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TOTAL POLYPHENOLS AND DPPH FREE RADICALS SCAVENGING ACTIVITY IN SIX LEAFY VEGETABLES OF BANGLADESH

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ABSTRACT

Vegetables are the most important sources of essential bioactive compounds providing health benefits. To seek out the potential cheap sources of dietary bioactive compounds, ethanol extracts of six commonly consumed Bangladeshi leafy vegetables were screened for polyphenols and DPPH free radical scavenging activity. Among the extracts, *Lagenaria siceraria* showed the highest total polyphenol content (21.45 mg gallic acid equivalent (GAE)/g extract), followed by *Basella alba* (15.51 mg GAE/g) and *Coriander sativum* (14.37 mg GAE/g) whereas *Centella asiatica* showed the lowest polyphenol content (9.62 mg GAE/g extract), aftreward *Chenopodum album* (12.93 mg GAE/g) and *Pisum sativum* (13.17 mg GAE/g). *Pisum sativum* showed the most potent DPPH free radical scavenging activity with an IC_{50} 76.64 µg/ml subsequently *Lagenaria siceraria* 123.78 µg/ml. From the given results it can be concluded that *Pisum sativum* followed by *Lagenaria siceraria* are most potential sources of antioxidants among the six leafy vegetables.

Key words: Leafy vegetables, Total polyphenols, DPPH, Ethanol extracts.

INTRODUCTION

In Bangladesh, Vegetables are the cheapest and more available food items of daily diet. But most of the peoples are not conscious about the nutritional value of these vegetables because of the lack of research and specific data.

Polyphenols are the Secondary metabolites mainly secretes by plants known as phytochemicals may have beneficial effects on human health and provide protection against such chronic diseases as cardiovascular diseases, neurodegenerative disorders, and cancers [1-5].

DPPH is purple colored, stable free radical, reduced into the yellow-colored diphenylpicryl hydrazine. DPPH is widely used to test the ability of compounds to act as free radicals scavengers or hydrogen donors and to evaluate antioxidant activity of foods [6].

The various methods used to measure antioxidant activity of food products can give varying result depending on the specificity of the free radical being used as a reactant. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant components, but applies to the overall antioxidants capacity of the sample.

Bangladesh is bestowed with gentle climatic condition conducive for the growth of different leafy vegetables known for their nutritional values. A measure of the total polyphenols and DPPH free radical activity will help us to understand the functional properties of these vegetables. Ex:…for their nutritional value. A measure of the……..of these vegetables. So this research will be helpful to understand the nutritional value of the six leafy vegetables. The objectives of this study to determine the total phenolic content and also the DPPH free radical scavenging ability of the six leafy vegetables.

MATERIALS AND METHODS

Collection and preparation of plant materials

The leafy vegetables of *Centella asiatica*, *Chenopodum album*, *Coriander sativum*, *Pisum sativum*, *Lagenaria siceraria* and *Basella alba* were collected from

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the local market of Khulna city, Bangladesh. After washing with distilled water the collected vegetables were shed dried. The dried sample was ground to powder by grinding machine and stored in air tight containers. Ethanol was used as solvent.

Twenty five grams of powder of each sample was taken in 100 ml of ethanol and kept in airtight bottle. After 7 days, the ethanol was filtered by Whatman No. 1 filter paper. The filtrate was air dried and the solid extracts were kept in the refrigerator. For the preparation of lipophilic extract, 10 g powder was shaken vigorously by hand with 100 ml of 100% ethanol and after filtration the filtrate was air dried to obtain ethanol extract. Finally 10 mg of the solid extract was dissolved in 1ml of 100% ethanol used to conduct the experiments.

Determination of Total phenolic content (TPH)

The total concentration of phenolics (TPH) in ethanol extracts was determined (in triplicate) according to the Folin-Ciocalteu method [7] with gallic acid (GA) as the standard and expressed (mg) as gallic acid equivalents (GAE)/g of extract [8].

Determination of DPPH free radical scavenging activity

The reaction mixture (total volume, 3 ml), consisting of 0.5 ml of 0.5 M acetic acid buffer solution at pH 5.5, 1 ml of 0.2 mM DPPH in ethanol, and 1.5 ml of 50% (v/v) ethanol aqueous solution, was shaken vigorously with the ethanol extracts [9]. After incubation at room temperature for 30 min, the amount of DPPH remaining was determined by measuring the spectrophotometer with absorbance at 517 nm. Mean values were obtained from triplicate experiments.

Results are expressed as mean ± SD for a given number of observations (n = 3). The level of significance was set at *p* value of 0.05.

RESULTS

Total phenolic content (TPH)

Total Phenolic Contents (TPH) of ethanolic extract of six leafy vegetables was shown in Table 1. The TPH content was expressed as gallic acid (GA) equivalent. The TPH content ranged from 9.62 to 21.45 mg GAE/g of extract. The amount was highest in the extract of *L. siceraria* (21.45 mg GAE/g) followed *B. alba* (15.51 mg GAE/g) and C. sativum (14.37 mg GAE/g) whereas it was lowest in the extract of *C. asiatica* (9.62 mg GAE/g) followed by *C. album* (12.93 mg GAE/g) and *P. sativum* (13.17 mg GAE/g).

DPPH Free Radical Scavenging Activity

The DPPH free radical scavenging activities of six leafy vegetables are also shown in Table 1. The DPPH free radical scavenging ranged from 46.17 to 92.84 % at 200 µg/ ml of extract. At this concentration *P. sativum* showed the highest DPPH radical scavenging activity (92.84%) followed by *L. siceraria* (84.84%) and *B. alba* (71.63%). *C. album* exerted the lowest activity (46.17%).We also done the dose-dependent DPPH free radical scavenging activity of highest two value at 25, 50,100,150 and 200µg/ml concentration by the ascorbic acid (AA) standard. The dose-dependent DPPH free radical scavenging activity of *P. sativum* and *L. siceraria* are shown in Fig .1. The DPPH free radical scavenging activity was increased by the increasing of the concentration of the two vegetables. The comparison of IC_{50} values for DPPH free radical scavenging activity of the two most potent leafy vegetables are shown in Fig. 2. Here we also found the positive result. For *P. sativum* was 76.64µg/ml and for *L. siceraria* was 123.78µg/ml. The correlation between total polyphenols and DPPH free radical scavenging activity is shown in Fig. 3. The correlation co- efficient r is 0.57.

Fig 1. Dose-dependency of DPPH free radical scavenging activity of *P. sativum* and *L. siceraria* (n=3).

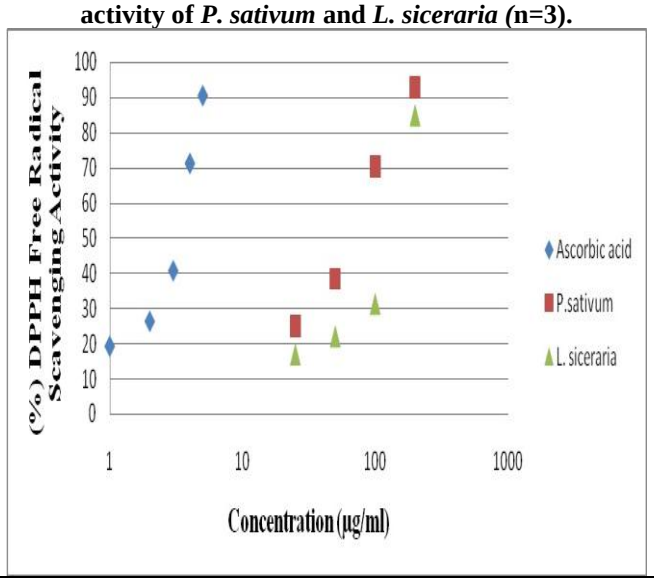

Fig 2. Comparison of IC_{50} values of *P. sativum and L. siceraria.* The lowest value shows the highest activity

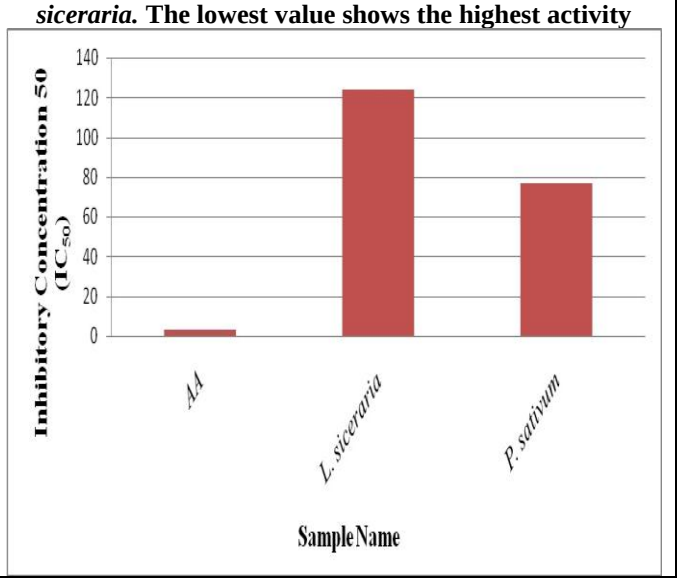

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Fig 3. Correlation between total polyphenols and DPPH free radical scavenging activity at 200 µg/ml of extracts of Leafy vegetables (r = 0.57)

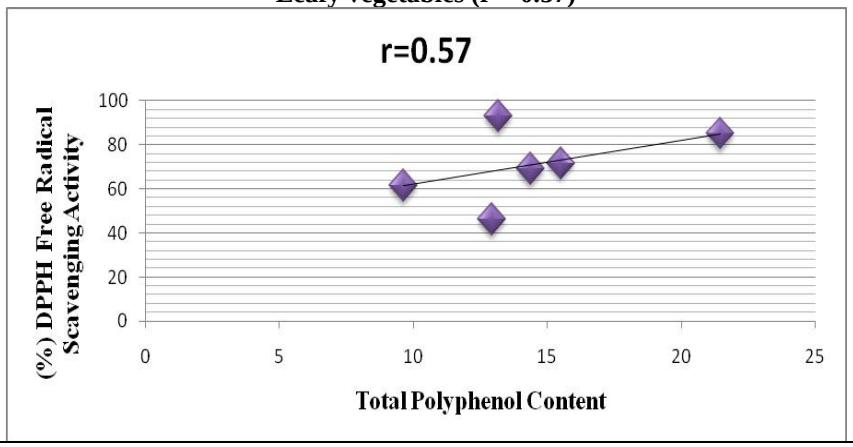

Table 1. Total phenolic content (TPH) and DPPH Free radical scavenging activity of the ethanolic extracts of six leafy vegetables .The data are presented as mean ±SD of at least three experiments (n=3)

Name of the Sample	Total phenolic content (mg GAE/g)	(%) DPPH Free Radical Scavenging Activity (200µg/ml)
B. alba	15.51 ± 0.70	71.63 ± 0.57
C. album	12.93 ± 0.58	46.17 ± 1.06
C. asiatica	9.62 ± 0.43	61.43 ± 1.13
C. sativum	14.37 ± 0.65	68.91 ± 1.65
L. siceraria	21.45 ± 0.97	84.84 ± 1.41
P. sativum	13.17 ± 0.59	92.84 ± 0.11

GAE=Gallic acid equivalent, SD=Standard deviation, n=no of experiment

DISCUSSION AND CONCLUTION

Based on my experimental results among the six leafy vegetables, *L. siceraria* showed the highest content of total polyphenols followed by *B. alba, C. sativum*, *P. sativum*, *C. album* and *C. asiatica*. Whereas the *P. sativum* can scavenge the large amount of DPPH free radical followed by *L. siceraria, B. alba, C. sativum*, *C. asiatica* ,*C. album.* From the comparison of the two tests we suggest that from among the six leafy vegetables *L. siceraria* and *P. sativum* are the most potent two leafy vegetables that are related to Hossain *et al* [8].

For the confirmation of our result we also done other related experiment of the two most potent vegetables .We also done the dose dependent test of the *P. sativum* and *L. siceraria* .The experiment was conducted at four different concentrations; 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml. The DPPH free radical scavenging activity increased due to the increased of the concentration that proved by Hossain *et al* [8]. The IC_{50} value of ethanolic extract of *P.sativum* was found as 76.64 µg/ml, while the IC_{50} value of *L. siceraria* was found as 123.78µg/ml.The low IC_{50} value of ethanolic extract of *P. sativum* is due to presence of high polyphenols in it.

Hossain *et al* [8] conducted an experiment on Bangladeshi fruits and correlation co- efficient between total polyphenol and DPPH radical scavenging activity was 0.73. In our experiment, the correlation co-efficient between total polyphenol and DPPH radical scavenging activity is 0.57. Probably unknown components rather than polyphenols present in the extracts of vegetables might contribute in part to their antioxidant activity. To take the all consideration of our result we can grade our vegetables according to the antioxidant potentiality *P. sativum*> *L. siceraria*> *B. alba*> C. *sativum*> *C. album*>*C. asiatica.*

Besides, due to complexity of oxidation-anti-oxidation process, no single testing method is capable of providing a complete anti-oxidative profile [10]. Therefore a multi-method approach is required to asses a complete anti-oxidative activity. This study was an attempt to determine the total phenolic content and DPPH free radical scavenging activity, dose-dependency of DPPH free radical scavenging activity and correlation between total polyphenol and DPPH free radical scavenging activity.

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