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In vitro antioxidant activity of methanolic extract of *Helianthus annuus* seeds

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Abstract
Helianthus annuus seeds belonging to the family *Asteraceae* have been investigated for the presence of various chemical components and evaluation of biological activities of the crude extracts of methanol with special emphasis to the phenolic component with anti-oxidant activity. The methanolic extract of *Helianthus annuus* seeds demonstrates a remarkable amount of phenolic content (54 µg/ml of GAE/100g of extract) by using Folin-Ciocalteau reagent. *In vitro* antioxidant activity of methanolic seed extract of *H. annuus* was determined by DPPH free radical scavenging assay, which showed significant antioxidant activity compared to standard antioxidant (ascorbic acid). A direct correlation between total phenol and antioxidant activity indicates polyphenols are the main antioxidants. The present study shows that *Helianthus annuus* seeds have radical scavenging activity and can be considered as good sources of natural antioxidants for medicinal and commercial uses.

Keywords: *Helianthus annuus*, Asteraceae, DPPH Scavenging activity, Antioxidant.

Introduction
The *Helianthus annuus* seed is the fruit of the sunflower which belongs to the family named asteraceae. By the pattern of seed husks sunflower seeds are mainly differentiated. The seeds are called black oil sunflower seeds when the husk is solid black. The crops are known as oilseed sunflower crops. By pressing the seeds the oil of sunflower seed can be extracted. Due to the good nutritional content of sunflower seed, it is a potential protein supplement for human diet. However the primary use of sunflower seed is not for edible protein, it is for oil because certain attributes of sunflower seed oil have particularly attractive to the food industry. It is the rich source of vitamins specially vitamin E.
Phenolic compounds are derived from phenylalanine and considered to be secondary metabolites. Phenolic possess a unique structure containing an aromatic ring with one or more hydroxyl groups [1]. Flavonoids, tannins, benzoic acids and cinnamic acid derivatives are the examples of phenolic derivatives which can be found in plants [2]. About 8000 plant phenolics are found naturally and most of them are flavonoids. Flavonoids also have unique structure which is based on the C_{15} heterocyclic nucleus of flavones and they usually vary in the number of phenols, such as phenolics quinines, phenolics acid and phenyl propanoids [3].
Antioxidants are considered important nutraceuticals on account of many health benefits. Due to the high antioxidant activity, *Helianthus annuus* seeds might be considered in the management and prevention of degenerative diseases associated with free radical damage. The requirement of a standard assay is very important in order to compare the results of different laboratories and validation of the conclusions. 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) is a stable free radical which has an unpaired valence electron at one atom of Nitrogen Bridge. Scavenging of DPPH radical is the basis of the popular DPPH antioxidant assay [4]. Due to simplicity, flexibility, high throughput, TLC technique is used for qualitative antioxidant test.
In previous studies, there have been analyze chemical composition of *H. annuus* seeds. There is very limited data about phenolic content and anti-oxidant activity of Helianthus annuus seeds. So the present study was undertaken to evaluate extensively the total phenolic content with antioxidant activity of *H. annuus* seeds.

Materials and methods
Collection of seeds
The air dried seeds of *H. annuus* was collected at their matured form from Bangladesh Agriculture Research Institute, Gazipur, Bangladesh in June 2014. Undesired plant parts or

additives were removed after the collection. Then it was dried through air but absences of sun light for several days. The seeds were ground into fine powder with the help of electric grinder. Then the powder was stored in air tight container and placed in a cool, dry and dark place.

Preparation of extract

300 g dried powder was weighed and taken in an aspirator (2.5 L). Before placing powder into the aspirator, the jar was washed properly with acetone and then dried. 800 ml of solvent i.e. methanol was added gradually. The container with its content was sealed and kept for 20 days with occasional shaking and stirring. The major portion of the extractable compounds of the plant materials were dissolved in the solvent. Then whole mixture was filtered through cotton wool and the filtrate was concentrated by evaporation in dry and clean air. The entire process was repeated using ethyl acetate and petroleum ether as the solvent to obtain ethyl acetate and petroleum ether extract. But here 225 g of dried seeds was taken with 675 ml ethyl acetate and petroleum ether separately. And it was kept for 15 days [5].

Chemicals and reagents

Methanol, folin-Ciocalteau reagent, gallic acid, DPPH solution (1, 1-Diphenyl-2-picryl-hydrazyl) are used for the experiment.

Phytochemical screening

Phytochemical screening performed to investigate various chemical compositions present in seeds. To perform the tests the following tests were used: Fehling test for carbohydrates, wagner's test for alkaloids, keller-killiani test for glycoside, foam test for saponins, coumarin test for steroids and terpenoids, alcoholic test for flavonoids, ferric chloride test for tannins, molisch test for gum. Among them the presence of carbohydrate, alkaloid, steroid and terpenoids were found. These were identified by characteristic color changes using standard procedures [6].

Determination of Total Phenolic Conponent (TPC)

By using Folin- Ciocalteau reagent total phenolic component were determined colorimetrcially which was described by Taga *et al.* (1984). In 10ml methanol 150 mg extract was dissolved and 2 ml of this solution was filled up with 0.3% HCl to 5 ml. A 100-µl aliquot of the resulting solution was added to 2 ml of 2% Na_2CO_3 and 100 µl of Folin-Ciocalteau reagent was added after 2 minute. The absorbance was measured at 750 nm using a spectrophotometer after 30 minutes. Total phenolic component were quantified by measuring the calibration curve total phenolic content was quantified. The calibration curve was obtain from measuring the absorbance of a known concentration of gallic acid standard. The final results were expressed as micrograms gallic acid equivalents per 100 ml extract [7].

Antioxidant activity

Flavonoids are known to be highly effective antioxidant by scavenging oxygen radical, by having interesting anti-cancer, hypo lipidemic, anti-ageing, and anti-inflammatory activities. Moreover, the protective effects of flavonoids in biological systems are attributed to their capacity to scavenge free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce alpha tocopherol radicals, and inhibit oxidases [8].

Preparation of extract solution

The antioxidant activity of seed extract was determined by *in vitro* method (DPPH free radical scavenging assay). The free radical scavenging capacity of the methanolic extract of *H. annuus* was determined using DPPH. DPPH solution (0.004% w/v) was prepared in methanol. Methanol extract of *H. annuus* was mixed with methanol to prepare the stock solution (10 mg/100 ml). The concentration of this *H. annuus* methanolic extract solution was 10 mg/100 ml from stock solution, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.625 µg/ml, 7.812 µg/ml, 3.906 µg/ml, 1.953 µg/ml, 0.96 µg/ml solution was prepared respectively. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing *H. annuus* methanolic extract and after 10 min, the absorbance was taken at 517 nm using a UV–visible spectrophotometer. Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (10 mg/100 ml) of methanolic and ethyl acetate extract of *H. annuus.* Control sample was prepared without adding any extract and ascorbic acid, methanol was used as blank, ascorbic acid solution was used as a standard.

The Scavenging activity of DPPH (%) was calculated by using the following equation–

$$\% \text{ DPPH radical scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

In antioxidant activity assays, different concentrations of seed extract were used to find out the concentrations which could include 50% inhibition to calculate IC_{50}.

Statistical Analyses

The data was statistically analyzed by using one-way ANOVA. The values are reported as mean ± SD.

Results

Total Phenolic Contents (TPC)

Follin–Ciocalteu method was used to determine total phenolic content and results were showed in gallic acid equivalents (Figure 1). Our experiment showed the results of the total phenolic content 54 µg/ml for methanolic extract of *H. annuus* seeds. (Table 1)

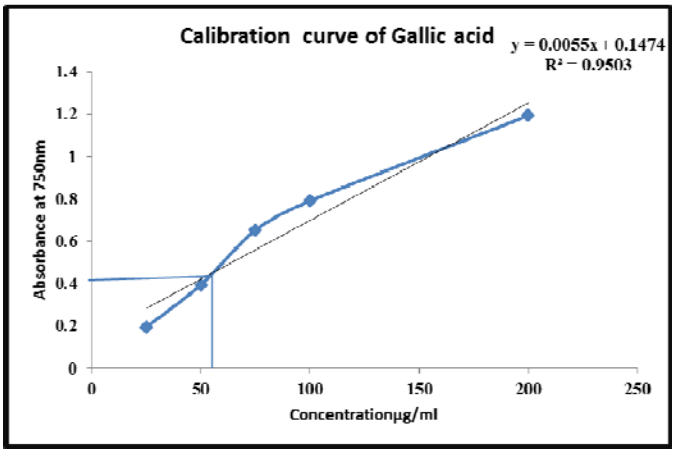

Fig 1: Standard curve of Gallic Acid.

Table 1: Determination of total phenolic content of methanolic extract of *H. annuus* seeds.

Sample	Absorbance	Average	Phenolic content µg/ml
Methanolic	0.478		
	0.486	0.489	54µg/ml
	0.489		
	0.487		

Antioxidant test

In this study, the antioxidant capacity of methanolic extract of *H. annuus* seeds and ascorbic acid was systematically

evaluated. Antioxidant activity of selected plant extract compared with standard antioxidant ascorbic acid showed that selected plants have potential antioxidant activity like ascorbic acid**.** Methanolic extract of *H. annuus* showed 51.57% DPPH scavenging activity and IC_{50} was 0.814 where as 1 in ascorbic acid**.** (Table 2)

Table 2: Radical scavenging activity of methanolic extract and standard antioxidant on DPPH free radical.

Sample	IC_{50} (µg/ml) Mean ± SD	% Inhibition
Methanol extract	90 ± 11.56	51.57
Ascorbic acid	73.26 ± 17.80	46.66

Discussion

Calculating the total phenolic content in plant species is important. Phenolics and polyphenolic compounds are directly related to antioxidative action and they involve in the main class of natural antioxidants present in plants [9]. Phenolic compounds in sunflower seeds have been worked out by various scientists previously [10]. Because of differences in analytical methodologies, sample material and origin and the development of novel sophisticated methods, it is hardly possible to compare with the data obtained in the present study. The total phenolics contents in sunflower seeds was found 2700 mg/100 g on dry weight basis which was previously done by Fiska *et al.* (2006) [11].

Our study investigated for the relationship between phenolic compounds and antioxidant activity and found a significant relationship among those (R^2=0.9503). From the results of our study it can be definite that there is a direct relationship between total phenolics contents and antioxidant activity of *H. annuus* seeds. It can be interpreted that, if seeds have more phenolics contents they also have significant antioxidant potential and vice versa. Previous study done by also reported different results about the relationship between phenolic content and antioxidant activity. Some previous studies found correlation between the total phenolic contents and the antioxidant activity while some found no such relationship. During germination of Pangium edule there was a parallel increase in between phenol content and antioxidant activity which was done by Andarwulan (1999) [12].

An increase in the antioxidant activity of lupin seed was also observed by Tsaliki *et al.* (1999) [13]. Javanmardi *et al*. (2003) also found a linear positive relationship between the antioxidant activity and total phenolic content of the tested *Ocimum* accessions [14]. Maillard and Berset (1995) showed no correlation between phenolic contents and antioxidant activity in malts and suggested that other compounds are also responsible for the antioxidant activity [15]. Another study done by citrus residues also showed no relationship between antioxidant activity and phenolic composition. Further research should be needed to find out the proper relationship between phenolic content and antioxidant activity of *Helianthus annuus* seeds.

Conclusion

From the results of our study it is clear that due to the presence of total phenolic contents of *Helianthus annuus*, it possess significant antioxidant activity. A strict linear relationship was also observed in the study between total phenolics and antioxidant activity. As sunflower seeds have major medicinal effects, therefore the potency of these extracts could provide a chemical basis for health benefits claimed. It needs further studies to assess their potential components as effective natural remedies.

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