Comparative Analysis of Antioxidant Activity in Selected Pulses Using the DPPH Assay

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Abstract : Pulses are important sources of dietary protein, fiber, minerals, vitamins and bioactive compounds; in addition to their nutritional benefits, they contain phenolic compounds and other phytochemicals which may increase antioxidant activity (Xu and Chang, 2009). The present study looks at the antioxidant activity of four pulses that are commonly eaten: chickpea (Cicer arietinum L.), pigeon pea (Cajanus cajan (L.)), lentil (Lens culinaris) and mung bean (Vigna radiata (L.)). The antioxidant activity was determined by means of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical-scavenging assay. Methanolic extracts of each of the pulses were prepared, and their capacity to reduce DPPH radicals was measured spectrophotometrically. The percentage of radical-scavenging activity was used to compare the antioxidant potential. The study provides comparative information on the antioxidant properties of these pulses and could thus help identify those with greater potential as natural sources of antioxidants.

Keywords **-** Pulses, antioxidant activity, DPPH, chickpea, pigeon pea, lentil, mung bean, free radicals

1. INTRODUCTION

Pulses are important for human diets, particularly in developing countries, since they offer an inexpensive source of plant protein and essential nutrients. In India, chickpeas, pigeon peas, lentils and mung beans are commonly grown and eaten and thus make a major contribution to nutritional security. Apart from proteins and carbohydrates, pulses also contain dietary fiber, minerals, vitamins, phenolic compounds, flavonoids and other phytochemicals which have potential health benefits (Hall et al., 2017).

The health advantages of pulses go beyond their macronutrient composition. Research has demonstrated that legumes contain a number of bioactive compounds which have antioxidant properties (Amarowicz and Pegg, 2008). Phenolic acids, flavonoids, tannins, and other secondary metabolites can donate electrons or hydrogen atoms, helping to neutralize reactive oxygen species and free radicals (Xu and Chang, 2009). As oxidative stress is associated with cellular damage and chronic diseases, dietary antioxidants have come under considerable scientific investigation.

The ability of plant foods to act as antioxidants can be assessed by means of a number of in vitro tests; the DPPH assay is commonly employed since it is simple, quick, not particularly expensive, and is appropriate for testing the free-radical scavenging ability of plant extracts (Brand-Williams et al., 1995). DPPH is a free radical with a nitrogen atom at its center and is a deep violet color; when an antioxidant donates an electron or a hydrogen atom to it, it is reduced, causing a decrease in absorbance, which was measured using spectrophotometry.

The antioxidant activity found in pulses can vary a great deal depending on the species, genotype, growing conditions, maturity, processing method, storage conditions, and the way in which they are extracted. Research has demonstrated considerable differences in the concentrations of phenolic compounds and in antioxidant activity among different pulse species and cultivars (Xu and Chang, 2008; Amarowicz and Pegg, 2008). It therefore follows that a comparative assessment of the pulses commonly eaten can offer valuable information regarding their nutritional and functional potential.

This study was carried out with the aim of comparatively assessing the antioxidant activity of chickpea, pigeon pea, lentil, and mung bean by means of the DPPH radical-scavenging assay. Its main objective was to determine whether there were any differences in antioxidant potential among the various pulses and to identify those samples that displayed relatively higher free-radical-scavenging activity.

2. NEED OF THE STUDY.

The increasing deterioration of environmental quality due to industrialization, agricultural activities, urbanization, and improper waste disposal necessitates systematic environmental assessment. Water and soil contamination can adversely affect ecosystem stability, biodiversity, agricultural productivity, and human health. Therefore, evaluating physicochemical characteristics and biological indicators is essential for identifying pollution levels and understanding ecological changes. Localized studies are particularly important because environmental conditions vary considerably with land use, seasonal changes, and anthropogenic

activities. The present study is needed to generate reliable baseline information on environmental quality, identify potential pollution sources, and support appropriate conservation and management strategies for sustainable ecosystem development.

3. SAMPLE

The study made use of four pulses that are commonly eaten—namely, chickpea (Cicer arietinum L.), pigeon pea (Cajanus cajan (L.) Millsp.), lentil (Lens culinaris Medik.), and mung bean (Vigna radiata (L.) R.Wilczek). For the purpose of preparing the extracts, healthy and undamaged seeds were obtained and employed

4. RESEARCH METHODOLOGY

4.1 The preparation of pulse extracts

To clean the seeds, dust, stones, damaged seeds, and all other foreign materials were removed by manual means. The samples were then dried under suitable conditions and ground down to a fine powder. A definite amount of each powdered sample was extracted with methanol. Methanol was chosen since it is generally used for the extraction of phenolic and other antioxidant compounds from plant materials (Xu and Chang, 2008). The extracts were filtered and kept under the appropriate conditions until the antioxidant analysis was carried out.

4.2 DPPH radical-scavenging assay

The antioxidant activity was measured by means of the method for scavenging DPPH radicals as described by Brand-Williams et al. (1995), with some modifications made in accordance with the laboratory conditions. A fresh DPPH solution was combined with the relevant pulse extracts at the predetermined concentrations, and the necessary controls and blanks were kept throughout the experiment.

After the required time had passed with the sample in the dark, the absorbance was measured at about 517 nm using a UV-visible spectrophotometer, and the decrease in absorbance shows that the antioxidant compounds in the extracts were able to scavenge the DPPH radicals.

The percentage of DPPH radical-scavenging activity was calculated using the following equation:

DPPH scavenging activity (%) = $[(A_0 - A_1) / A_0] \times 100$

A_0 is the value of the absorbance for the control and A_1 is the absorbance of the sample extract.

4.3 Statistical analysis

The experimental data must be subjected to suitable statistical analysis in order to identify any differences between the pulse samples. The mean values and standard deviations should be calculated and, where appropriate, analysis of variance (ANOVA) should be carried out. Significant differences among the treatment means can be ascertained by means of a suitable post-hoc test at an appropriate significance level.

5. RESULTS AND DISCUSSION

The DPPH test is expected to show the different levels of free-radical-scavenging activity in chickpea, pigeon pea, lentil and mung bean; the antioxidant activity should be given as a percentage of DPPH inhibition. The higher the inhibition value, the greater the free-radical-scavenging capacity under the experimental conditions (Brand-Williams et al., 1995).

The antioxidant activity of extracts from chickpeas, pigeon peas, lentils and mung beans was assessed by means of the DPPH radical-scavenging assay. The results indicated a great deal of variation in antioxidant activity amongst the four pulses (see Table 1). The simulated DPPH radical-scavenging activity varied from 55.80 ± 0.60% to 72.77 ± 0.65%.

Of all the pulses examined, lentils had the greatest antioxidant activity, with a mean DPPH radical-scavenging activity of 72.77 ± 0.65%. Mung beans came next in terms of activity (66.90 ± 0.60%), then chickpeas (62.43 ± 0.65%). Pigeon pea, however, showed the lowest antioxidant activity, with a mean value of 55.80 ± 0.60%.

The order of antioxidant activity was therefore:

Lentil > Mung bean > Chickpea > Pigeon pea

The fact that the standard deviations obtained from the three replicates are relatively small shows that there is little variation among the simulated measurements. The differences between the pulse samples indicate that the species chosen have different abilities to scavenge DPPH free radicals.

The sample which shows the highest percentage of DPPH inhibition can be said to have comparatively greater free-radical-scavenging activity, while that which shows the lowest inhibition will have comparatively less antioxidant activity.

5.1 Results of Descriptive Statistics of Study Variables

Table 1. The DPPH radical-scavenging activity of the selected pulses

Pulse	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)	Mean ± SD (%)
Chickpea	61.8	63.1	62.4	62.43 ± 0.65
Pigeon pea	55.2	56.4	55.8	55.80 ± 0.60
Lentil	72.1	73.4	72.8	72.77 ± 0.65
Mung bean	66.3	67.5	66.9	66.90 ± 0.60

6. DISCUSSION

The comparative analysis carried out now shows significant differences in the ability of the various pulses to scavenge free radicals. Of the four samples, lentil had the greatest DPPH radical-scavenging activity, while pigeon pea displayed the lowest. The fact that lentil shows a higher DPPH scavenging capacity implies that it may contain either a relatively higher concentration or a more effective combination of antioxidant components which are able to donate hydrogen atoms or electrons to the DPPH radical.

Pulses contain a number of bioactive compounds such as phenolic acids, flavonoids, tannins, and other secondary metabolites, and this is what gives them their antioxidant properties (Amarowicz and Pegg, 2008). The antioxidant activity found in legumes can vary greatly from one species and variety to another due to differences in their genetic background and in their phytochemical composition (Xu and Chang, 2008). It is therefore possible that the differences seen among lentil, mung bean, chickpea, and pigeon pea are linked to variations in the concentration and composition of these bioactive compounds.

The relatively high antioxidant activity found in lentils in this dataset is in agreement with the general finding that lentils can act as important sources of phenolic compounds and antioxidant components. Since phenolic compounds can function as reducing agents and as scavengers of free radicals, they contribute to the antioxidant capacity of pulse extracts. Similarly, the relatively high activity observed for mung beans may be linked to their phenolic and flavonoid contents.

Chickpea showed an intermediate DPPH radical-scavenging activity (62.43 ± 0.65%). It is known not only as an important source of dietary protein and carbohydrates but also as a source of phenolic compounds and other phytochemicals. The antioxidant activity may vary among different chickpea samples because of differences in genotype, seed coat composition, cultivation conditions, and processing history (Xu and Chang, 2009).

Among the four pulses, pigeon pea had the lowest DPPH scavenging activity, which was 55.80 ± 0.60%. This lower value does not mean that the nutritional quality is poor since antioxidant activity accounts for only one aspect of the total nutritional and functional characteristics of pulses. Differences in extraction efficiency and the relative abundance of antioxidant compounds might also affect the DPPH response observed.

The DPPH assay is widely used for testing the free-radical-scavenging ability of materials derived from plants since it is simple and quick (Brand-Williams et al., 1995). A decrease in the absorbance of DPPH shows that the compounds in the extract are able to reduce the stable DPPH radical. Yet the DPPH activity measured in an in vitro assay cannot be directly regarded as an equivalent indication of antioxidant activity in the human body since factors such as bioavailability, digestion, metabolism, and the interactions between dietary compounds can affect the biological effects of antioxidants.

The fact that different results were obtained in the present study also show how important it is to choose appropriate pulse crops for use as functional foods. Since lentil and mung bean exhibited relatively greater DPPH activity in the current dataset, further research into their phenolic and flavonoid contents should be carried out. Additional antioxidant tests, such as ABTS, FRAP, and reducing power assays, would provide supplementary information and enable a more thorough assessment of antioxidant potential.

The results show that there are differences in antioxidant activity among the various pulse species and that pulses can serve as a useful source of naturally occurring antioxidant compounds; yet further investigation employing real experimental measurements, a greater number of samples, phytochemical characterization, and suitable statistical analysis is needed in order to verify these findings.

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