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Effect of solvent types on the antioxidant activity and total flavonoids of some Bangladeshi legumes

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Abstract

In this study, an attempt was made to estimate the antioxidant activity of some popular Bangladeshi legumes by employing DPPH radical scavenging assay, total phenolic content (TPC), and ferric reducing antioxidant assay (FRAP). The extraction efficiency and total flavonoid content (TFC) of hyacinth bean (*Lablab niger*), kidney bean (*Phaseolus vulgaris*), black gram (*Vigna radiate*), mung bean (*Vigna mungo*), and green pea (*Pisum sativam*) by different solvents were also examined. Extraction was done using 80% acetone, ethanol, and methanol as solvent. The maximum and minimum DPPH scavenging activity was shown by mung bean (27.67±1.53%) and Hyacinth bean (4.33±1.53%), respectively using ethanol as solvent. The highest and lowest FRAP value was recorded 89.60±0.80 µg AAE/mL for green pea and 35.11±1.39 µg AAE/mL for mung bean, respectively with ethanol solvent. For TPC, green pea with ethanol solvent showed the highest value (1045.92±21.30 mg GAE/100 g), and mung bean with ethanol solvent exhibited the lowest value (415.92±1.44 mg GAE/100 g). For TFC, black gram with ethanol solvent exhibited the highest value (342.21±3.05 mg QE/100 g), while mung bean with acetone solvent exhibited the lowest value (77.38±0.80 mg QE/100 g).

1. Introduction

Legumes are mostly cultivated for their pulse, for animal forage and silage, and as a soil amendment that improves the soil. About 5% of the cropped area of Bangladesh is occupied by legume foods. In Bangladesh, legumes ranked the second largest cultivated area after rice and they play vital roles in rainfed agriculture. Khesari (*Lathyrus sativus*), chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), mung bean (*Vigna radiata*), cowpea (*Vigna unguiculata*), and black gram (*Vigna mungo*) are the main pulses cultivated and add to over 95% of the total production of pulses in the nation (BBS, 2014).

Legumes are significant sources of micronutrients and macronutrients. They also played a significant part in the conventional diet in many areas around the world. Besides their nutritional value, legumes are classified as healthy foods that encourage healthy health and have medicinal properties (Roy *et al.*, 2020). In recent years, the search for natural plant-based antioxidants has risen significantly. Antioxidant compounds derived from natural sources, including fruits, rice, olive seeds, tea, beans, spices, and vegetables, have been studied (Alam *et al.*, 2020; Hossain *et al.*, 2020a; Hossain *et al.*, 2021; Hossain *et al.*, 2020b; Sarkar *et al.*, 2020; Sarkar *et al.*, 2021; Zzaman *et al.*, 2021). Many of these bioactive components are present in many plants, including pulses' fruits, woods, leaves, stems, roots, barks, herbs, and seeds (Ahmad *et al.*, 2016). Unfortunately, very few research works were carried out to determine the antioxidant activity of pulses. The effects of different solvent extraction methods on the antioxidant activity of pulses were also got less attention.

Recent studies have shown that legumes have strong antioxidant activities (Takahashi *et al.*, 2005). These studies reported that pulses could be eaten for disease prevention and health promotion as an excellent dietetic source of natural antioxidants. By extracting free radical substances, antioxidants inhibit these chain reactions and avoid several oxidative reactions (Shenoy and Shirwaikar, 2002). They are thought to play a part in stopping chronic diseases such as heart disease, cancer, rheumatoid arthritis, stroke, and cataracts from developing. Oxidative stress occurs when the development of harmful molecules known as free radicals is beyond the protective capacity of antioxidant defences (Cai *et al.*, 2004). Pulses have been considered

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in the everyday diet as folk remedies and beverages and have been commonly used for treating antidotes, edemas, diuretics, antifebriles, carminatives and more (Roy *et al.*, 2021). Phytochemical constituents typically present in food plants are phenolic compounds, which include phenols and phenolic acids, derivatives of hydroxycinnamic acid, and flavonoids. The organoleptic and nutritious value of raw and refined plant foods was closely correlated with phenolic substances. The phenolic components present in plants are excellent natural sources of antioxidants.

However, their solubility in solvents with varying polarities is often determined by variations in the composition of phenolic compounds. Hence, both the solvent type and the extraction methods may have a substantial effect on the production of antioxidants and total flavonoids from plant materials (Hossain *et al.*, 2020a). Several results are found regarding the optimization of phenolic compounds' extraction conditions and the antioxidant ability of particular plant foods (Hossain and Hossain, 2021). Nonetheless, the appropriate approach is usually different for various plant matrices as seen by some studies (Rababah *et al.*, 2010). Many studies have documented the impact of various solvents and extraction techniques on the quality and quantity of natural antioxidants. Different types of solvents like methanol, ethanol, and acetone have been widely utilized to extract phenolic from fresh products. In this context, the objectives of this study were to estimate the extraction efficiency of methanol, ethanol, and acetone, and to determine the antioxidant activity, and total flavonoids of different pulses available in Bangladesh.

2. Materials and methods

2.1 Location of the study

The entire research work was done at the Food Chemistry laboratory of Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh.

2.2 Chemicals and equipment

The analytical grade chemicals used in this analysis were obtained from industrial suppliers. Ethanol (C_2H_6O), Methanol (CH_3OH), Acetone (C_3H_6O), 2,2-diphenyl-1- picrylhydrazyl (DPPH), and Folin-Ciocalteu Phenol- Reagent were purchased from Sigma Aldrich (USA); Sodium carbonate (Na_2CO_3), Potassium di-hydro phosphate (KH_2PO_4), and Monobasic dihydrate ($NaH_2PO_4.2H_2O$) were obtained from Merck (India); and Ferric Chloride ($FeCl_3$), Trichloroacetic acid ($C_2HCl_3O_2$), and Potassium ferricyanide [$K_3Fe(CN)_6$] were collected from Merc (Germany).

Oven Dryer (Model ON-01E), UV Spectrophotometer (PG Instruments Ltd., Model - T60 U), Vortex Mixer (Model - VM-2000), Incubator (AAH 26016U), Shaking incubator (Model SI-100), Blender (Panasonic, Model - MJ-M176P), and Hot water bath (NE2-9D Bennett Scientific) were used as equipment.

2.3 Preparation of samples

From the regional supermarket of Sylhet, Bangladesh, Hyacinth Bean (*Lablab niger* Medik.), Kidney bean (*Phaseolus vulgaris* L.), Black Gram (*Vigna mungo* (L.) Hepper), Mung Bean (*Vigna radiata* (L.) R. Wilczek), and Green Pea (*Pisum sativum* L.) were purchased. Beans were then oven-dried at 60°C for 24 hrs and blended using a mechanical blender.

2.4 Extraction procedure

Extraction was performed with a minor adjustment relying on Hussain *et al.* (2012) method. At first, 1 g of the ground sample was added separately with 15 mL of 80% (v/v) aqueous methanol, ethanol and acetone and kept at ambient temperature (25±2°C) for 2 hrs with continuous stirring using an orbital shaker (model SI-100, Germany) at 150 rpm before centrifugation for 10 mins at 4000 rpm. The supernatant obtained was stored at -4°C for further experiment. Figure 1 exhibits the bird's eye view of the entire research design.

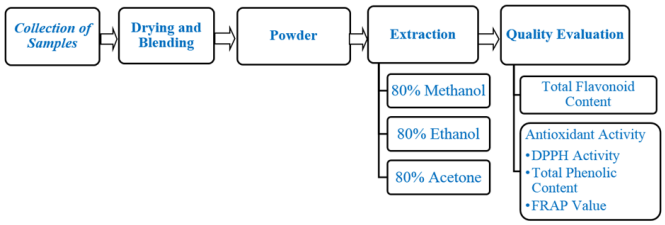

Figure 1. Schematic diagram of research design

2.5 DPPH radical scavenging activity

The Brand-Williams *et al.* (1995) method was slightly modified for the DPPH radical scavenging assay. In a tube, 1 mL of the aliquot from each of the five extracts was added separately with 4 mL of DPPH solution. After that, the tubes were vortexed and allowed to stand in the dark for 30 mins. Then, the absorbance of the mixtures was taken using a T60 U Spectrophotometer at 517 nm. An aliquot-free DPPH solution was used as a control, and the results were expressed in percentage. The following equation was used to estimate the DPPH radical scavenging activity.

$$\text{DPPH radical scavenging effect (\%)} = \left(1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ control}\right) \times 100\%$$

2.6 The total phenolic compounds (TPC)

The total polar phenolic compound was estimated by following the Rahman *et al.* (2016) method with some modifications. Approximately 0.5 mL from five extracts were separately placed in a 10 mL flask. The Folin-Ciocalteu reagent (0.5 mL) was then applied to the solvent and shook for 3 mins. In order to prepare a 10 mL solution, one mL of saturated sodium carbonate (Na_2CO_3) was added, and the remaining amount was filled up with distilled water. Solutions' absorbances were measured with a Spectrophotometer against a reagent blank at 725 nm. Pure gallic acid (GA) was used as a standard for calibration curve preparation. The results were expressed in mg GAE/ gm w/w using the equation $y = 0.0004x + 0.0013$; $R^2 = 0.999$.

2.7 Ferric reducing antioxidant power (FRAP)

The modified method of Oyaizu (1986) was used to determine the total antioxidant activity of the extracts by ferric reducing antioxidant power (FRAP). An aliquot of 0.3 mL from five extracts was added separately and vortexed with 0.85 mL of 0.2 M phosphate buffer of pH 6.6 and 1%, 0.85 mL of potassium ferricyanide. After incubating the mixture at 50°C for 20 mins, 0.85 mL of trichloroacetic acid (10%) was added and vortexed well. Finally, 2.85 mL of double-distilled water and 1%, 0.57 mL of Fe_2Cl_3 were mixed and incubated at 25°C for half an hour. After the second incubation, absorbance was taken at 700 nm by operating a Spectrophotometer. A Blank was prepared in parallel, where distilled water was added instead of the aliquot. The standard ascorbic acid was developed by serial aqueous dilution of stock solution. The standard curve was prepared by fitting the absorbance versus its corresponding standard ascorbic solutions. The outcomes were estimated in µg ascorbic acid correspondent antioxidant capability/100 g w/w (µg AAE/100 g w/w) using the equation $y = 0.176x + 0.005$; $R^2 = 0.998$.

2.8 Determination of total flavonoid compound

The total flavonoids were determined by the aluminium chloride colourimetric assay of Pothitirat *et al.* (2009). Approximately 1.5 mL of the extracts were placed in a 10 mL flask. Following that, 2% $AlCl_3$ solution and 6 mL of distilled water were added to the flask and vortexed. After half an hour, the solutions' absorbances were measured using a T60-U Spectrophotometer (Germany) at 415nm against a blank. The equation $y = 0.003x + 0.025$; $R^2 = 0.999$ was used as a standard curve to determine the total flavonoid compounds and expressed as equivalent per gram.

2.9 Statistical analysis

The result was reported as mean ± standard deviation for three replications of each treatment. The data were analyzed statistically using the Minitab-19 statistical software. Mean and Standard Deviation (SD) were measured by Tukey One Way ANOVA Test. The least significant difference was estimated at a 95% level of confidence ($p < 0.05$).

3. Results and discussion

3.1 Antioxidant activities of different pulses

Free radicals that are produced in the human body are mainly related to cancer aetiology and several other chronic diseases. Food sources like legumes which are rich in antioxidants may have a protective effect against different diseases. Consequently, five different legumes were used in this study to estimate their antioxidant capability.

3.1.1 DPPH scavenging activity

The application of DPPH has given a convenient and fast way to assess the function of antioxidants. It is a secure, chemical radical which does not degrade in water or other alcoholic solutions. Sarkar *et al.* (2020) stated that the free radical scavenging activities of the extracts mainly depend on the antioxidants' efficiency to end up losing hydrogen and their structural make-up. When an electron or a free radical substance is obtained, it loses its absorption, which creates visible changes in colour from purple to yellow. This can retain several samples in a brief period and is sufficiently capable of detecting active compounds at low concentrations (Kumoro *et al.*, 2009). DPPH scavenging activity was the maximum for mung bean (27.67±1.53%) with ethanol solvent, and the lowest (4.33±1.53%) was for Hyacinth bean when ethanol was used as a solvent (Figure 2). It may vary from sample to sample. From the data, it can be seen that for all the samples except mung bean (*Vigna* radiate), the efficiency of ethanol solvent is less compared to acetone and methanol. Acetone has the highest value for all the samples except the kidney bean (*Phaseolus vulgaris*). The findings from Figure 2 show that methanol extracts have more scavenging activity in most cases. Methanol has the highest value only for the kidney bean (*Phaseolus vulgaris*) and shows the medium value for the rest of the samples. It is well known that different extracts pose different antiradical activities due to different functional groups present in them. In the antioxidant activity, the location of the hydroxyl group, the involvement of other functional groups as double bonds, and the structure of hydroxyl groups and ketones play a crucial role (Memon *et al.*, 2007). The results are in agreement with the previous results, where it was

found that the DPPH radical scavenging activity may vary with the types of extraction solvent because of the polarity of the solvent (Dawidowicz and Olszowy, 2012).

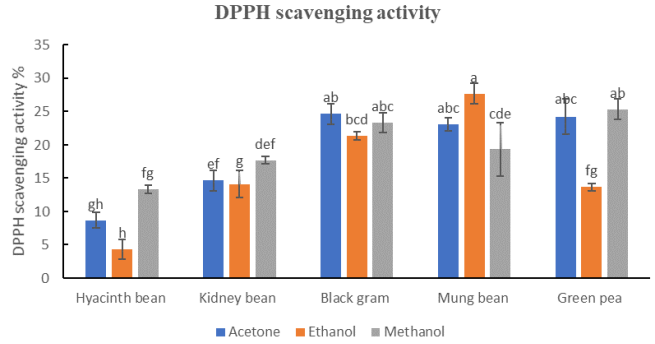

Figure 2. DPPH scavenging activity of different pulses for different types of extracts. Bars with different notations per sample are significantly different (P<0.05) according to Tukey Method.

3.1.2 Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) assay is one of the most commonly used methods in which antioxidants act as reducers in redox-linked colour reactions. Here, the ferric ion is reduced to ferrous ion at low pH that induces the development of colour from a colourless ferric-probe complex. The highest value of Ferric reducing antioxidant power was 89.60±0.80 µg AAE/mL in the case of green pea with ethanol solvent, the lowest 35.11±1.38 µg AAE/mL was for Mung bean with ethanol solvent (Figure 3). The extract's reducing power reflects its antioxidant capacity. The antioxidant properties in the sample help the Fe^{3+} ferricyanide complex to be reduced to the Fe^{2+} form, which is investigated by evaluating Perl's Prussian blue formation at 700 nm (Yang *et al.*, 2010). From the data, we can see that all three solvents give the almost same value for both hyacinth bean (*Lablab niger*) and Kidney bean (*Phaseolus vulgaris*). For green pea (*Pisum sativam*), methanol gives a lesser value than acetone and ethanol. Different plant materials have different responses towards the extraction solvents due to their functional groups. The results are in line with the previous studies (Loizzo *et al.*, 2010; Hossain *et al.*, 2020b).

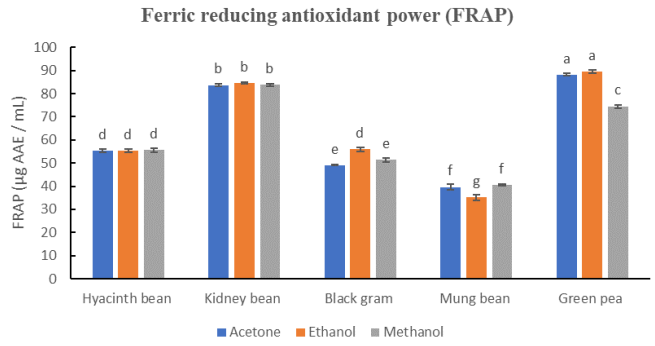

Figure 3. Ferric reducing antioxidant power (FRAP) of different pulses for different types of extracts. Bars with different notations per sample are significantly different (P<0.05) according to Tukey Method.

3.1.3 Total phenolic content

Plant phenolic agents mainly exert their positive health benefits by their antioxidant action. Shahidi and Naczk (2004) reported that phenolics can reduce the concentration of oxygen, intercept the singlet oxygen, block the beginning of the chain reaction, bind catalysts of metal ions, decompose non-radical species with primary oxidation items, and break the chains to prevent the continuous absorption of hydrogen from compounds. Phenolic compounds boost the total antioxidant function in plant foods. In this study, spectrometric absorption methods were used to determine the total phenolic content of different extract solutions, where the reaction of the Folin-Ciocalteu reagent with the sample extracts and the comparison with the regular gallic acid equivalent solutions were taken into account following the previous studies (Cook and Samman, 1996). Phenolic compounds act as antioxidants by terminating the free radicals. The maximum amount of Phenolic content was found in green pea using ethanol extract (1045.92±21.30 mg GAE/ 100 g), and the minimum was 415.92±1.44 mg GAE/ 100 g in the case of mung bean (Figure 4). Many non-phenol molecules, together with carbohydrates and terpene, may be due to the existence of water extracts in contrast with other extracts. The probable complex production of other phenolic compounds soluble in methanol, ethanol, and acetone in the extract may also trigger this.

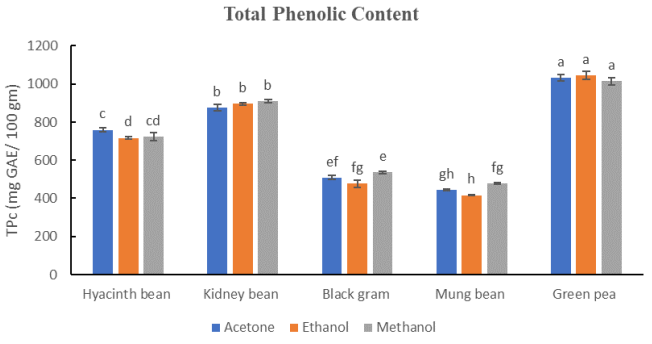

Figure 4. Total phenolic content of different pulses for different types of extracts. Bars with different notations per sample are significantly different (P<0.05) according to Tukey Method.

3.2 Total flavonoid content

Different kinds of flavonoids, like flavones, and condensed tannins are widely used as secondary metabolites of plants. Epidemiological researches indicate that the consumption of flavonoids rich foods protects against illness linked to oxidative stress. They are consistently found in the human diet as constituents

of vegetables and fruits. Nevertheless, very few studies have been published on the detection of flavonoids in legumes. For instance, only a number of research works have been reported on common beans (Romani *et al.*, 2004) and peas (Troszyńska *et al.*, 2002).

It was found for flavonoids content that methanol gives significantly the lowest value for mung bean (*Vigna radiate*) and highest value for Black gram (*Vigna mungo*). Acetone extract solution shows the lowest value for mung bean (*Vigna radiate*) and the highest for kidney bean (*Phaseolus vulgaris*). Ethanol shows the highest value for Black gram (*Vigna mungo*) and the lowest for hyacinth bean (*Lablab niger*) Green pea (*Pisum sativum*) (Table 1). The effect of solvent on total flavonoid content is similar to total phenolic content. Methanol is well known for its polarity and capacity to extract flavonoids. Methanol is also known to work more efficiently in the case of low molecular weight polyphenols. The findings of this study are similar to those of previous studies (Siddhuraju and Becker, 2003; Ojha *et al.*, 2018).

Table 1. Total flavonoid content of legumes samples for different solvents

Samples	Extraction Solvent	Flavonoid content (mg QE/100 g)
Hyacinth bean	Acetone	218.44 ± 6.87^{fg}
	Ethanol	93.17 ± 11.80^{j}
	Methanol	213.09 ± 4.98^{gh}
Kidney bean	Acetone	262.13 ± 12.44^{d}
	Ethanol	237.74 ± 1.73^{ef}
	Methanol	288.26 ± 11.06^{c}
Black gram	Acetone	245.37 ± 8.43^{de}
	Ethanol	386.51 ± 3.05^{a}
	Methanol	363.96 ± 5.92^{b}
Mung bean	Acetone	69.14 ± 0.81^{k}
	Ethanol	87.83 ± 2.14^{jk}
	Methanol	99.05 ± 4.11^{j}
Green pea	Acetone	194.14 ± 11.72^{h}
	Ethanol	220.63 ± 2.09^{fg}
	Methanol	170.19 ± 6.56^{i}

Values are presented as mean±SD. Values with different superscript are significantly different ($P < 0.05$) according to Tukey Method.

4. Conclusion

This study revealed that the effects of extraction solvents on antioxidant activity are samples specific. For DPPH radical scavenging activity, acetone and methanol solvent seemed to be more efficient than ethanol. But when it comes to the FRAP, three of them are almost close in value though ethanol exhibited better performance than methanol and acetone. Acetone and methanol seemed to be effective for phenolic extraction. Methanol showed inconsistency at flavonoid extraction, gave both the highest and lowest value for different samples. Mung bean (*Vigna mungo*) gave the highest DPPH scavenging activity but the lowest FRAP, total phenolic content, and flavonoid content. Green pea (*Pisum sativam*) shows the highest FRAP value and total phenolic content, where black gram (*Vigna radiate*) was seemed to have the highest flavonoid content. However, further studies could be done for specific samples and certain solvent in various ratios.

Conflict of interest

The authors declare no conflict of interest.

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