* protease inhibitors), impairment of hydrolytic functions and of transport at the enterocyte site (lectins), formation of insoluble complexes which cannot be adsorbed, decrease of bioavailability of some nutrients (phytates, polyphenols), and the increase of the production of gases in the colon (α-galactosides). Anti-nutritional factors are naturally-occurring compounds that exert anti-nutritional effect, anti-physiological effect and limit the nutrients availability to living organism [14].

Protease inhibitors are proteins of low molecular weight forming stable complexes with digestive enzyme, irreversibly inhibiting their activity. The presence of protease inhibitors in food decreases the apparent nutritional quality of proteins in the diet by affecting the ability of body digestive enzymes to degrade dietary protein, and thus limiting the intake of amino acids needed to construct new proteins. [3]. Moreover, the control of proteases activity, considered to play a decisive role in a wide range of biological processes and misfunctioning related to cancer progression, may be considered as anticarcinogenic mechanism [11]. Several *in vitro* and *in vivo* studies have provided evidence that certain protease inhibitors of legume seeds are effective at preventing or suppressing carcinogen-induced transformation [10].

Lectins are a class of proteins of that bind carbohydrates without modifying them. Originally, the term lectin was restricted to soluble, multivalent proteins capable of agglutination and historically was limited to proteins of plant origin. However, today the term lectin is used in a broad sense to denote all types of carbohydrate-binding proteins that do not catalyze reactions with their ligands [8]. There are numerous lectins available in the nature, and the fact that they can be easily prepared in the purified form and accessible for chemical manipulation makes them potential tools for biological research. The applications of lectins ranges from identification of microorganisms, cell surface biology studies to cancer research and they are serve as probes for the characterization and isolation of simple and complex sugars [13].

Among polyphenols, tannins will be extensively described in this review. The content of tannins in dry bean seeds varies from 0.00 to 0.93% [15]. They are compounds of intermediate to high molecular weight up to 30,000 Da. In bean seed, the major amount of tannins is located in the seed coat, with low or negligible amounts located in the cotyledons. Tannins are known to interact with proteins forming complexes which, in turn, decrease the solubility of proteins and make protein complexes less susceptible to proteolytic attack than the same proteins alone [16]. Besides, they impair starch and disaccharide assimilation, and interact with proteolytic enzymes inhibiting their activity. Other toxic effects of tannins can be categorized as: depression of food intake, inhibition of digestive enzymes, increased excretion of endogenous protein, digestive tract malfunctions and toxicity of absorbed tannin or its metabolites. The objective of the present study is to determine and quantify the presence of nutritional and antinutritional compounds in the seeds of *Artocarpus heterophyllus* (jack fruit), Canavalia *ensiformis (*jack bean*), Lens culinaris* (lentil) *and Pisum sativum* (pea).

EXPERIMENTAL SECTION

Source of material

Seeds of *Canavalia ensiformis, Lens lentil, Artocarpus heterophyllus* and *Pisum sativum* were collected locally and used for the study. The present study was conducted at the Research Centre, Mount Carmel College, Bangalore.

Preparation of plant extract

The collected seeds were washed thoroughly with tap water followed with distilled water for the removal of dust and soil particles. The seeds were then washed in an alcoholic solution followed by a wash in sodium hypochlorite solution 0.2% (v/v) for 5 min and cut into small pieces with a blender.

Extraction and Determination of Soluble Protein and carbohydrate content

Samples of ground seeds were diluted in a solution of sodium phosphate buffer 0.1 mol L-1 pH 7.0, shaken during 1 h at 4ºC, and then centrifuged at 12,000 x g for 15 min at 4ºC. The precipitate was discarded and the supernatant

was used for the estimation.Protein concentration was determined by the method of Bradford [2], using BSA as standard. The total carbohydrate content was estimated by phenol sulphuric acid method with D-glucose as the reference sugar [5].

Proteolytic activity

Proteolytic activity was determined by incubation of 0.1 ml of crude seed extract with 0.5 ml. of 1% casein solution (w/v), prepared in phosphate buffer (0.1M) at a pH of 7.6 and a temperature of 37 degree Celsius for 20 minutes. Reaction was terminated by the addition of 1.5 ml of 5% TCA solution (v/v). After centrifugation at 2000 x g, the absorbance of the supernatant was measured at 280 nm using a UV spectrophotometer.

Protease inhibitor activity

Samples were incubated with 0.1 ml trypsin solution (1.0 mg/ mL) and phosphate buffer (0.1M) at a pH of 7.6 for a temperature of 37 degree Celsius for 10 minutes. 0.5 ml of 1% casein solution was added to the solution and the reaction mixture was incubated for 10 minutes. The reaction was arrested by adding 1.5 ml of 5% TCA solution (v/v).

Detection of lectins

The crude extracts (200 mL) were mixed with 200 mL of NaCl solution (0.15 mol L-1) containing CaCl2 (0.1 mol L-1) and MnCl2 (0.1 mol L-1).Then, 100 mL of human erythrocytes at 2% were added to the mixture and incubated at 37ºC for 30 min and exposed to room temperature for 30 min. The tubes were centrifuged at 8,000 x g and analyzed for the presence of erythrocyte agglutination.

Determination of total phenolic contents and tannins

10 g of sample was mixed with 100 mL boiling water by magnetic stirrer for 4 h. Then the extract was filtered through Whatman No.1 paper, and the filtrate was used for determination of total phenolics. Total phenolics were determined using Folin-Ciocalteu reagent (Singleton and Slinkard 1977). 200 mL of samples were taken into test tubes, and then 1.0 mL Folin-Ciocalteu's reagent and 0.8 mL sodium carbonate (7.5%) were added. The absorbance of all samples was measured at 760 nm after incubating at 30C for 1.5 h. Results were expressed as milligram of gallic acid equivalent per gram of fresh weight.

The total content of tannins was determined using a method based on the precipitation of tannins with casein. The content of tannins was determined by the FC method and expressed as the difference between the initial content of polyphenols and the content after precipitation with casein.

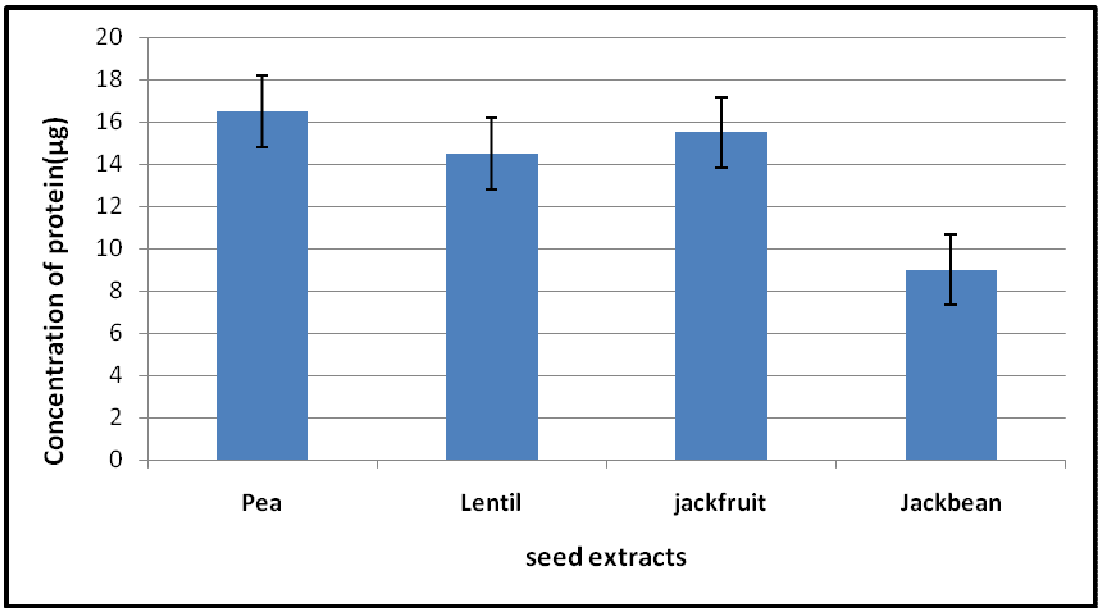

Figure 1: Soluble Protein levels in different seed extracts

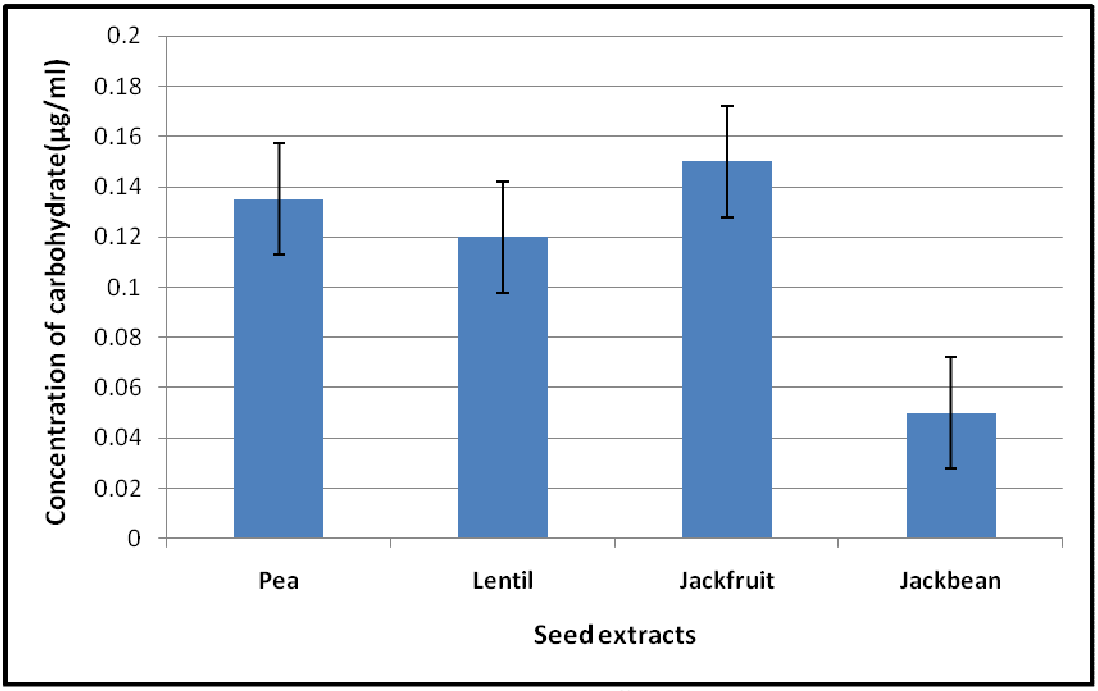

Figure 2: Carbohydrate levels in different seed extracts

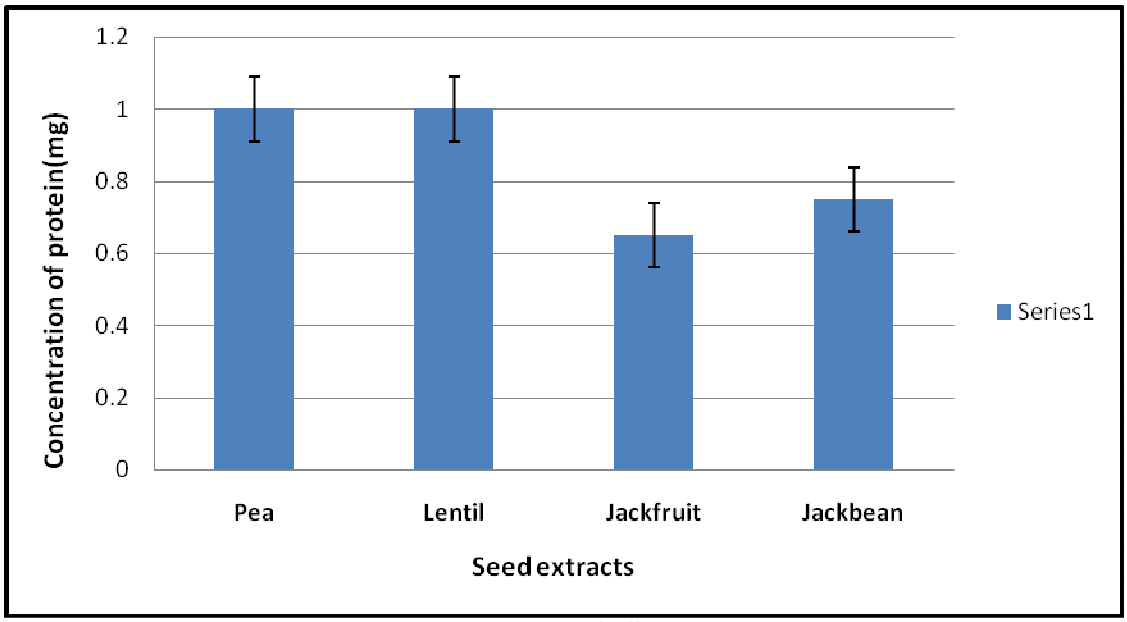

Figure 3: Proteolytic activity of different seed extracts

RESULTS AND DISCUSSION

Figures 1 and 2 show the results of soluble protein and carbohydrate concentration in the seed extracts. Of the four seed extracts, soluble protein content was high in pea seeds followed by jack fruit, lentil and least in jack bean seeds. The carbohydrate content was highest in Jack fruit seeds followed by pea, lentil and lowest in jack bean seeds, showing that the pea and jack fruit seeds have high potential for extraction of these compounds and also serve as good sources of dietary proteins and starch.

Figures 3 and 4 show the results of proteolytic and protease inhibitor activity of the seed extracts. All the four seed extracts showed low proteolytic activity as they did not cleave casein into its smaller peptides and the protein concentration was considerably high.

Jack fruit seeds showed a low protease inhibitor activity compared to the other three extracts as trypsin was least inhibited in the presence of jack fruit seed extract in the reaction mixture and hence there was a decrease in the

protein concentration. The low proteolytic and inhibitory activity of the extracts indicates their possible role in nutrition.

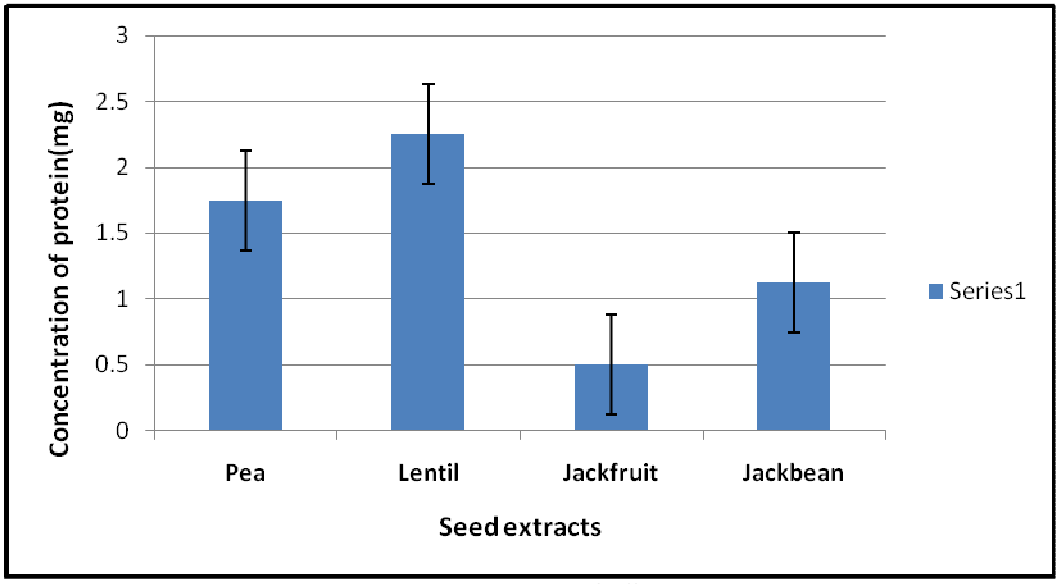

Figure 4: Protease inhibitor activity of different seed extracts

	Blood Groups			
Seed extracts	**O**	**A**	**B**	**AB**
Pea	+	+	+	+
Jackfruit	+	+	+	+
Lentil	+	-	+	+
Jackbean	-	-	-	-

Table 1: Erythroagglutination activities of the seed extracts

Table 1 indicates the results of erythroagglutination activities of the seed extracts.

Pea and jack fruit showed hemagglutination for all the four blood groups whereas jack bean had no effect in bringing about hemagglutination. Lentil showed agglutination for all blood groups except A. These results show that pea and jackfruit seeds have a great potential application in medical research and diagnosis due to its high content of lectins.

Table 2 indicates the total concentration of phenolics and tannins in the seed extracts.

Seed extracts	Phenols (mg GAE/100 g)	Tannins (mg GAE/100 g)
Pea	**205.25**	**122.30**
Jackfruit	**406.14**	**198.38**
Lentil	**119.06**	**137.45**
Jackbean	**233.95**	**117.25**

Table 2: Total concentration of phenolics and tannins in the seed extracts

The Folin-Ciocalteu method for the determination of the total phenolic and tannin content showed that Jackfruit seeds presented the highest amounts of phenolics and tannins. Phenolic compounds of all studied seeds were not significant compared to toxic values found in the literature indicating the non toxic nature of these antinutritional compounds.

CONCLUSION

A major reason for the cause of metabolic diseases like obesity, type 2 diabetes, coronary heart disease and cancer is considered to be unhealthy diet lacking essential nutritional components. . Hence it becomes important to promote an increased contribution of natural dietary plant products in order to take advantage of their components that are nutritious and provide most of the ingredients that help to improve heath. In conclusion to the above studies, the beneficial effect of plant products on human health, when consumed in significant amounts, is attributable to their

nutritive compounds and it is likely that the bioactive antinutritional compounds present in the plants also play an important role as well.

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