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# Analysis of Antioxidant Activity of Selected Ethnic and Conventional Leafy Vegetables of Bangladesh by DPPH Assay

**Afsana Anwar, Sneha Sarwar*, Jyosna Khanam, Ontora Khatun, Sheikh Nazrul Islam**

Institute of Nutrition and Food Science, University of Dhaka, Dhaka, Bangladesh

## Abstract

Human body continuously produces different types of oxidants through various cellular metabolic activities. Antioxidants are substances that delay or prevent oxidative damage. So they are inevitable to combat reactive oxygen species in the body which are solely responsible for numerous degenerative non communicable diseases. Plants and different leafy vegetables are rich sources of antioxidant rich vitamins. The aim of this study is to analyze the antioxidant activity of selected ethnic and conventional leafy vegetables of Bangladesh. The DPPH free radical scavenging method is used to undertake the study. It is an effective method for analyzing the antioxidant activity of the plant material. Inhibition percentage and $IC_{50}$ (concentration required to obtain 50% antioxidant effect) value are used to express the antioxidant activity by DPPH method. Methanolic extracts of ten selected ethnic and conventional vegetables were analyzed for their potential antioxidant activity. Their percent inhibitions in different concentrations (200µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml) were determined. According to this study, at highest concentration maximum inhibition was done by Sojne pata *(Moringa oleifera),* which was almost 75%. Sojne pata (*Moringa oleifera)* showed the highest antioxidant activity with an $IC_{50}$ value of 374.8µg/ml and Mulashakh (*Raphanus sativus* L.) showed the lowest antioxidant activity with an $IC_{50}$ value 2057.3 µg/ml. This study helps to identify the contribution of conventional and ethnic vegetables in regular diet regarding antioxidant activity and paves the way for further research regarding health implications.

## Keywords

Conventional Vegetables, Ethnic Vegetables, Antioxidant Capacity, DPPH Assay, $IC_{50}$ Value

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## 1. Introduction

An antioxidant is any substance that delays or prevents oxidative damage [1] The main function of antioxidant is to scavenge reactive oxygen species [2]. Human body produces different kinds of oxidants during metabolism. Those oxidants are mainly reactive oxygen species (ROS) for instance superoxide anion ($O^{2-}$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical (HO•), hydrogen radical (H•) are continuously produced in our body [3]. Excess ROS and reduced endogenous antioxidant system are responsible for oxidative stress [4]. Oxidative stress can damage cellular membranes, lipids, proteins, lipoproteins, and deoxyribonucleic acid (DNA) [5-6]. Oxidative stress is

* Corresponding author
E-mail address: afsana21anwar@gmail.com (A. Anwar), snehasarwar4@gmail.com (S. Sarwar), jkhanam93@gmail.com (J. Khanam), ontora.du.96@gmail.com (O. Khatun), sheikhnazrul@du.ac.bd (S. N. Islam)

responsible for inducing and aggravating complications in non communicable diseases such as diabetes, cancer, cardiovascular disease and neurological diseases [7-9].

Diet that contains a lot of leafy vegetables can be a major source of antioxidants. Fat soluble vitamins such as Vitamin A, Vitamin E, Water soluble vitamin i.e. Vitamin C, Pro-vitamin such as B- carotene, phenolic compounds, polyphenols, lycopenes etc. are found in whole grains, fruits and vegetables [10]. Natural and dietary antioxidants are high in demand for application as nutraceuticals, bio-pharmaceuticals as well as food additive due to consumer preference [11].

Leafy vegetables may help to alleviate oxidative stress by scavenging reactive oxygen species due to their antioxidant properties [10, 12].

Therefore, the objective of the study is to evaluate in vitro antioxidant activity of selected ethnic and conventional leafy vegetables.

# 2. Methods and Materials

## 2.1. Chemicals Used

Primary solvent Methanol was HPLC grade DPPH was bought from Sigma-Aldrich. Ascorbic acid and hydrochloric acid (HCl) were analytical grade.

## 2.2. Collection of Samples

Ten vegetables (Local, English and Scientific name in Table 1) were selected for the study. Among them, three are consumed by the ethnic people of Chittagong hill tract area and seven are widely consumed across the country. The ethnic vegetables were collected from Rangamati and Bandarban area whereas commonly consumed vegetables were collected from Dhaka New market, Abdullahpur and Tongi Bazar, Dhaka, Bangladesh. Collecting from different markets, a homogenous mixture was made for each vegetable.

**Table 1.** Selected ethnic and conventional plants.

| Types of vegetables | SL no | Local name | English name | Scientific name |
|---|---|---|---|---|
| | 1 | Ozone shakh | . | *Spilanthes calva* |
| Ethnic plants | 2 | Lelom pata | . | *Premnaobtiusifolia* |
| | 3 | Maisa pagong | . | *Eryngium foetidum* |
| | 4 | Sojne pata | Drum stick leaf | *Moringa oleifera* |
| | 5 | Pudina pata | Mint leaf | *Mentha viridis* |
| | 6 | Dhoniyapata | Coriander leaf | *Centella asiatica*(L)Urban |
| Conventional plants | 7 | Mula shakh | Radish leaf | *Raphanus sativus* L. |
| | 8 | Lau shakh | Bottle gourd leaf | *Lagenaria siceraria (Molina)* Standl. |
| | 9 | Peyaz koli | Spring Onion | *Allium cepa* L. |
| | 10 | Badha kopy | Cabbage | *Brassica oleracaeae*var. capitata L. |

## 2.3. Preparation of Plant Extract

Approximately 2 grams of the powdered sample from each sample were weighed and transferred to a 250 ml conical flask. After that, 42.5 ml methanol and 7.5 ml 1N hydrochloric acid (HCl) was added. Then it was soaked at room temperature for a day with intermittent shaking. No.1 Whitman filter paper was used to filter the sample and the filtrate was isolated. The filtrate was then evaporated by a rotary evaporator to concentrate the extracts [12].

## 2.4. Standard Curve Calibration

Stock solutions of ascorbic acid were made of 1mg/ml concentration. Dilution of stock solution was carried out with 200µg, 400 µg, 600 µg and 800µg concentration. 2 ml of the diluted standard solution of every concentration was taken and 2 ml of 0.1mM methanolic DPPH solution was added. The mixture was stirred for 25 second and kept in dark for 30 min for the reaction to occur.

## 2.5. Method of determination of Antioxidant Activity by DPPH Assay

A modified method described by Gupta was used to evaluate the antioxidant activity of the methanol extract of the sample [13-14]. The DPPH solution changes its colour from deep violet to colourless or pale yellow when reacts with reducing agent.

In this assay, 2 ml of 0.1 mM methanolic DPPH solution was added to 2 ml of extract sample solution at different concentrations (200 µg/ml, 400 µg/ml, 600µg/ml, 800 µg/ml). Then it was treated similarly as the standard sample. A blank sample was prepared. Then the absorbance was measured after half an hour, against the blank. Absorbance was taken at 517 nm with a Double Beam Thermo Scientific UV/Visible spectrophotometer.[12]

The percentage of DPPH radical scavenging activity of each

extract was calculated as:

$$\% \text{ of inhibition} = \frac{A0 - A}{A0} \times 100$$

Where,

$A_0$ is the absorbance of the control solution (containing control solution except extract).

A is the absorbance of the DPPH solution containing extract.

The DPPH radical scavenging activity% was plotted against the extract concentration (µg/ml) to determine the concentration of extract necessary to decrease DPPH radical scavenging by 50% known as $IC_{50}$. $IC_{50}$ value of each extract was estimated by linear regression [12].

### 2.6. Statistical Analysis

All the analyses were done in triplicates. Then data were averaged and reported as Mean ± Standard Deviation (SD). Data analysis was done by SPSS Version 20.0 and Microsoft Excel 2013.

## 3. Result and Discussion

The selected ten vegetables were grouped into 2 categories: ethnic and conventional. Three ethnic and seven conventional vegetables were analyzed. They are differently known throughout the country.

Since the main mechanism of antioxidant in food is to neutralize free radical, synthetic organic solvent, methanol was used as solvent at room temperature. In this study, DPPH method, the simplest method, was used for evaluating the antioxidant potential of an extract or other biological sources. [15]. This method was developed by Blois with the viewpoint to determine the antioxidant activity in a like manner by using a stable free radical 2, 2-diphenyl-1picrylhydrazyl (DPPH; $C_{18}H_{12}N_5O_6$, M=394.33) [16].

The assay is based on the measurement of the scavenging capacity of antioxidants towards it. In DPPH assay, a hydrogen atom is received by the nitrogen odd electron from antioxidants to produce the corresponding hydrazine [16]. DPPH is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole so that the molecules do not dimerise, like most other free radicals. $IC_{50}$ value is defined as the concentration of substrate that causes 50% loss of the DPPH activity [17].

Their percent inhibitions in different concentrations (200µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml) were analyzed. According to Table 2, it can be delineated that with increasing concentration there was an increase in free radical scavenging capacity in each of the samples. At lowest concentration (200 µg/ml) greatest inhibition was observed by Ozone shakh *(Spilanthes calva)* which was 42.43% and lowest inhibition was observed by Pudina pata *(Mentha viridis)* which was about16.30%. But at maximum concentration (800 µg/ml) used, there was almost four folds increase (i.e. almost 64%) in inhibition percentage exerted by Pudina pata *(Mentha viridis)*. At highest concentration (800 µg/ml) maximum inhibition was done by Sojne pata *(Moringa oleifera)* which was almost 75% but even at that concentration Lelong *(Premnaobtiusifolia)* exerted lowest inhibition which was only about 30%.

**Table 2.** DPPH free radical scavenging activity of selected ethnic and conventional plants.

| Sample name | | % inhibition at different concentration(µg/ml) | | | |
|---|---|---|---|---|---|
| | | 200 µg/ml | 400 µg/ml | 600 µg/ml | 800 µg/ml |
| Ethnic plants | Ozone shakh *(Spilanthes calva)* | 42.43±2.16 | 44.97±1.48 | 48.18±0.39 | 51.81±0.42 |
| | Lelong*(Premnaobtiusifolia)* | 17.31±0.86 | 20.68±0.38 | 27.28±1.05 | 30.11±0.12 |
| | Maisapagong*(Eryngiumfoetidum)* | 16.40±1.87 | 23.03±0.32 | 27.50±0.43 | 36.50±1.36 |
| | Sojnepata*(Moringa oleifera)* | 35.63±1.78 | 54.15±1.45 | 69.08±0.35 | 74.88±0.11 |
| | Pudina*(Mentha viridis)* | 16.30±0.55 | 29.42±1.31 | 43.38±0.58 | 64.21±0.244 |
| Conventional plants | Dhoniya*(Centella asiatica*) | 27.72±0.36 | 29.73±1.12 | 39.60±0.41 | 54.28±2.27 |
| | Lau shakh*(Raphanus sativus* L.) | 26.11±0.68 | 29.12±0.11 | 31.34±0.17 | 52.51±0.49 |
| | Mulashakh*(Lagenaria siceraria*) | 22.28±0.13 | 25.05±2.07 | 29.68±0.12 | 31.32±0.19 |
| | Peyazkoli*(Allium cepa* ) | 27.32±0.22 | 29.07±0.12 | 43.38±0.58 | 47.99±0.82 |
| | Badhakopy*(Brassica oleracaeae*var) | 27.79±0.22 | 28.32±0.33 | 47.85±0.51 | 51.06±2.94 |

Values were calculated from the average of three replicates. Mean ± SD is reported.

$IC_{50}$ value was analyzed it is known that, lowest $IC_{50}$ value indicates the strongest antioxidant activity [1]. According to Figure 1, $IC_{50}$ value of the selected conventional and ethnic vegetables ranged from 374.8µg/ml to 2057.3 µg/ml. Sojne pata (*Moringa oleifera*) showed the highest (374.8 µg/ml) antioxidant activity whereas Mulashakh (*Raphanus sativus* L.) showed the lowest (2057.3 µg/ml) antioxidant activity.

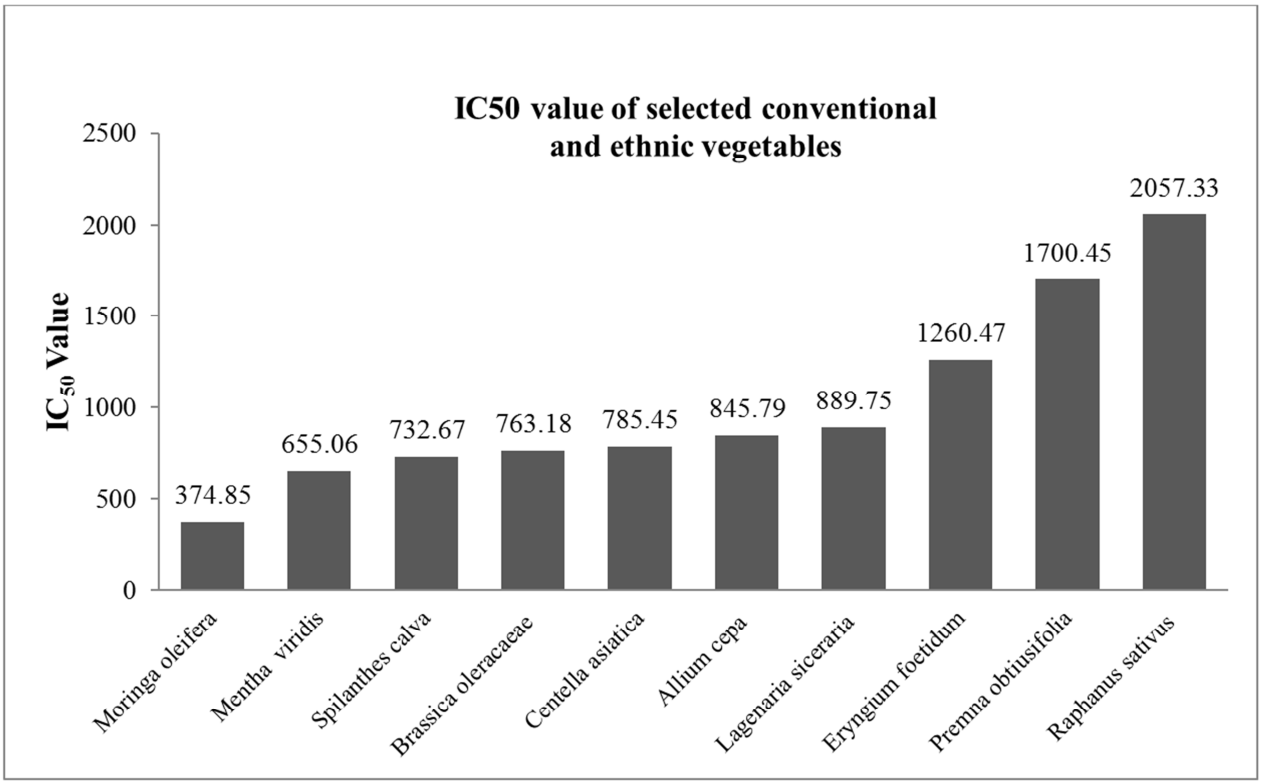


**Figure 1.** $IC_{50}$ value of selected ethnic and conventional plants.

$IC_{50}$ of the studied ethnic and conventional vegetables were in order of Sojne pata (Moringa oleifera) > Pudina pata (Mentha viridis) > Ozone shakh (Spilanthes calva) > Badhakopy (Brassica oleracaeaevar) > Dhoniya (Centella asiatica) > Peyazkoli (Allium cepa) > Lau shakh (Raphanus sativus L.) > Maisapagong (Eryngium foetidum) > Lelong (Premnaobtiusifolia) > Mulashakh (Lagenaria siceraria).

## 4. Conclusion

From the present study it can be concluded that these conventional and ethnic vegetables can be a potent source of antioxidants in the regular diet. Among the vegetables analyzed, *Moringa oleifera* exhibited the highest antioxidant activity. *Mentha viridis*, *Brassica oleracaeae, Centella asiatica*, *Lagenaria siceraria, Allium cepa*, *Spilanthes calva* can also be fair sources of antioxidant in diet. These vegetables can be used as neutraceutical and to prepare pharmacological products. Regular consumption of these vegetables may play a role in preventing oxidative stress and related diseases and aging. However, further researches on potential anti oxidative components of these vegetables, their in vivo different activity should be undertaken.

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