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# Determination of antioxidant potential of *Cucurbita pepo* Linn. (An edible herbs of Bangladesh)

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**Abstract**
For initial screening 1, 1-diphenyl-2-picryl hydrazyl (DPPH) was applied on the TLC plate which indicated the presence of antioxidant components. The anti-oxidant potential of the ethanolic extract of leaves and stems of *Cucurbita pepo* Linn. was determined from their scavenging activity of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) as well as from phenolic and flavonoid content. The ethanolic extract possessed a significant DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging activity with an $IC_{50}$ value of 30 µg/ml compared to standard ascorbic acid which was 8 µg/ml. The total phenolic content of ethanolic extract of Cucurbita pepo Linn. revealed to be 17.49 mg GAE/g of dried plant material. The total flavonoid content of ethanol extract of Cucurbita pepo Linn. revealed to be 25.43 mg QE/g of dried plant material.

**Keywords:** Antioxidant activity, Ethanolic extract, *Cucurbita pepo* Linn. DPPH

## Introduction
Antioxidants play an important role in nutritional by lengthening the shelf life of food and reducing nutritional losses and formation of harmful substances. The safety of synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are now in doubted [1,2].Thus, attention is now increasingly paid to the development and utilization of more effective and non-toxic antioxidants of natural origin. A great number of natural medicinal plants have been tested for their antioxidant activities and results have shown that the raw extracts or isolated pure compounds from them were more effective antioxidants *in vitro* than BHT or vitamin E [3]. So, medicinal plants can be a potential source of natural antioxidants [4].
*Cucurbita pepo Linn.* an edible herbs of Bangladesh belongs to the family cucurbitaceae commonly known as Misti Cumra, Pumpkin, Vegetable marrow, Crookneck squash, Vegetable spaghetti, Crookneck squash, Crookneck squash, Spaghetti melon, Squash. The plants are typically 1-2.5 feet high, 2-3 feet wide, and have yellow flowers [5]. The leaves of cucurbita pepo Linn. simple and Foliolate and sessile or subsessile. Stem is striate, sparsely pubescent and with capitate glandular hairs; tendrils present. Traditionally the pumpkin seeds are used in treating parasites and worms, bladder infections. Leaves are used as a painkiller, a treatment for nausea, and a boost to haemoglobin content of the blood [6]. The herbs are also used in traditional folk medicine to treat cold and alleviate ache [7]. Upon sufficient literature survey on the different plants (*Citrullus lanatus*, *Momordica charantia*, *Cucumis sativus*) of Cucurbitaceae family it is found that the seed, leaves and stem was used for research work. In case of Cucurbita pepo several researcher found active component from the seed and pulp of *Cucurbita pepo* Linn. that have antidepressant, antiulcerant, hypoglycemic, hypolipidemic and cytotoxic effects. But the plant's leaves and stems was not much scientifically explored. That's why to judge antioxidant potential of the herbs, ethanolic extract of leaves and stem was used.

## Materials and Method
### Collection of plants
For this present investigation the plant *Cucurbita pepo* Linn. (Family: Cucurbitaceae) was collected from Khulna, Bangladesh on October, 2013 and was identified by experts at Bangladesh National Herbarium, Mirpur, Dhaka.

### Preparation of ethanolic extract
The collected plant parts were separated from undesirable materials or plants or plant parts and were washed with water. They were sun-dried for one week.

The plant parts were grinded into coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. The leaves and stems were extracted by cold extraction method. 250gm grinded powder was soaked in 1000 ml of 98% of ethanol in a glass container for fifteen days accompanying regular shaking and stirring. The extract was separated from the plant debris by filtration by a piece of clean, white cotton material & it was done two times. The filtrate (Ethanol extract) obtained was evaporated under the air of ceiling fan and in a water bath until dried. After drying the filtrate, dried adhesive powdered mass was obtained. This powdered mass was designated as crude extract.

**DPPH Scavenging Activity**

Apparatus required for this study are Test tubes, Beakers, Sonicator, Pipette, Thermometer, UV spectrophotometer and Electronic balance. Reagents required are Ethanol, 0.004% DPPH ethanolic solution and Ascorbic acid (as positive control).

The aliquots of the different concentrations (5-100µg/mL) of the extract were added to 6 mL of a 0.004% w/v solution of DPPH. Absorbance at 517 nm was determined after 30 min, and $IC_{50}$ (Inhibitory conc. 50%) was determined. $IC_{50}$ value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals [8]. For sample or standard solution, 7 test tubes were taken to make aliquots of 7 conc. (5, 10, 20, 40, 60, 80, 100 µg/ml) for plant extract (sample) and ascorbic acid (standard) each. Plant extract and ascorbic acid were weighed and dissolved in ethanol to make the required concentrations by dilution technique. Here ascorbic acid was taken as positive control. DPPH was weighed and dissolved in ethanol to make 0.004% (w/v) solution. To dissolve homogeneously sonicator was used. 2 mL of different concentration of plant extracts and ascorbic acid was taken in each test tube. After making the desired concentrations 6 ml of 0.004% DPPH solution was applied on each test tube by pipette. The room temperature was recorded and kept the test tubes for 30 minutes in dark to complete the reaction. DPPH was also applied on the blank test tubes at the same time where only ethanol was taken as blank. After 30 minutes, absorbance of each test tube was determined by UV spectrophotometer at 517 nm.

% of inhibition was calculated as

% inhibition = [(Blank absorbance - Sample absorbance) / Blank absorbance] X 100

**Total Phenolic Content Assay**

Apparatus required for this study are Test tubes, Beakers, Sonicator, Pipette, Centrifuge apparatus, UV spectrophotometer and Electronic balance. Reagents required are Ethanol, Ethanol, Distilled water, Folin-Ciocalteu (FC) reagent, Gallic acid, 7% $Na_2CO_3$

Total phenolic content was determined by Folin-Ciocalteu's reagent [9]. Grinded plant material (0.5 g) was mixed with 50 mL of 80% aqueous ethanol and sonicated for 20 min. An aliquot of 2 mL was taken from it and centrifuged for 15min. Standard gallic acid solutions were prepared by serial dilution to get concentrations of 500, 250, 125, 62.5, 31.25, and 15.62 µg/ml. Gallic acid solutions of each concentration and extract solution of 1mL were transferred to 25mL volumetric flasks and 9mL distilled water was added. Folin-Ciocalteu's reagent of 1mL was added to each volumetric flask with continuous shaking. After 5min, 10mL of 7% $Na_2CO_3$ was added and adjusted with distilled water to make the final volume of 25mL. It was kept for 30min to complete any reaction that occurs. The absorbance was measured at 750 nm against blank. Blank was prepared by following the same steps mentioned above without adding extract solution or gallic acid. Standard curve of gallic acid was prepared by plotting absorbance versus concentration. The total phenolic content of extract was determined using the standard calibration curve of gallic acid and the value was expressed as gallic acid equivalent (GAE)/ g of dried plant material.

**Total flavonoid content assay**

Reagents required for this study are Crystalline Aluminum Chloride, Crystalline Sodium Acetate and Distilled water. Apparatus required are Test tubes Beakers, Pipette, UV spectrophotometer, Electronic balance, Cotton and Volumetric flask. The total content of flavonoids was evaluated from the regression equation of the calibration curve ($R^2$: 0.996, y = 0.040x + 0.002), expressed in quercetin equivalent as milligrams per gram of dry extract (mg QE/g of dry extract) [10, 11].133 mg Crystalline Aluminum Chloride ($AlCl_3$) and 400 mg Crystalline Sodium Acetate were dissolved in 100 ml distilled water to prepare the $AlCl_3$ reagent. To prepare the blank solution 5 ml analyzed solution mixed with 2.5 ml of extracting solvent. The total flavonoid content of ethanol extracts was determined according to Moreno *et al* 2000 [12]. A 5 ml sample of each extract (extract solution, 1 mg/ml) was mixed with $AlCl_3$ reagent (133mg crystalline Aluminium Chloride and 400 mg crystalline sodium Acetate were dissolved in 100 ml distilled water). The mixture was vortexed and the solution was allowed to stand for 40 minutes for reaction at room temperature. The absorbance was measured spectrophotometrically at 430 nm against a blank. The calibration curve was prepared by preparing quercetin solutions at concentrations 0.25, 0.50, 0.75, 1 µg/ml in ethanol. The total flavonoid content was determined as mg of Quercetin equivalent per gram using the equation obtained from a standard Quercetin calibration curve. Total flavonoid values are expressed in terms of Quercetin equivalent (QE) per gm of dry extract.

**Results and Discussion**

The ethanolic extract of leaves and stems showed DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging activity with an $IC_{50}$ value of 30 µg/ml compared to standard ascorbic acid which was 8 µg/ml. From the DPPH scavenging assay it can be said that the plant have free radical scavenging properties.

**Table 1:** DPPH scavenging assay of standard (ascorbic acid) and *Cucurbita pepo* Linn**.**

| Sample | Concentration (µg/ml) | Absorbance at 517 nm | | Average Absorbance±SD | % Inhibition | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|---|
| | | Sample-1 | Sample-2 | | | |
| Blank | 0 | 0.806 | 0.809 | 0.8075±0.002 | | |
| Ascorbic acid (Standard) | 5 | 0.495 | 0.498 | 0.4965±0.002 | 38.51 | |
| | 10 | 0.315 | 0.314 | 0.3145±0.001 | 61.05 | |
| | 20 | 0.211 | 0.212 | 0.2115±0.001 | 73.81 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 40 | 0.148 | 0.144 | 0.146±0.003 | 81.92 | ~ 8 |
| | 60 | 0.078 | 0.075 | 0.0765±0.002 | 90.53 | |
| | 80 | 0.071 | 0.073 | 0.072±0.001 | 91.08 | |
| | 100 | 0.052 | 0.055 | 0.0535±0.002 | 93.37 | |
| *Cucurbita pepo* L. | 5 | 0.691 | 0.693 | 0.692±0.001 | 14.36 | ~ 30 |
| | 10 | 0.529 | 0.531 | 0.530±0.001 | 34.41 | |
| | 20 | 0.486 | 0.488 | 0.487±0.001 | 39.73 | |
| | 40 | 0.324 | 0.326 | 0.325±0.001 | 59.78 | |
| | 60 | 0.243 | 0.245 | 0.244±0.001 | 69.80 | |
| | 80 | 0.126 | 0.128 | 0.127±0.001 | 84.28 | |
| | 100 | 0.051 | 0.053 | 0.052±0.001 | 93.55 | |

**Table 2:** UV Absorbance of gallic acid (standard) at 750 nm.

| Concentration (µg/ml) | Absorbance 1 | Absorbance 2 | Average ± SD |
|---|---|---|---|
| 500 | 0.485 | 0.491 | 0.488±0.004 |
| 250 | 0.45 | 0.445 | 0.448±0.004 |
| 125 | 0.421 | 0.427 | 0.424±0.004 |
| 62.5 | 0.351 | 0.353 | 0.352±0.001 |
| 31.25 | 0.251 | 0.254 | 0.253±0.002 |
| 15.62 | 0.158 | 0.157 | 0.158±0.001 |

Values are expressed as mean ±SD (n=2)

The amount of total phenol was determined with the Folin-Ciocalteu reagent. Gallic acid was used as standard and the total phenolic content was expressed as mg GAE/100g of dried plant material using the standard curve equation: y = 0.065x+0.123, $R^2$= 0.946, Where y is absorbance at 750 nm and x is total phenolic content in the samples. The total phenolic content of ethanol extract of *Cucurbita pepo* Linn. revealed to be 17.49 mg GAE/g of dried plant material. The total flavonoid content of ethanol extract of *Cucurbita pepo* Linn. revealed to be 25.43 mg QE/g of dried plant material.

**Table 3:** UV absorbance of sample at 750 nm.

| No. | 1 st reading | 2 nd reading | Average ±SD |
|---|---|---|---|
| 1 | 1.25 | 1.27 | 1.26±0.014 |

Values are expressed as mean ±SD (n=2)

**Table 4:** UV Absorbance of Quercetin (standard) at 430 nm.

| Concentration ( µg/ml) | Absorbance 1 | Absorbance 2 | Average±SD |
|---|---|---|---|
| 1 | 0.159 | 0.162 | 0.161±0.002 |
| 0.75 | 0.125 | 0.129 | 0.127±0.003 |
| 0.5 | 0.081 | 0.078 | 0.080±0.002 |
| 0.25 | 0.039 | 0.046 | 0.043±0.005 |

*Values are expressed as mean ±SD (n=2)*

**Table 5:** UV absorbance of sample at 430 nm.

| No. | 1 st reading | 2 nd reading | Average±SD |
|---|---|---|---|
| 1 | 1.021 | 1.018 | 1.019±0.002 |

Values are expressed as mean ±SD (n=2)

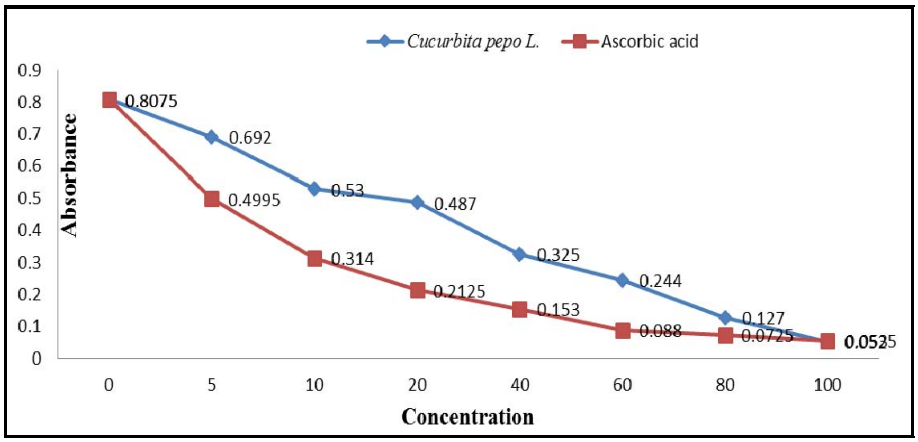


**Fig 1:** DPPH Scavenging Assay of *Cucurbita pepo* Linn. (Absorbance vs. Concentration)

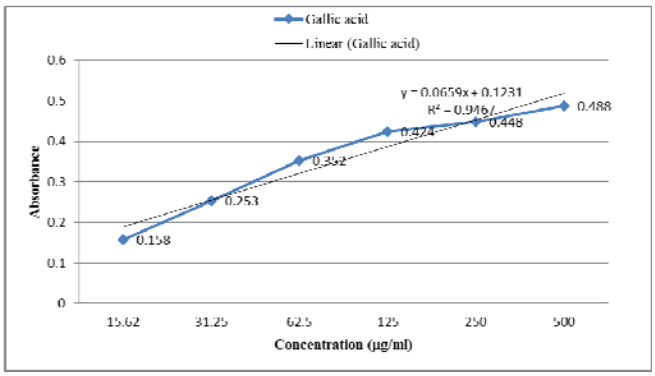


**Fig 2:** Total phenolic content determination of *Cucurbita pepo* Linn. with the help of gallic acid standard calibration curve.

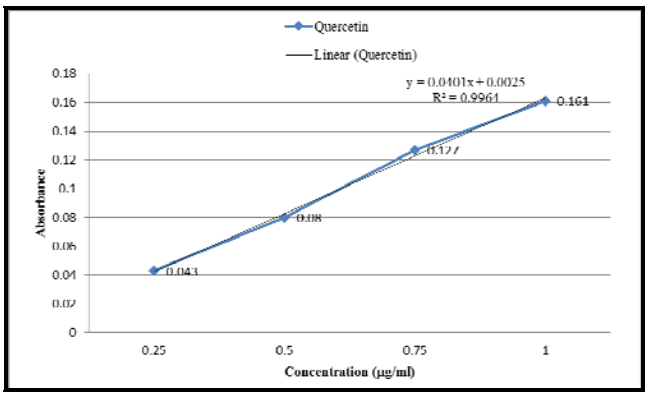


**Fig 3:** Total flavonoid content determination of *Cucurbita pepo* Linn. with the help of Quercetin standard calibration curve.

Studies have attributed that antioxidant properties are due to the presence of phenols and flavonoids [13, 14].The presence of phytoconstituents, such as phenols, flavonoids and tannin in plants, indicates the possibility of antioxidant activity and this activity will helps in preventing a number of diseases through free radical scavenging activity. Many studies focused on the biological activities of phenolic compounds, which have potential antioxidants and free radical scavengers [15].

**Conclusion**

The present investigation indicated the presence of antioxidant properties of edible herbs *Cucurbita pepo* Linn. Further advanced investigations are required to isolate bioactive compounds which demands further investigations like LC-MS, GC-MS, and XRD to isolate bioactive compounds as well as to characterize its structure.

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