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COMPARATIVE EVALUATION OF ONION GERMPLASM (*Allium cepa* L.) OF BANGLADESH FOR PHYSICAL AND ANTIOXIDANT PROPERTIES

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ABSTRACT

Determination of antioxidant activity is one of the key elements for understanding the biological properties of a widely used spice onion (*A. cepa*). In contrast, physical properties help understand the textural quality of onion necessary for postharvest operations of sorting and grading in particular. Considering the physicochemical importance of onion, the present study focused on the determination of physical attributes and antioxidant content of the onion germplasm in Bangladesh using visual methods and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, respectively. The five-onion germplasms (BARI Piaz-2, BARI Piaz-3, BARI Piaz-4, BARI Piaz-5, Faridpuri Vati) collected from selected onion growers of the Department of Agricultural Extension (DAE) of the southwestern part of Bangladesh were used as treatments for physicochemical analysis at the Horticulture laboratory of the Agrotechnology Discipline, Khulna University, Khulna, Bangladesh during the period from February to August 2019. The laboratory study was laid out in Completely Randomized Design (CRD) with three replications. Most of the physical characteristics of onion were highest in germplasm-1 than those of the other germplasm tested. The results showed that the DPPH free radicals were scavenged by all onion germplasm extracts in a concentration-dependent pattern. The highest IC_{50} value (238.10 ppm) was noticed in germplasm-3 and the lowest in germplasm-5 (161.29 ppm) with no statistical difference from germplasm-4 (172.41 ppm). The lower IC_{50} value indicating that onion germplasms-5 and -4 extracts were more potent in scavenging free radicals than the other onion germplasms. Thus, the low IC_{50} value facilitated the germplasm-5 and -4 to be enriched with antioxidant compounds significantly in higher amounts than the others.

Keywords: Antioxidant, DPPH assay, IC_{50}, Onion germplasm, Physicochemical properties

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INTRODUCTION

Onion (*Allium cepa* L.) belongs to the family Alliaceae is one of the most widely used spices all over the world (Kuete, 2017). Its edible part is bulb which varies in size, shape, color, and pungency. It can be grown in any type of soil, but well-drained, fertile silty to sandy loam soils rich in humus are best for onion cultivation (Azad et al., 2018; Rashid, 2010). Information about the physical properties of onion is crucial for postharvest operations involving sorting, grading, packaging and storage (Emana et al., 2017). Naturally, onion bulb has several pigmented layers with papery scales enclosing fleshy leaf scales, intermediate parenchyma tissues and underneath epidermis (Rashid et al., 2019). Physical properties of different onion cultivars differ with their proximate composition and the effect of moisture content being the most obvious (Abhayawick et al., 2002). Kaveri and Thirupathi (2015) described that both geometrical and physical properties of the Co 4 onion varieties are the necessary parameters and required for the analysis of the behaviour of the products during processing and storage. Crop cultivars, agronomic practices and environmental factors have great effects on yield and quality of onion crops (Khan et al., 2002). All these essential appendages of onion are equally important for both the physical and chemical quality enhancement (Ng et al., 2000). Bahnasawy et al. (2004), and Vijaya and Srivastava (2006) researched on physical and mechanical properties of onion constituting weight and size of bulb, weight of dried scale, linear dimensions, shape index, surface area, friction angle, coefficient of static friction, etc. Bahnasawy et al. (2004) reveal that the equatorial and polar diameters ranged from 5.12 ± 0.33 to 6.20 ± 1.5 cm for three popular cultivars of onion viz. Additionally, they mentioned that the coefficient of friction ranged from 0.67 to 1.34 for all three cultivars. Giza 6 (white), Beheri (red) and Giza 20 (yellow) in Egypt. Vijaya and Srivastava (2006) reported that Pusa Red onion variety was larger in size than the Agrifound Dark Red and NP-53. Also, Pusa Red variety was found to be denser (270 kg/m) than the other two varieties.

Onion comprises a wide range of medicinal properties such as anti-microbial, anti-spasmodic, anti-cholesterolaemic, hypotensive, hypoglycemic, anti-asthmatic, anti-cancer, and antioxidant (Griffiths et al., 2002). Antioxidant is the type of molecule that neutralizes harmful compounds called free radicals causing damage to living cells and spoiling food materials. Antioxidant properties are part of a refined array of secondary compounds that have evolved to help scavenge free radicals and reduce the risk of cancer and cardiovascular diseases (Stajner et al., 2006, Jadid et al., 2017). Antioxidants are absorbed and metabolized in the body in several ways. The natural antioxidants in foods, fruits, vegetables, beverages, spices and supplements have received much attention for their nutritive value in recent years. Antioxidants counteract the damaging effects of reactive oxygen species (ROS) produced within the organisms and delays the oxidation reaction of an oxidizable substrate. The production of free radicals within the body due to oxidative stress is quite common that might cause different inflammatory diseases and disorders (Pham-Huy et al.,

2008; Jadid et al., 2017). At this time human body synthesized endogenous antioxidants that counteract the available free radicals. The later uric acid, ubiquinol and glutathione were added to this system. However, human diet provides endogenous antioxidants such as ascorbic acid, β- carotene and vitamin E (Kim et al., 2007). It might cause molecular damage when free radicle and antioxidant production imbalances within the cell (McCord, 2004).

Bangladesh is one of the overpopulated countries in the world and most of the people are suffering from malnutrition problems due to lack of consumption of nutritious food. The daily per capita intake of vegetables is only 62 g which is far below the minimum consumption (220 g) according to Food and Agriculture Organization of the United Nations (FAO) and per capita deficiency of vegetables is 158 g per day. However, people usually can purchase onion to serve their culinary purposes but most of them do not know that it can also supply antioxidant molecules in body to fight against free radicals. The climate of Bangladesh is suitable for onion production, but it is not cultivated as much as we need due to the dominance of cereal crops especially paddy. It is possible to motivate the farmer and consumer for expediting production and consumption of onion by conveying the information of its exclusive physicochemical properties to them. Physical and chemical properties (flavor intensity) varies with cultivars and environment (Randle and Lancaster, 2002). Small-sized bulb tends to increase flavonoids content (Williams and Grayer, 2004; Mogren et al., 2006; Okamoto et al., 2006). Researchers claimed that local cultivars of onion contained a better nutritional properties (antioxidant, volatile compounds, soluble solids, flavors, pungency, phenolic compounds etc.). So, it is high time to give attention to utilize local onion cultivars and boost up onion production that will help to fulfill spices demand as well as to supply antioxidant-rich foods to the Bangladeshi peoples. Limited number of research was done previously to address the above-mentioned properties determination. Therefore, the current study was concerned with the determination of physical characteristics and antioxidant content of the onion germplasm using DPPH assay.

MATERIALS AND METHODS

Experimental site, design, and planting materials

The experiment was carried out at the Horticulture Laboratory of the Agrotechnology Discipline, Khulna University, Khulna, Bangladesh during the period from February to August 2019. To assess the physical and antioxidant properties of onion germplasms, a laboratory experiment was laid out in Completely Randomized Design (CRD) with three replications. The five-onion germplasms (BARI Piaz-2, BARI Piaz-3, BARI Piaz-4, BARI Piaz-5, Faridpuri Vati) were collected from selected onion growers of Department of Agricultural Extension (DAE) of the southwestern part of Bangladesh which was used as the treatments of this experiment.

Determination of physical properties

The onion bulb weight was measured by an electric balance (Electric weighing machine, K Tek Analytics, India). The equatorial diameter and thickness of onion bulb were determined by an electronic digital slide caliper (Model: 14-648-17, Fisher brand, Traceable, Pittsburgh, PA). The equatorial diameter is the maximum width of the onion in a plane perpendicular to the polar diameter. By using a sharp knife the dried scale was peeled out from onion. Then the skin weight was taken by keeping it in the chamber of an electric balance (Electric balance, Swastik Systems and Services, New Delhi, India) and the reading was taken in gram (g)The percentage was determined using the following equation:

$$Edible\ or\ non-edible\ parts\ (\%) = \frac{Weight\ of\ edible\ or\ non-edible\ parts}{Weight\ of\ whole\ bulb} \times 100$$

Determination of antioxidant content

The onion samples were washed, dried and ground into fine powder and then subject to maceration process. A suitably diluted stock solutions (dry sample 0.04 g with 10 ml methanol) were spotted on pre-coated silica gel thin-layer chromatography (TLC) plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar compound of the extract. The plates were dried at room temperature and were sprayed with 0.02% 1, 1-diphenyl-2-picrylhydrazyl (DPPH) in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 minutes and the color changes (yellow on purple background) were noted. DPPH formed deep pink color when it was dissolved in ethanol. When it is sprayed on the chromatogram of the extract (Chloroform: Methanol: Water= 40:10:1), it forms pale yellow color which indicates the presence of antioxidants in the methanolic extract of five onion germplasms.

Preparation of ascorbic acid standard

At first ascorbic acid (10 mg) was dissolved in 100 mL methanol to obtain a solution of 100 ppm concentration. Solution was also made into several final concentrations of 1.57, 3.13, 6.25, 12.5, 25, 50, 100, 200, 300 and 400 ppm for further use in antioxidant activity assay.

1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH) Radical Scavenging Assay

The free radical scavenging activity of the five onion germplasm extracts was analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to Jadid et al. (2015). The DPPH solution was prepared in methanol and subsequently added to various concentrations of the onion extracts (1.57, 3.13, 6.25, 12.5, 25, 50, 100, 200, 300 and 400 ppm). The absorbance changes of each test tube were determined by UV spectrophotometer at 517 nm. Ascorbic acid was used as a positive control (standard). 10 mg of ascorbic acid (SAP Chemical) were dissolved in 100 mL

methanol (BRATACO Chemical) to obtain a solution with a concentration of 100 ppm. Solution was then prepared into several final concentrations for being used subsequently in antioxidant activity assay. These measurements were performed in blank and percentage of inhibition was calculated using the following equation:

$$Inhibition\ (\%) = \frac{Blank\ absorbance - extract\ absorbance}{Blank\ absorbance} \times 100$$

The IC_{50} values were calculated using a linear regression model (y=ax+b) and used to specify the antioxidant activity of five onion germplasms.

Statistical analysis

Recorded data from the experiment were analyzed statistically using the GLIMMIX procedure of SAS and mean separation was done with Tukey-Kramer adjustment at $p \leq 0.05$ (SAS software Version 9.4, SAS Institute Inc, Cary, NC). The graph was illustrated using SigmaPlot 10.0 (SigmaPlot®10.0, Systat Software Inc.).

RESULTS AND DISCUSSION

Physical characteristics of onion germplasm

Different germplasms caused significant variation in weight of onion bulb (Table 1). The weight of onion bulb was the highest in germplasm-1 (94.13 g), while it was the lowest in germplasm-5 (6.20 g). Also, germplasm effect was significant for the thickness of onion bulb. The germplasm-1 had the longest thickness bulb (4.55 cm) which was statistically similar to the germplasm-2. In contrast, the shortest thickness of bulb (2.55 cm) was measured from germplasm-5. Similar trend was also observed in equatorial diameter of onion bulb. The equatorial dimeter was varied from 2.05 to 6.07 cm. The highest equatorial diameter in germplasm-1 (6.07 cm) while the lowest was found in germplasm-2 (2.05 cm). Different germplasms had significant effect on the weight of edible portion. The edible portion weight was the highest with germplasm-1 (93.04 g) and the lowest weight was observed in germplasm-4 (5.49 g). In contrast, the maximum weight of non-edible portion was found in germplasm-3 (1.86 g), whereas, the minimum weight was found in germplasm-2 (0.36 g). The second-lowest non-edible portion was recorded from germplam-5 (0.68 g) followed by germplam-4 (0.96 g) and germplasm-1 (1.09 g), respectively. The percentage of edible portion was the highest in germplasm-1 (98.80%), which was statistically similar to the germplasm-2 (Figure 1), while the lowest edible portion was measured in germplasm-5 (88.56%), which did not statistically differ from germplasm -3 and germplasm-4. However, the percentage of non-edible portion was the maximum in germplasm-5 (11.43%) whereas the minimum in germplasm-2 (1.05%) with no difference from germplasm-1.

The results suggested that physical parameters varied due to germplasms differences. Azad et al. (2017) reported that onion cultivars showed different shape and size due to variability in edaphic factors of the onion growing areas as well as genenic

makeup. Similarly, Bahnasawy et al. (2004) and Vijaya and Srivastava (2006) observed that physical properties varied due to the disparity of onion cultivars. Also, they mentioned that the shape and size are so important for onion in assessing the solubility of processing as well as their retail value.

Table 1. Effect of onion germplasms on physical characteristics

Treatments	Weight of onion bulb (g)	Thickness of onion bulb (cm)	Equatorial diameter of onion bulb (cm)	Weight of edible part (g)	Weight of non-edible part (g)
Germplasm-1	94.13 a	4.55 a	6.07 a	93.04 a	1.09 b
Germplasm-2	34.43 b	4.35 a	3.80 b	33.90 b	0.36 d
Germplasm-3	22.74 c	3.65 b	3.52 b	21.04 c	1.86 a
Germplasm-4	13.95 d	3.65 b	3.50 b	12.68d	0.96 b
Germplasm-5	6.20 e	2.55 c	2.05 c	5.49e	0.68 c
Significance	**	**	**	**	**

Means followed by the same letters within column do not differ significantly whereas means having dissimilar letters differ significantly and Tukey-Kramer adjustment for multiple comparisons. ** significant at $p < 0.01$,Germplasm-1 (BARI Piaz-2), germplasm-2 (BARI Piaz-3), germplasm-3 (BARI Piaz-4), germplasm-4 (BARI Piaz-5), germplasm-5 (Faridpuri Vati).

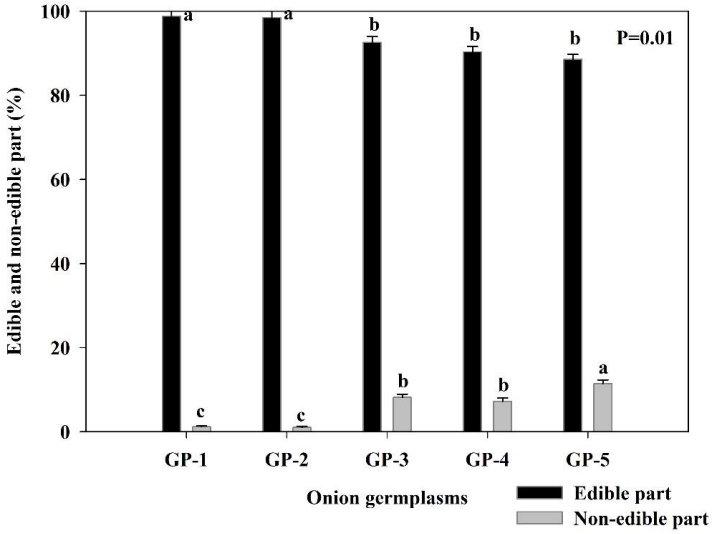

Figure 1. Effect of onion germplasms on edible and non-edible part [GP-1 (BARI Piaz-2), GP-2 (BARI Piaz-3), GP-3 (BARI Piaz-4), GP-4 (BARI Piaz-5), GP-5 (Faridpuri Vati).

Radical scavenging activity determination

The percentage of inhibition was calculated to assess the antioxidant activity of the onion germplasm extracts which can obstruct free radicles (Table 2). Nine varying concentrations (1.57, 3.13, 6.25, 12.5, 25, 50, 100, 200, 300 and 400 ppm) of different onion extracts showed various percentage of inhibition. The scavenging activity of each onion germplasm extract was increased with the rise of concentration. The 400 ppm onion extract showed the best antioxidant activity. Among the onion germplasms extract, the germplasm-5 (96.59%) was the highest inhibition followed by germplasm-4, germplasm-2, germplasm-3 and germplasm-1, respectively. At the highest concentration (400 ppm), the scavenging activity of germplasm-5 and-4 was higher than the ascorbic acid (standard).

The DPPH assay was used to assess the free radical scavenging activity of five onion germplasms extract (Figure 2). It is a rapid and efficient method to determine the free radical scavenging activity. The DPPH forms a stable diamagnetic molecule after accepting an electron or hydrogen radicle (Jadid et al., 2017). The color changes from purple to yellow specifies a reduction in absorbance of DPPH radicle. This evidences that antioxidant found in the extract interacting with the free radicals (Kedare and Singh, 2011). Levels of radical scavenging activity increase from onion core to skin (Kim and Kim, 2006).

Table 2. Effect of concentration in inhibition (%) of five onion germplasms extract

Concentration (ppm)	Inhibition (%)					
	Germplasm					Ascorbic acid
	1	2	3	4	5	
1.57	4.26 e	4.73 e	2.95 e	4.26 f	15.38 e	28.99 f
3.13	9.62 de	8.28 e	4.49 e	7.10 f	23.90 e	36.09 de
6.25	13. 37 d	9.70 e	6.153 e	17.04 f	35.97 d	42.01 d
12.5	19.10 d	23.78 d	16.92 d	20.35 f	39.40 cd	57.39 c
25	31.88 c	27.57 cd	28.04 c	32.30 e	49.11 c	83.43 b
50	39.64bc	33.60 c	34.67bc	48.52 d	54.91 c	89.70 a
100	47.53 b	40.71 c	40.47 b	58.46 c	60.59 c	91.83 a
200	54.63 b	65.05 b	48.75 b	69.98 b	78.79 b	92.96 a
400	69.45 a	82.12 a	68.82 a	95.34 a	96.59 a	94.02 a
Significance	**	**	**	**	**	**

Means followed by the same letters within column do not differ significantly whereas means having dissimilar letters differ significantly and Tukey-Kramer adjustment for multiple comparisons. ** significant at $P < 0.01$, Germplasm-1 (BARI Piaz-2), germplasm-2 (BARI Piaz-3), germplasm-3 (BARI Piaz-4), germplasm-4 (BARI Piaz-5), germplasm-5 (Faridpuri Vati).

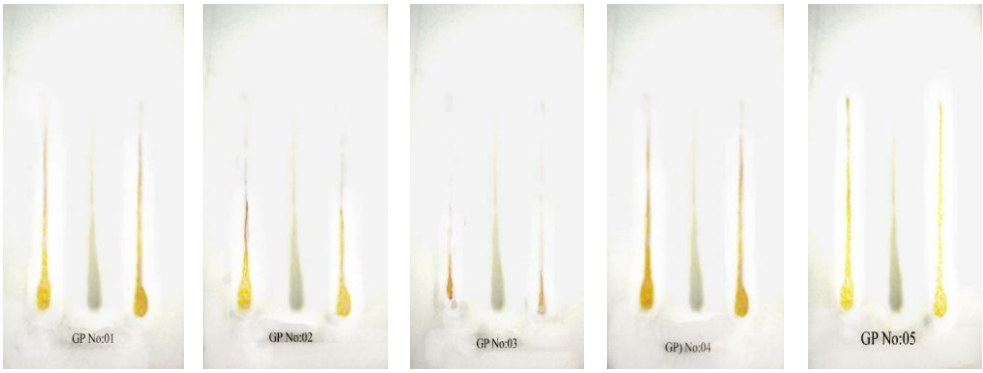

Figure 2. TLC plate showing free radical scavenging activity of five onion germplasms extract after applying DPPH.

The IC50 value of DPPH radical scavenging activity

The results showed that germplasm-5 and germplasm-4 extract exhibited the highest antioxidant activity (161.29 ppm and 172.41 ppm) than the other germplasms extract (Figure 3). The IC50 value was measured to assess the concentration of the extract required to inhibit 50% of radical. The lower the IC50 value, the higher the antioxidant activity of the extract (Li et al., 2009). Similarly, Thampi and Jeyadoss (2015) reported that green onion leaves showed more IC_{50} value than onion bulb at higher concentration (1000 ppm) which is parallel to this study. Also, Hashem et al. (2018) found IC_{50} was higher in onion than garlic (*Allium sativum*) extracts from a comparative study of commonly used spice in Bangladesh. Antioxidant contents of onion cultivars varied among the sample extracts due to several causes. Alike findings were also reported by Yang et al. (2004) where they stated that antioxidant activity depends not only on the onion cultivars but also on the processing or on the different heat treatments applied to the onion.

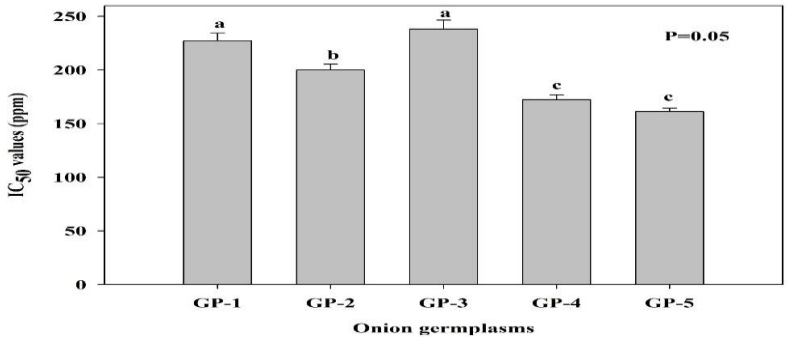

Figure 3. Effect of onion germplasms on IC_{50} value of DPPH radicle scavenging activity [GP-1 (BARI Piaz-2), GP-2 (BARI Piaz-3), GP-3 (BARI Piaz-4), GP-4 (BARI Piaz-5), GP-5 (Faridpuri Vati).

CONCLUSION

In nutshell, the criteria for the selection of the onion may be weight, length, diameter, and edible portion. Most of the physical characteristics were highest in germplasm-1. The germplasm-5 followed by germplasm-4 exhibited high antioxidant activity. They acted in a concentration-dependent manner. Peoples can consume onion germplasm-5 or germplasm-4 as a supplementary source of antioxidant. These different properties explored from the study would be directive for the spicy lovers in choosing of Bangladeshi onions to secure maximum health benefits and this finding suggests that onion germaplsm-4 and -5 intake will promote our immunity to high level against free radical-induced fatal diseases like cardiovascular complexity, cancer, neurological disorders, diabetes, atherosclerosis and inflammatory joint disease. Further study is recommended to incorporate more germplasms and to determine the quality parameters viz. total phenols, anthocyanin, flavonoid, carotenoid content.

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