PHYSICO-CHEMICAL CHARACTERISTICS OF TOMATO (*Lycopersicon esculentum* Mill.) GERMPLASM AVAILABLE IN SOUTH WESTERN REGION OF BANGLADESH

A THESIS
BY
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DECLARATION

This thesis is entirely based on the candidate's own investigation except for quotations and citations which have been duly acknowledged and no part of this paper has been accepted nor is it being previously or concurrently submitted for any degree.

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DEDICATED TO

MY BELOVED PARENTS

&

All FARMERS

OF BANGLADESH

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ABSTRACT

The research work was carried out to determine the physico-chemical characteristics of 13 selected tomato germplasm collected from the south-western region of Bangladesh during December 2012 to May 2013. The experiment was laid out in a Completely Randomized Design (CRD) with three replications. A significant variation among the germplasm in relation to fruit characteristics was observed. The highest values found in germplasm No. 6 in respect of total weight of fruit, length of fruit, width of fruit and depth of fruit among the 13 selected germplasm. The highest seed weight of fruits was observed in germplasm No. 2. The highest titrable acidity (0.953%) was observed in germplasm No. 12. The highest vitamin C (24.33 mg /100 g) content of fruit pulp was determined from germplasm No. 3 while germplasm No. 13 was best in respect of pH (4.833), germplasm No. 3 in total soluble solids (6.033%) content, germplasm No. 5 in carotenoids (0.848 mg /100 g) content, germplasm No. 2 in anthocyanin (0.125 mg/100 g) content and germplasm No. 11 in flavonoids (5.270 g/100 g) content of fruit pulp. The highest antioxidant was found in germplasm No.7 and that was lowest in germplasm No. 9.

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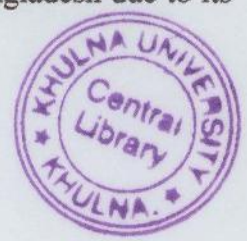
INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important edible and nutritious vegetable crops in Bangladesh. It belongs to the family Solanaceae. It is cultivated in almost all home gardens and also in the field for its adaptability to wide range of soil and climate in Bangladesh. It ranks next to potato and onion in respect of production in Bangladesh. It is widely cultivated in tropical, sub-tropical and temperate climates and thus it ranks second in terms of world vegetable production (Anonyms, 2011). The leading tomato producing countries are China, United States of America, India, Egypt, Turkey, Iran, Mexico, Brazil and Spain (Anonyms, 2011).

In Bangladesh, tomato is cultivated all over the country due to its adaptability to wide range of soil and climate (Ahamed, 1995). The cultivated area under tomato in Bangladesh is 24,696.35 hectares, total production 2,32,000 metric tons having an average yield of 9.38 tons per hectare (BBS, 2011), which is very low compared to other countries like India (19.5 t ha^{-1}), Italy (50.7 t ha^{-1}), USA (81.0 t ha^{-1}), China (48.1 t ha^{-1}), Egypt (39.5 t ha^{-1}), Iran (35.8 t ha^{-1}), Spain (74.0 t ha^{-1}), Brazil (60.7 t ha^{-1}), Mexico (30.5 t ha^{-1}) and Turkey (33.1 t ha^{-1}) (Anonyms, 2011). Tomato productions in Nigeria become more than doubled in the last ten years and the production in 2001 alone was about 879,000 metric tons (FAO, 2002).

The low yield potentiality of this crop is responsible for unavailability of quality seeds of improved varieties, improper management of fertilizers, irrigation, disease control and lack of suitable pruning practices. Tomato cultivated in all parts of Bangladesh due to its adaptability to wide range of soil and climate (Hoque *et al.,* 1999).



The best growing areas of tomato in Bangladesh are Chittagong, Comilla and Rajshahi (Sharfuddin and Siddque, 1985) and it ranks fourth in respect of production and third in respect of area (BBS, 2011). It contains a number of nutritive elements almost double compared to apple and shows superiority with regard to food values (Barman, 2007).

Food value of tomato is greatly dependent on its chemical composition such as dry matter, titrable acidity, total sugar, total soluble solids and ascorbic acid etc. Tomatoes and their products provide an essential source of vitamin C, potassium, and antioxidants (primarily lycopene). Lycopene, present at high concentrations in tomato and tomato products, has attracted considerable attention because of epidemiological evidence that suggests this compound may provide protection against cancer and other degenerative diseases (Weisburger, 2002).

Producing cultivars with big amount of lycopene has been a goal of breeders for a long time, primarily because of the increased red colour of such cultivars, but more recently because of the enhanced health benefit for humans. The quality of fresh ripe tomatoes is influenced by growing conditions and genetic factors.

The fruit ripening process results in biochemical changes that enhance fresh fruit quality, such as carotenoids accumulation and development of flavour volatiles. However, the ripening process also initiates degradative processes, such as fruit softening. The texture of the ripe fruit has significant effect on quality and influences consumer preferences, storability, shelf life, pathogen resistance and transportability. Although a clear definition of quality for agricultural products does not exist, it could be described as the group of characteristics that consumers wish to find in the product.

The processing industries demand concrete specifications from its providers which affects the demanded quality. Since most quality factors are related to physicochemical properties, it is possible to develop quality evaluation methods based on these properties, in most of the cases. Data on different tomato cultivar properties are extremely valuable to the processing industry as a tool for choosing the better cultivars to each different tomato product it intends to produce. Although, cultivar (cv.) is probably the most important factor affecting the quality of processed tomato products, other major parameters are tomato maturity and growing.

The nutritional value of tomato is due to the beneficial effects of some health promoting constituents (vitamins, fibre and carotenoids), which in general inhibit oxidative processes and in particular, help to prevent some types of cancer and cardiovascular diseases (Muratore *et al.*, 2005). Tomatoes are good dietary sources of lycopene, β-carotene, ascorbic acid and polyphenols (Nguyen and Schwartz, 1999; Ruiz *et al.*, 2001; Sahlin *et al.*, 2004) thus they could help in alleviating the solve of micro-nutrient deficiency in the world, which is put at more than two billion (Kennedy *et al.*, 2003).

Bangladesh is one of the over populated country in the world where most of the people live below the poverty line. It is difficult to fulfill their nutritional demands. To meet the nutritional demand of the people of Bangladesh, tomato crops can play a vital role as a source of vitamin C, potassium, and antioxidants (primarily lycopene) and other nutrients. As it grows easily in our country, so it is possible to take it at a cheap rate.

South western region as well as costal part of Bangladesh is poor in vegetable cultivation due to increase in salinity level. For this reasons the poor people or small farmers are suffering from malnutrition. It is essential to increase the cultivation of vegetables which are rich in nutrition such as vitamin C, carotenoid, potassium, and antioxidants (primarily lycopene) and other nutrients.

By determining the physical and chemical characteristics of tomatoes it can be possible to obtain its nutritive value and other characteristics. Such types of findings will be used to increase the production of it by inspiring the farmers and other concern people.

So, the present study was undertaken with the following objectives:

- To determine the physico-chemical characteristics of some selected tomato germplasm.
- To assess the antioxidant level of the selected tomato germplasm.

REVIEW OF LITERATURE

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important and popular vegetables in Bangladesh. It ranks third in the world's vegetable production, next to potato and sweet potato, placing itself first as processing crop among the vegetables. It is a cheap source of vitamin-C and minerals. Tomato covers about 9.80% of the area under total winter vegetables in Bangladesh and its yield was 06.98 t/ha in the country during the year 2005-06 (BBS, 2007).

Adequate supply of nutrient can increase the yield, fruit quality, fruit size, keeping quality, colour and taste of tomato (Shukla and Naik, 1993). Micro nutrients also play an important role in tomato production. Among the micro elements, boron and zinc play an important role directly and indirectly in improving the yield and quality of tomato in addition to checking various diseases and physiological disorders (Magalhaes *et al.*, 1980)

Tomato is one of the most popular vegetables in the world. In Lithuania people harvested approximately 18000 t of tomatoes every year and about 25000 t are consumed (Viskelis *et al.*, 2005). Tomatoes and their products provide an essential source of vitamin C, potassium, and antioxidants (primarily lycopene). Lycopene, present at high concentrations in tomatoes and tomato products, has attracted considerable attention because of epidemiological evidence that suggests this compound may provide protection against cancer and other degenerative diseases (Weisburger, 2002).

Producing cultivars with big amount of lycopene has been a goal of breeders for a long time, primarily because of the increased red colour of such cultivars, but more recently because of the enhanced health benefit for humans.The quality of fresh ripe tomatoes is influenced by growing conditions and genetic factors. The fruit ripening process results in biochemical changes that enhance fresh fruit quality, such as carotenoids accumulation and development of flavour volatiles. However, the ripening process also initiates degradative processes, such as fruit softening. The texture of the ripe fruit has significant effect on quality and influences consumer preferences, storability, shelf life, pathogen resistance and transportability.

During the last 50 years, the fruit of the cultivated tomato (*Lycopersicon esculentum*) has become a popular and highly consumed food (Canene-Adams *et al.*, 2005). Tomato contains several components that are beneficial to overall health, including vitamin E, trace elements, flavonoids, phytosterols, and several water- soluble vitamins (Agarwal and Rao, 1998 and Verhoeyen *et al.*, 2002). Tomato is also a rich source of folate, vitamin C, and potassium (Mancini *et al.*, 1995 and Blum *et al.*, 2005). Moreover, the antioxidant activity of lycopene as well as several other carotenoids and their abundance in tomato, makes it a rich source of antioxidants (Agarwal and Rao, 1998 and Verhoeyen *et al.*, 2002). In addition, vitamin A precursors, such as beta- and gamma-carotene are present in modest levels in tomatoes.

2.1 Morphological characteristics

Plant height

Hossain *et al.* (2010) reported that Plant height is one of the most important characteristics of tomato plant. He indicated that there were no significant leave of variation among the tomato genotypes and varieties grown at Rangpur. Considering all the varieties and genotypes at both locations BARI tomato-7 was the tallest plant; TM-13, TM-155 and BINA tomato-5 were almost similar; while TM-110 was the shortest genotype. The range of plant height was varied from 69.93 cm to 92.40 cm.

Individual fruit weight

Single fruit weight is one of the most important yield attributing characters of tomato. The results of single fruit weight showed statistically significant variation. Hossain *et al.* (2010) observed the range of single fruit weight was varied from 21.54g to 60.92g. Considering all the varieties and genotypes BARI tomato-7 gave the highest weight of single fruit; TM-13 was identical second highest value of single fruit weight; TM-155, TM-152 and BINA tomato-5 gave similar value of single fruit weight; while TM-110 gave the lowest value of single fruit weight. Ereifej *et al.* (1997) also found similar results.

Length of fruits

Hossain *et al.* (2010) observed that TM-13 was the tallest mutant. The fruit length of BARI tomato-7 was next in compare the TM-13. TM-133 and TM-152 were alike mutants. TM-105, TM-110, were almost similar. The highest value of fruit length was observed in TM-13 (5.14 cm), where as the lowest value of fruit length was observed in TM-110 (3.35 cm). Similar results were also found by Rathore (1992).

Breadth of fruits

Hossain *et al.* (2010) fruit breadth of BARI tomato-7 was superior among varieties/mutants. TM-13, TM-133, TM-155, TM-152, TM-110, TM-105, were almost similar mutants. The highest value of fruit breadth was observed in BARI tomato-7 (4.02 cm), where as the lowest value of fruit breadth was observed in TM-105 (3.35 cm), respectively.

Moisture content

Hossain *et al.* (2010) stated that the highest moisture content was observed in TM-105 (89.02%). TM-110 has the second highest value of moisture content and it was quite similar to TM- 105; while TM-13, TM-133, TM-152, TM-155 and BINA tomato-5 had the similar value of moisture content. The lowest moisture content was found in BARI tomato-7 (82.46%) and (81.30%). Similar result was also reported by Rathore (1992).

Dry matter content

Hossain *et al.* (2010) reported that BARI tomato-7 has the highest dry matter content (17.54%), while TM-13, TM-133, TM-152, TM-155 and BINA tomato-5 had the similar value of dry matter content; TM-105 has the lowest value of dry matter content (10.60%). Davis and Hobson (1981) also found the less variation in dry matter content among the different variety.

2.2 Chemical characteristics

Vitamin C content

According to Hossain *et al.* (2010) vitamin C content showed statistically significant variation among the genotypes and varieties and the range of vitamin C content varied from 17.32 mg 100^{-1}mg to 26.59 mg 100^{-1}mg. similar result was also founded by Kallo (1985).

Duttaroy (2011) reported that raw tomatoes content 12.7 mg vitamin C, tomato juice content 18.3 mg and tomato soup content 27.3 mg vitamin C. According to data of USDA SR-21 tomato content 22.9 mg *vitamin* C. From USDA National Nutrient data base tomato content 13 mg /100 g vitamin C.

Purkayastha, and Mahanta (2010) the Solam Garima cultivar of Meghalaya had the highest vitamin C content of 21.54 mg/100 g, followed by Pau-2374 (19.375 mg/100 g) which in-turn closely followed by Sel-3 having a value of 18.532 mg/100 g. The Sel-2 cultivar had the least, with a value of 12.688 mg/100 g. They also found that Vitamin C is a very heat-labile component and its retention is affected by thermal processing. So, the decreasing trend of ascorbic acid content in blanched and dehydrated tomatoes is ascribed to heat labile nature of the vitamin.

pH

According to Hossain *et al.* (2010) considering all the varieties and genotypes TM-133(pH 4.01) gave the highest value of pH. TM-152(pH 3.86) and TM-155 (pH 3.74) gave similar pH value they were chronologically next to TM-133; while TM-105 (pH 3.72) gave the lowest pH value. Saimbhi *et al* (1989) reported similar results.

The brine pH reduced from between 6.44 and 7.90 before processing to a range of 4.80 to 5.04 in the canned product on evaluation after the 60 days storage (Makanjuola *et al.*, 2012). As regards pH of tomato paste held at different storage temperatures, a decreasing trend was observed during the storage period. The overall decrease in pH was maximum (4.63%) and minimum (2.19%) in samples stored at 25 C and at -10 C respectively. The temperature influences the decrease in pH. Change in pH is directly related to change in acidity of samples (Safdar *et al.*, 2010). Similar findings were reported by Ahmad (1997) during his study on tomato concentrate.

Titratable acidity

The range of titratable acidity content varied from 0.256% to 0.353 % (Hossain *et.al.* 2010). High acid values are required for best flavor and it is mainly because of the presence of citric and malic acids (Adedeji *et al., 2006*). Ereifej *et al.* (1997) also reported 0.1 to 0.5 percent titrable acidity in tomatoes.

According to Guold and Berry (1972), a tomato variety for processing should have acid contents ranging from 0.35to 0.55%. Acids present in foods not only improve the palatability of many fruit products but also influence their nutritive value by playing a significant role in the maintenance of acid-base balance in the body.

The acids influence the flavor, brightness of color, stability, consistency and keeping quality of the product. In addition, acidity of the tomato juice greatly influences the processing time and temperature of the product (Adedeji *et al.*, 2006).

Purkayastha and Mahanta (2010) found that titratable acidity (% citric acid) of five tomato cultivars was Solam Garima (0.8216 ± 1.61%), VR-415 (0.4536 ± 2.01%), Pau-2374 (0.6192 ± 0.83%), Sel-2 (0.3867 ± 1.01%) and Sel-3 (0.3560 ± 0.09%).

The present data indicates that, the Solam Garima and Pau-2374cultivars as having higher acid values than VR-415, Sel-2 and Sel-3cultivars, which implies better flavour.

Moneruzzaman *et al.* (2008) stated that the half ripens tomato pulp contained highest quantity of total titrable acidity (0.48%) followed by full ripe (0.47%) and mature green tomatoes (0.44%) at 9th day of observation. It was found that the titrable acidity content of tomato juice was increased with the advancement of ripening of fruit and reached at peak stage on 9th day and there after again started to decrease. Similar result was also found by Sinaga (1986) and Siddiqui *et al.* (1986).

Total soluble solids

The value of total soluble solids content varied from 4.79% to 6.02%. (Hossain *et al., 2010)*. Similar result was also found by Rathore (1992). Saimbhi *et al.* (1989) showed that, the ratio of TSS/acid determines the taste and flavour of the varieties. Low TSS/acid

ratio makes the variety acidic and unsuitable for table purpose. Sel-3 has the highest TSS/acid ratio of 12:360 and Solam Garima had the lowest ratio.

The soluble solids content is one of the most important quality parameters in processing tomato. Fifty to Sixty five percent of soluble solids contents are sugars, glucose and fructose and their amount and proportion influences the organoleptic quality of tomatoes (Adedeji *et al.,* 2006). The TSS content is important criterion in determining the suitability of varieties for processing. High TSS is desirable to yield higher recovery of processed products. Guold and Berry (1972) worked on tomatoes for canning and formulated that for varieties suitable for processing, TSS content should be more than 5%.

Carotenoid content

Bertin and others also investigated tomato quality changes during ripening process and reported negative correlations between β-carotene and lycopene contents and fruit firmness (Bertin *et al.,* 2001). Viskelis *et al.* (2005) observed that the highest content of β-carotene was found in red-ripe fruits of cv. 'Rutuliai' (1.22 mg 100 g^{-1}). The content of β-carotene in red-ripe tomatoes of 'Admiro' F1and 'Kassa' F1was 0.89 and 0.83 mg 100 g^{-1}, respectively.

Reducing and non-reducing sugar content

According to Hossain *et al.* (2010) found that considering all the genotypes and varieties TM-133 have the highest reducing sugar content. BINA tomato-5 and TM-13 gave same results; TM-105, TM-110 and TM-152 were similar. While TM-152 has the lowest reducing sugar content. The range of reducing sugar content varied from 2.36% to 2.74%. Similar trend of reducing sugar content was also reported by Kallo (1985).

Hossain *et al.* (2010) stated that considering all the genotypes and varieties TM-133 has the highest non reducing sugar content. BARI tomato-7, BINA tomato-5, TM-13, TM-152 were chronologically next to TM-133; TM-110 and TM-155 were similar. While

TM-105 has the lowest non reducing sugar content. The range of non reducing content varied from 0.95 % to 1.19 %.

Nitrogen, phosphorus and potassium content

Hossain *et al.* (2010) stated that TM-133 has the highest nitrogen content at the both locations. TM-13, TM-105, TM-152, BARI tomato-7, TM-110 and BINA tomato-5 were chronologically next to TM-133. While TM-155 has the lowest nitrogen content. The value of nitrogen content ranged from 0.49 % to 0.70 %. Considering all the genotypes and varieties TM-133 has the highest phosphorus content at the both locations. BINA tomato-5 was next to TM-133; BARI tomato-7 and TM-155 were similar; while TM-110 has the lowest phosphorus content. The value of phosphorus content varied from 0.48 % to 0.66 %.

Kallo (1985) found that tomato fruits contained 0.2-0.8 g 100^{-1}g of dry fruit weight. Kallo (1985) found that tomato fruits contained (0.4-0.8) g 100^{-1}g of dry fruit weight. Davis and Hobson (1981) observed almost the similar result. Considering all the genotypes and varieties TM-133 has the highest potassium content at the both locations. While TM- 110 has the lowest potassium content. The value of potassium content varied from 0.36 % to 0.63 %.

MATERIALS AND METHODS

The experiment on physico-chemical characteristics of tomato was carried out during the period from December 2012 to May 2013. In this study, 13 germplasm were examined which were collected randomly from the south western region of Bangladesh. The collected fruits were brought to the Molecular Horticulture Laboratory of the Agrotechnology Discipline of Khulna University, Khulna. In the laboratory the fruits were studied to determine their physico-chemical characteristics.

3.1 Experimental design

The experiment was laid out in a Completely Randomized Design (CRD) with three replications. After collection of tomato they kept in ambient temperature for the study of physico-chemical characteristics.

3.2 Experimental materials

Mature green tomato of thirteen germplasm was selected as the experimental materials for the investigation. These fruits were collected from different places of south western region of Bangladesh. The list of tomato germplasm with their specific collection area has been presented in Appendix **IV**.

3.3 Methods of studying parameters

By using the following methods morphological and chemical parameters of the collected tomato germplasm were studied.

3.4.1 Morphological Parameter

3.4.1.1 Weight of tomato fruits

The weight of tomato fruit was measured by an electric balance. At first, the balance was adjusted to zero mark. The tomatoes were cleaned and weighted by keeping the tomato on the chamber of the balance. Then the reading was taken in gram (g).

3.4.1.2 Size of tomato fruits

Length, width and depth of the tomato fruit were estimated to determine the size of tomato by slide callipers. The values of these parameters were taken in centimetre (cm).

3.4.1.3 Seed weight of tomato fruits

The seed weight was measured by an electric balance. At first, the balance was adjusted to zero mark. The seed were cleaned and weighted by keeping the seed on the chamber of the balance. Then the reading was taken in gram (g).

3.4.1.4 Skin colour of tomato fruits

The tomato was clean with tap water and it was dried up with tissue paper. Skin colours were identified by visual observation comparing with colour chart.

3.4.2 Chemical characteristics

The methods for the estimation of pH, TSS, titrable acidity, vitamin C, carotenoids, anthocyanin, flavonoids and antioxidant of tomato pulp were followed as described by Mazumdar and Majumdar (2001) and Saini *et al.* (2006). The data were analyzed by fresh weight basis

3.4.2.1 Determination of pH of tomato pulp

The pH of tomato pulp was measured by a desktop pH meter (Model: HANNA instrument pH 211, Microprocessor pH meter).

Preparation of standard buffer solution

pH 7 and pH 4 buffer tablet were dissolved in water and made up the mark of 100 ml with distilled water.

Extraction of tomato juice

For the determination of pulp pH, 5 g of fresh pulp was taken in a conical flask with 10 ml of distilled water. Then the pulp was crashed by using blender and extract was filtrated through two layer of cloth.

Procedure

The standard buffer solution of pH 7 was taken into a clean and dry beaker. The electrode assembly of the pH meter was dipped into the solution. The temperature correction knob was set to 28°C and the fine adjustment was made by asymmetry potential knob to pH 7. After washing with distilled water the electrode assembly was dipped into a solution of standard pH 4 and adjusted to the required pH by fine asymmetry potential knob. The electrode assembly was raised, washed twice with distilled water then rinsed with tomato juice and finally it was dipped into the juice of tomato. pH was recorded from the meter.

3.4.2.2 Determination of total soluble solids (TSS)

Equipment

Hand refractometer

Procedure

A drop of juice from the pulp of tomato was placed on the prism of the refractometer and percentage of total soluble solid was obtained from direct reading. Temperature correction was made as described by Ranaganna (1979).

3.4.2.3 Determination of titratable acidity in tomato pulp

Reagents:

A. Sodium hydroxide (NaOH): 0.1 normality (N).

B. Indicator: Phenolphthalein ($C_{20}H_{14}O_4$) [approx. 0.5 % in 80% ethanol (W/V)].

Equipment:

Ten millilitre pipette, 25 ml burette, small glass funnel, dropper, blender, 250 ml measuring cylinder, burette stand and beaker.

Procedure:

- 30 g of tissue sample by weight was taken. This was extracted with distilled water by thorough crushing in blender. Extract was filtered and the filtrate was made upto 100 ml with distilled water.
- 15 ml of aliquot was taken into a conical flask. Three to four drops of phenolphthalein solution were added to this aliquot and shaken well.
- A burette was filled with the sodium hydroxide solution after washing and rinsing.
- Titration was done and end point was determined by the appearance of pink colour and its persistence for at least 10 to 15 seconds.

Calculation

In term of citric acid 1 ml of 0.1N NaOH solution can neutralize 0.064 g of citric acid. Therefore percentage of titratable acidity in the sample as equivalent of citric acid

$$\% \text{ Titratable acidity} = \frac{d \times 0.064 \times c}{a \times b} \quad X\ 100$$

Where,

a = Weight of sample.

b = Volume of aliquot taken for examination.

c = Volume made with distilled water.

d = Average burette reading for sample.

3.4.2.4 Determination of vitamin C (ascorbic acid) content

Apparatus: Volumetric flask 250 ml, conical flask 250 ml, beaker 150 ml, blender, burette 250 ml and pipette.

Reagents used for the estimation of vitamin C content

6% meta phosphoric acid (HPO_3): It was prepared by dissolving exactly 60 g of meta phosphoric acid in 160 ml of acetic acid and 500 ml of distilled water was added to it. For proper mixing the solution with distilled water hot plate and magnetic stirrer was used. Finally the volume was made to 1000 ml. Finally the solution was filtered and stored.

3% meta phosphoric acid (HPO_3): It was made by dissolving exactly 30 gms of meta phosphoric acid in 80 ml of acetic acid and 500 ml of distilled water was added to it. For proper mixing the solution with distilled water hot plate and magnetic stirrer was used. Then the volume was made to 1000 ml. Finally the solution was filtered and then stored it in a well labelled bottle.

Preparation of dye solution: Accurately 50 mg of 2,6- dichloro-phenol indophenol dye and 42 mg of sodium bicarbonate was weighed on a chemical balance. Both the chemicals were dissolved in 150 ml of distilled water. The solution was heated gently on a water bath to make it homogenous and raise the volume to 200 ml after cooling at room temperature. Then the filtrates were transferred to an air tight container and placed it in a refrigerator.

Preparation of standard ascorbic acid solution: 100 mg of ascorbic acid was weighed on a chemical balance and dissolved in 3% meta phosphoric acid and the volume was made to 500 ml.

Standardization of dye solution: 5 ml of standard ascorbic acid solution was taken in a clean beaker to which 5 ml of 3% meta phosphoric acid solution was added with the help of a pipette. This solution was titrated against the dye till a faint pink colour was obtained which persisted for more than 15 seconds.

Estimation of ascorbic acid

30 g of fruit flesh was weighed and blended it with equal weight of 6% meta phosphoric acid for 3 to 4 minutes. 15 g of this slurry was used in a 100 ml volumetric flask and volume was made by adding 3% meta phosphoric acid. This juice was filtrated through a fast filter paper (Whatman No. 42).

Titration

The burette was filled with standardized 2, 6-dichlorophenol, indophenols dye reagent.10 cc of filtered solution was taken in a conical flask and titrated immediately against standard dye solution, till faint pink colour was observed which persisted for not less than 15 seconds.

Calculation:

Ascorbic acid (mg per 100 g) sample = V x T x 100 /W

Where,

V= Volume of dye used in titration against an aliquant diluted sample.

T= Value of standardized dye.

W= weight of pulp.

3.4.2.5 Determination of carotenoids of tomato pulp

Materials

Acetone AR grade 80%

Procedure

1. 1 g of finely cut tomato sample was weighed by an electric balance.
2. The tomato sample was mixed well into a clean mortar.
3. The tissue sample was grinded to a fine pulp with addition of 20 ml of 80 % acetone.
4. It was centrifuged at 5000 rpm for 5 minutes and transferred the supernant to a 100ml of volumetric flask.
5. The residue was grinded with 20ml of 80% acetone, centrifuged and transferred the supernant to the same flask.

6. The procedure was repeated until the residue was colourless. The pestle and mortar were washed thoroughly with 80 % acetone and the clear washing was collected in the flask.

7. The volume was made to 100 ml with 80% acetone.

8. The absorbance was read at 510 nm and 480 nm against blank.

Calculations

$$mg\ carotenoids\ /\ g\ tissue = 7.6(A.480) - 1.49(A.510) \times \frac{V}{1000 \times 10}$$

Where,

A= Absorbance of the specific wave length.

V=Final Volume of the carotenoids in 80% acetone

W= Fresh weight of the tissue extracted

3.4.2.6 Determination of anthocyanin of tomato pulp

Reagents

(A) Ethanol: 95%

(B) Hydrochloric acid (HCl): 1.5 N.

(C) Ethanol -hydrochloric acid mixture: It was prepared by mixing 85 parts of reagent A and 15 parts of Regent B by volume.

Procedure

1. 5 g of tissue sample by weight was taken. This was extracted with ethanol hydrochloric acid mixture. It was allowed to stand overnight at a temperature of about 4°C.

2. After extraction, the mixture was filtered through filter paper (Whatman No.1). The extraction was repeated for maximum recovery of the pigment.

3. The extract was then made up to 15 ml with the solvent.

4. 15 ml of aliquot from the above extract was taken. It was filtered through fine (milli-pore) filter paper.

5. The filtered aliquot was maintained in darkness for about 2 hours with cover of the container.

6. This was then made up to 25 ml with the solvent (for colour measurement). The optical density (O.D.) value of the solution was then measured through 535nm wavelength in a colorimeter against blank.

Calculation

$$Total\ Asbsorbance\ value\ of\ the\ sample\ (per\ 100g) = \frac{e \times b \times c}{d \times a} \times 100$$

Where,

a = Weight of sample

b = Volume made for colour measurement

c = Total volume made

d = Volume of aliquot taken for estimation

e = Volume for 535 nm

Anthocyanine (mg/100g) = Total absorbance/98.2

3.4.2.7 Determination of flavonoids of tomato pulp

Reagent

(A) 80% methanol.

Procedure

1. 10 g of sample was taken and crushed finely.
2. 100 ml of 80% methanol was added and kept it in a water bath for 10 hours at 40^0C.
3. The whole solution was filtered through a filter paper.
4. The filtrate was transferred to a crucible and then evaporated to dryness over a water bath at room temperature.
5. The final finding was weighed as flavonoids.

3.4.2.8 Determination of antioxidant of tomato pulp

3.4.2.9 A Qualitative assay

A suitably diluted (methanol used as solvent) stock solutions were spotted on pre-coated silica gel 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-picryl hydrazyl (DPPH) in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 minutes and the colour changes (yellow on purple background) were noted. DPPH formed deep pink colour when it was dissolved in ethanol. When it is sprayed on the chromatogram of the extract, it forms pale yellow colour which indicates the presence of antioxidants.

3.4.2.9 B Thin layer chromatography (TLC) technique

Apparatus used

- TLC plates containing fluorescent material.
- TLC jar.
- Fine capillary tube.
- Spirit lamp.
- Spray gun.
- Hot plate.
- Filter paper.

Reagents used

- DPPH (0.02 % w/v) solution in ethanol
- Distilled water.
- Methanol.
- Chloroform.
- Ascorbic acid (as standard substance)

Solvent system used

Groups	Solvent system	Ratio
Polar	$CHCl_3$: CH_3OH:H_2O	40:10:1

Methods

TLC plate preparation

Commercially available TLC plate is cut into appropriate size of 10 cm long and 7 cm wide.

Sample application

- A fine capillary tube was used as spotter for sample application in the TLC plates.
- A very little amount of plant extract was taken in a small vial and diluted suitably with methanol.
- The sample was spotted in uniform size (about 0.3 cm) on TLC plates.
- The sample was applied several times in each spot to get better chromatogram.
- Each spot was dried before applying another volume of solution to the same spot.
- Solvent front was scratched by a knife.
- Two plates were spotted in such a manner.

Development of chromatogram

- The chromatogram was developed by ascending technique.
- These plates were then kept in a jar containing a solvent system of chloroform and methanol and water (40:10:1).
- A filter paper was kept into the jar by wetting with solvent system to keep jar saturated and the jar was closed tightly.
- The plates were placed in the jar in such a manner that the sample spots were just above the solvent surface.
- The solvent were allowed to move up the plates by capillary action until they have traveled a distance of about 7-8 cm from the point of application of the sample on a 10 cm plate.
- The plates were then removed from the jar and dried with a current of air suitably.

Antioxidant positive components detection

- Two plates from polar solvent systems were observed visually under UV light and various regions were marked.
- After these, plates ware sprayed with 0.02% DPPH.

Observation

After applying DPPH on the TLC plate, yellow color on purple background was observed which indicated the presence of antioxidant components in the methanolic extract of thirteen tomato germplasm.

(Chloroform: Methanol: Water= 40:10:1)

Quantitative analysis:

Radical-scavenging activity of antioxidants

Apparatus used

- Test tubes
- Beakers
- Magnetic stirrer
- Thermometer
- UV spectrophotometer (double beam)
- Electric balance

Reagents used

- Methanol
- 0.004% DPPH methanolic solution
- Ascorbic acid (as positive control)

Method

- At first 10 test tubes were taken to make aliquots of 9 conc. (1.57, 3.13, 6.25, 12. 5, 25, 50, 100, 200 and 400) µg/ml for tomato extract & 7 test tubes were taken to make aliquots of 7 conc.(1.57, 3.13, 6.25,12.5, 25, 50,100) µg/ml for ascorbic acid.
- Tomato extract and ascorbic acid were weighed 3 times 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 magnetic stirrer was used.
- After making the desired concentrations, 6 ml of 0.004% DPPH solution was applied on each test tube by pipette and then 2 ml of different concentrations was mixed in each test tube.
- Test tubes were kept for 30 minutes in dark to complete the reactions.
- DPPH was also applied on the blank test tubes at the same time where only 2 ml 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.

- IC_{50} was determined from % inhibition vs. concentration graph.

3.10 Statistical analysis

The collected data from experiment were statistically analyzed by Analysis of Variance (ANOVA). Duncan's New Multiple Range Test (DMRT) was used to compare the means of different parameters and the means were calculated by using "MSTATC" programme in computer.

RESULTS AND DISCUSSION

The results of the study on physico-chemical characteristics of tomato germplasm are presented and discussed in this part. The data have been presented in the tables (1 to 2). A summary of the analysis of variance in respect of all the parameters have been shown in appendices (I to II).

4.1 Physico-chemical characteristics of tomato germplasm

In this experiment 13 tomato germplasm were studied to determine their morphological and chemical characteristics. The results of this experiment are presented and discussed under the following headings.

4.1.1 Morphological characteristics of tomato germplasm

Data on morphological characteristics of tomato germplasm are presented in Table 1. The morphological characteristics of tomato germplasm are described based on quantitative characteristics in this study.

4.1.1.1 Weight of individual tomato

The weight of tomato was significantly varied among the 13 germplasm (Appendix I). The germplasm No. 6 gave the maximum weight (262.017 g) which was followed by germplasm No. 5 (104.470 g) and No. 8 (131.677 g) while it was minimum in germplasm No.11 (40.940 g) preceded by germplasm No. 12 (47.937 g) and No. 13 (59.237 g) (Table 1). Average fruit weight of individual tomato was found 105.958g. The present result fairly agrees with the report of Hossain *et al.* (2010) and Ereifej *et al.* (1997).They observed that the range of single fruit weight was varied from 21.54g to 60.92g.

4.1.1.2 Length of tomato

Significant difference was found among the 13 germplasm in respect of length of tomato (Appendix I). The longest tomato fruit (7.047 cm) was found in germplasm No. 6 which was statistically similar to germplasm No. 5 (6.386 cm). The shortest tomato fruit (4.625cm) was measured from germplasm No. 2 preceded by germplasm No. 13 (4.681 cm)

Table 1. Morphological characteristics of tomato germplasm

Germplasm No.	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Fruit depth (cm)	Seed weight (g)	Skin colour
GP No.1, BARI Tomato-1(Manik)	68.637ef	5.522bcd	4.553ef	4.863efg	3.033h	Red
GP No.2, BARI Tomato-7 (Apurba)	103.773cd	4.625d	5.737bc	6.275bcd	6.857a	Orange
GP No.3, BARI Tomato-2 (Ratan)	117.617bcd	5.196cd	6.106bc	6.527b	4.447c	Red
GP No.4, BARI Tomato-14	99.797d	5.595bcd	5.324cde	5.520def	5.280b	Red
GP No.5, BARI Tomato-9 (Lalima)	140.470b	6.386ab	5.857bc	5.922bcd	2.850i	Light red
GP No.6, BARI Tomato-8 (Shila)	262.017a	7.047a	7.564a	7.479 a	3.180gh	Light red
GP No.7, Local Tomato-1 (Mintu super)	89.400dc	4.722d	5.512cd	5.629cde	3.787f	Orange
GP No.8, BARI Hybrid Tomato-6	131.677bc	5.582bcd	6.398b	6.455bc	4.410cd	Red
GP No.9, Local Tomato-2 (Sathi)	109.680cd	5.168cd	5.869bc	5.935bcd	4.280de	Orange
GP No.10, BARI Tomato-12 (Sidur)	106.270cd	5.472bcd	5.729bc	5.908bcd	3.200g	Bright red
GP No.11, BARI Tomato-11 (Jhumka)	40.940f	5.760bc	3.343g	3.287h	3.120gh	Red
GP No.12, BARI Hybrid Tomato-3(summer)	47.937f	5.466bcd	3.916fg	4.052gh	3.750f	Red
GP No.13, BARI Tomato-3	59.237f	4.681d	4.665def	4.736fg	4.247e	Red
Average	105.958	5.478	5.429	5.584	4.034	
Level of significance	**	**	**	**	**	
C.V %	12.07	7.90	7.15	6.55	1.83	

Note: In a column, figures having similar letters do not differ significantly whereas figures having dissimilar letters differ significantly as per DMRT.

and No.7 (4.722 cm) (Table 1). The average length of tomato fruit was found 5.478 cm. Present findings agreed with the findings of Hossain *et al.* (2010), they reported that the highest of length of tomato fruit was observed in TM-13 (5.14 cm), where as the lowest was observed in TM-110 (3.35 cm). Similar results were also reported by Rathore (1992).

4.1.1.3 Width of tomato

Significant variation was found in width of tomato among the 13 germplasm (Appendix I). The broadest tomato fruit (7.564 cm) was found in germplasm No. 6 followed by germplasm No. 8 (6.398 cm) and the narrowest fruit (3.343 cm) was measured from germplasm No. 11 which was statistically similar to germplasm No. 12 (3.916 cm) (Table 1). The average width of tomato was found 5.429 cm fruit.

The present result is in agreement with the findings of Hossain *et al.* (2010). They mentioned that the width of tomato was about 3.35-4.02 cm.

4.1.1.4 Depth of tomato

The depth of the tomato fruit was significantly varied among the 13 germplasm (Appendix I). The germplasm No. 6 gave the maximum depth (7.479 cm) followed by germplasm No. 3 (6.527 cm) while the depth was minimum in germplasm No. 11 (3.287 cm) which was statistically similar to germplasm No. 12 (4.052 cm) (Table 1). Average depth of tomato fruit was found 5.584 cm.

4.1.1.5 Seed weight of tomato

Significant difference was found among the 13 germplasm in respect of seed weight of tomato (Appendix I). The highest seed weight (6.857 g) was obtained from germplasm No. 2 which was followed by germplasm No. 4 (5.280 g) and No. 3 (4.447 g), while the lowest seed weight (2.850 g) was found in germplasm No. 5 preceded by germplasm No. 1 (3.033 g), No. 11 (3.120 g) and No. 6 (3.180 g) (Table 1). The average seed weight was found 4.034 g.

4.1.1.6 Skin colour of tomato

Similar skin colours were observed among the 13 germplasm comparing with colour chart. Skin colours of tomato were Red (GP No. 1, 3, 4, 8, 11, 12, 13), Bright red (GP No. 10), Light red (GP No. 5, 6) and Orange (GP No. 2, 7, 9).

Table 2. Chemical characteristics of tomato germplasm

Germplasm No.	pH of fruit pulp	Total soluble solid (%)	T-acidity (%)	Vitamin C (mg/100g)	Carotenoids (mg/100g)	Anthocyanin (mg/100g)	Flavonoids (g\100g)
GP No.1, BARI Tomato-1(Manik)	4.297cd	5.300b	0.804b	15.500e	0.300e	0.031e	1.883h
GP No.2, BARI Tomato-7 (Apurba)	3.873e	6.000a	0.734bc	13.600f	0.262f	0.125a	2.769d
GP No.3, BARI Tomato-2 (Ratan)	4.370c	6.033a	0.612d	24.333a	0.176k	0.031e	2.783d
GP No.4, BARI Tomato-14	4.117d	5.367ab	0.662cd	18.167cd	0.250g	0.003i	2.337ef
GP No.5, BARI Tomato-9 (Lalima)	3.550f	5.367ab	0.218e	10.500g	0.848a	0.034d	1.720i
GP No.6, BARI Tomato-8 (Shila)	3.870e	5.233b	0.254e	18.833c	0.451c	0.035cd	2.753d
GP No.7, Local Tomato-1 (Mintu super)	4.750ab	5.200b	0.671cd	8.167h	0.228j	0.006h	3.193c
GP No.8, BARI Hybrid Tomato-6	4.613b	5.100b	0.748b	16.167e	0.127m	0.016f	2.260f
GP No.9, Local Tomato-2 (Sathi)	4.297cd	5.133b	0.605d	17.500d	0.533b	0.014g	2.453e
GP No.10, BARI Tomato-12 (Sidur)	3.723ef	5.233b	0.666cd	15.167e	0.144 l	0.015fg	2.035g
GP No.11, BARI Tomato-11 (Jhumka)	3.617f	5.567ab	0.668cd	16.167e	0.244h	0.038c	5.270a
GP No.12, BARI Hybrid Tomato-3(summer)	3.883e	5.100b	0.953a	18.167cd	0.355d	0.085b	2.467e
GP No.13, BARI Tomato-3	4.833a	5.233b	0.930a	22.167b	0.240i	0.036cd	3.843b
Average	4.138	5.374	0.656	16.495	0.320	0.036	2.751
Level of significance	**	**	**	**	**	**	**
C.V %	2.11%	5.13%	3.91%	3.23%	5.85%	18.13%	2.28%

Note: In a column figures having similar letters do not differ significantly whereas figures having dissimilar letter differ significantly as per DMRT.

4.1.2.3 Titratable acidity of tomato pulp

The titratable acidity showed significant variation among the 13 germplasm (Appendix II). The highest percentage of titratable acid content (0.953%) was found in germplasm No. 12 followed by germplasm No. 13 (0.930%). The lowest percentage of titratable acid content (0.218%) was recorded from germplasm No. 5 which was statistically similar to germplasm No. 6 (0.254%) (Table2). Average titratable acidity of fruit pulp was 0.656%. Similar result in respect of titratable acidity of tomato pulp was obtained by Hossain *et al.* (2010), Guold and Berry (1972) and Purkayastha and Mahanta (2010). They observed that the range of titratable acidity content varied from 0.256% to 0.353 %.

4.1.2.4 Vitamin C (ascorbic acid) content of tomato pulp

There was a significant variation among the 13 germplasm in respect of vitamin C content of tomato pulp (Appendix II). The highest vitamin C content of tomato pulp (24.33 mg /100 g) was found in germplasm No. 3 followed by germplasm No. 13 (22.167 mg /100 g) and No. 6 (18.833 mg /100 g). The lowest amount of ascorbic acid content (8.167 mg /100 g) was observed from germplasm No. 7 which was statistically similar to germplasm No. 5 (10.500 mg /100 g) and germplasm No. 2 (13.600 mg /100 g) (Table 2). Average vitamin C content of tomato pulp was 16.495 mg /100 g.

Similar result in respect of vitamin C content was reported by Hossain *et al.* (2010). They opined about vitamin C content of tomato varied from 17.32 mg 100^{-1} g to 26.59 mg 100^{-1} g. Similar result was also founded by Kallo (1985). Duttaroy (2011) mentioned that raw tomatoes content 12.7 mg vitamin C, tomato juice content 18.3 mg and tomato soup content 27.3 mg vitamin C per 100 g.

4.1.2.5 Carotenoids content of tomato pulp

Carotenoids content of tomato pulp showed significant difference among the 13 germplasm (Appendix II). The maximum amount of carotenoids content of tomato pulp (0.848 mg /100 g) was found in germplasm No. 5 followed by germplasm No. 9 (0.533 mg /100 g) and No. 6 (0.451 mg /100 g). The minimum amount of carotenoids content (0.127 mg /100 g) was observed from germplasm No.8 preceded by germplasm No. 10

(0.144 mg /100 g) and No. 3 (0.176 mg /100 g) (Table 2). Average carotenoids content was 0.320 mg /100 g of tomato pulp.

The present result is in agreement with the findings of Viskelis *et al.* (2005). They observed that the highest content of β-carotene was found in red-ripe fruits of cv. 'Rutuliai' (1.22 mg 100 g^{-1}). The content of β-carotene in red-ripe tomatoes of 'Admiro' F1and 'Kassa' F1was 0.89 and 0.83 mg 100 g^{-1}, respectively.

4.1.2.6 Anthocyanin content of tomato pulp

The difference of anthocyanin content of tomato was significant among the 13 germplasm (Appendix II). The highest amount of anthocyanin of tomato pulp (0.125 mg /100 g) was observed from germplasm No. 2 followed by germplasm No. 12 (0.085 mg /100 g) and No. 11 (0.038 mg /100 g). The least amount of anthocyanin of tomato pulp (0.003 mg /100 g) was observed from germplasm No. 4 which preceded by germplasm No. 7 (0.006 mg /100 g) and No. 9 (0.014 mg /100 g) (Table 2). Average anthocyanin content of tomato pulp was 0.036 mg /100 g.

4.1.2.7 Flavonoids content of tomato pulp

There was a significant variation among the 13 germplasm in respect of flavonoids content of tomato pulp (Appendix II). The highest flavonoids content of tomato pulp (5.270 g /100g) was found in germplasm No. 11 followed by germplasm No. 13 (3.843 g /100g), No. 7 (3.193 g /100g), No. 3 (2.783 g /100g) and No. 2 (2.769 g /100g). The lowest flavonoids content of tomato pulp (1.720 g /100g) was found in germplasm No. 5 preceded by germplasm No. 1 (1.883 g /100g), No. 10 (2.035 g /100g) and No. 8 (2.260 g /100g) (Table 2). Average flavonoids content was 2.571 g /100 g of tomato pulp.

4.1.2.8 Anti oxidant content of tomato pulp

After applying DPPH on the TLC plate, yellow color on purple background was observed which indicated the presence of antioxidant components in the methanol extract of **thirteen tomato germplasm**

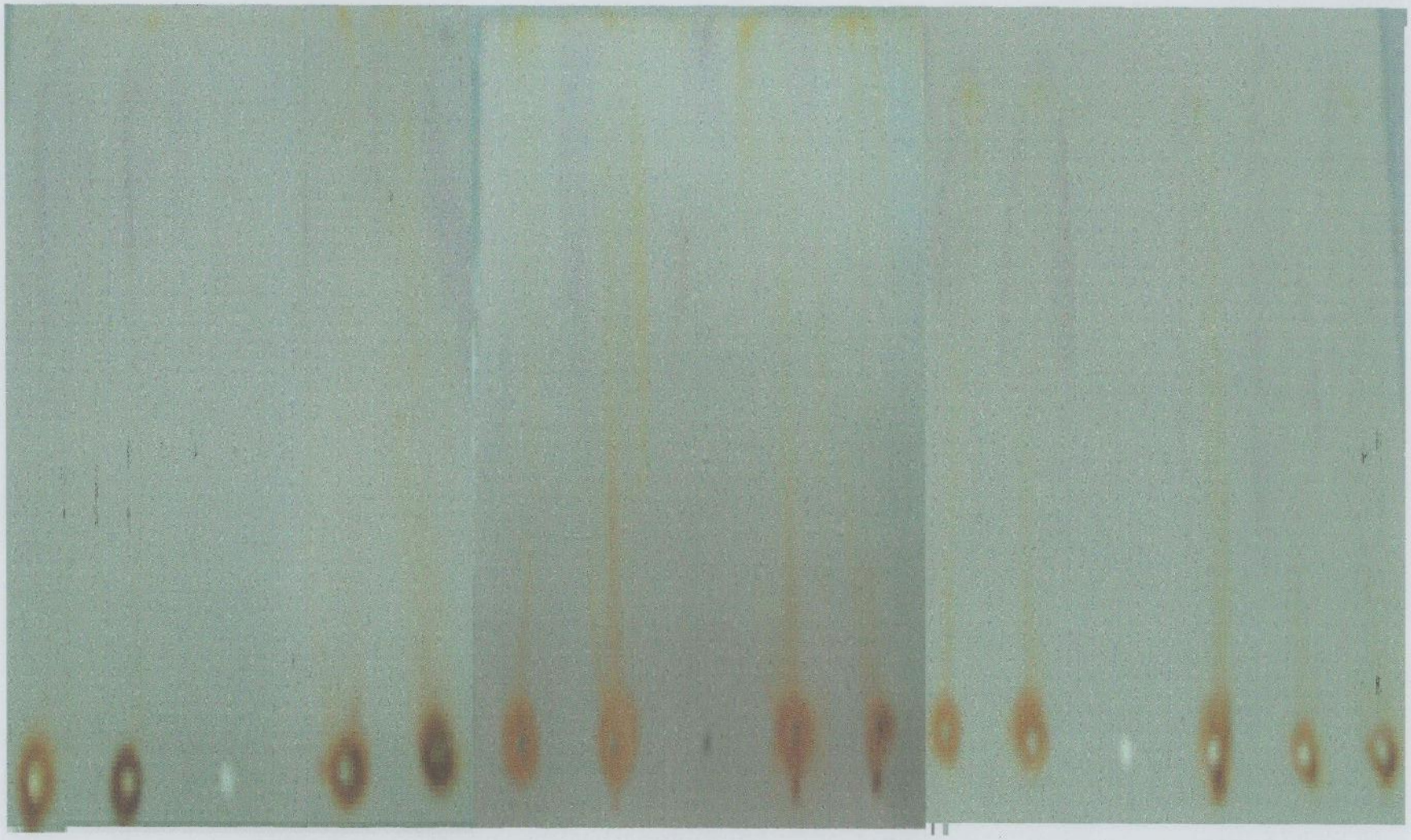


Figure 1. TLC plate for 13 tomato germplasm after applying DPPH

Table 3.1: Absorbance of blank solution

Blank	1st reading	2nd reading	3rd reading	Average
solution	0.81	0.81	0.81	0.81

Table 3.2: Absorbance of ascorbic acid (standard) at different concentration

Concentration	1st reading	2nd reading	3rd reading	Average	Inhibition (%)
1.57 µg/ml	0.59	0.60	0.61	0.60	26.01
3.13 µg/ml	0.53	0.54	0.55	0.54	33.41
6.25 µg/ml	0.49	0.50	0.48	0.49	39.58
12.5 µg/ml	0.36	0.37	0.35	0.36	55.61
25 µg/ml	0.14	0.14	0.14	0.14	82.73
50 µg/ml	0.08	0.08	0.08	0.08	89.27
100 µg/ml	0.06	0.06	0.07	0.06	91.49
200 µg/ml	0.05	0.05	0.053	0.05	93.67
400 µg/ml	0.041	0.04	0.043	0.04	94.82

Table 3.3: Absorbance of extract of germplasm No. 1 (BARI tomato – 1)

Concentration	1st reading	2nd reading	3rd reading	Average	% inhibition
1.57 µg/ml	0.78	0.78	0.78	0.78	3.20
3.13µg/ml	0.75	0.75	0.76	0.75	6.53
6.25 µg/ml	0.72	0.72	0.73	0.72	10.15
12.5 µg/ml	0.71	0.71	0.72	0.71	11.46
25 µg/ml	0.67	0.67	0.67	0.67	16.60
50 µg/ml	0.45	0.46	0.46	0.46	42.90
100 µg/ml	0.44	0.45	0.45	0.45	44.26
200 µg/ml	0.36	0.36	0.36	0.36	54.91
400 µg/ml	0.35	0.35	0.35	0.35	56.06

Table 3.4: Absorbance of extract of germplasm No. 2 (BARI tomato – 7)

Concentration	1st reading	2nd reading	3rd reading	Average	% inhibition
1.57 µg/ml	0.75	0.76	0.75	0.75	6.41
3.13µg/ml	0.71	0.72	0.72	0.72	11.09
6.25 µg/ml	0.70	0.71	0.71	0.71	12.45
12.5 µg/ml	0.68	0.67	0.68	0.68	16.15
25 µg/ml	0.65	0.66	0.65	0.65	18.74
50 µg/ml	0.46	0.46	0.46	0.46	42.54
100 µg/ml	0.45	0.45	0.45	0.45	44.14
200 µg/ml	0.30	0.31	0.31	0.31	61.65
400 µg/ml	0.19	0.19	0.19	0.19	76.20

Table 3.5: Absorbance of extract of germplasm No. 3 (BARI tomato – 2)

Concentration	1st reading	2nd reading	3rd reading	Average	% inhibition
1.57 µg/ml	0.79	0.79	0.79	0.79	1.84
3.13µg/ml	0.7	0.78	0.78	0.78	3.20
6.25 µg/ml	0.77	0.77	0.7	0.77	4.56
12.5 µg/ml	0.74	0.74	0.74	0.74	8.01
25 µg/ml	0.72	0.71	0.71	0.61	23.92
50 µg/ml	0.68	0.68	0.68	0.58	27.74
100 µg/ml	0.65	0.66	0.63	0.46	43.15
200 µg/ml	0.61	0.61	0.62	0.31	60.78
400 µg/ml	0.58	0.55	0.56	0.26	67.07

Table 3.6: Absorbance of extract of germplasm No. 4 (BARI tomato – 14)

Concentration	1st reading	2nd reading	3rd reading	Average	% inhibition
1.57 µg/ml	0.80	0.79	0.80	0.80	1.23
3.13µg/ml	078	0.78	0.78	0.78	3.20
6.25 µg/ml	0.77	0.76	0.76	0.76	5.30
12.5 µg/ml	0.65	0.65	0.65	0.65	19.23
25 µg/ml	0.53	0.53	0.53	0.53	33.78
50 µg/ml	0.44	0.45	0.44	0.44	44.63
100 µg/ml	0.43	0.42	0.43	0.43	46.97
200 µg/ml	0.28	0.27	0.27	0.27	66.09
400 µg/ml	0.17	0.17	0.17	0.17	78.79

Table 3.7: Absorbance of extract of germplasm No. 5 (BARI tomato – 9)

Concentration	1st reading	2nd reading	3rd reading	Average	% inhibition
1.57 µg/ml	0.79	0.80	0.79	0.79	1.60
3.13µg/ml	0.79	0.79	0.79	0.79	2.46
6.25 µg/ml	0.74	0.74	0.74	0.74	7.89
12.5 µg/ml	0.66	0.66	0.66	0.66	17.87
25 µg/ml	0.64	0.65	0.65	0.65	19.85
50 µg/ml	0.50	0.51	0.51	0.51	36.74
100 µg/ml	0.49	0.49	0.49	0.49	38.96
200 µg/ml	0.36	0.36	0.41	0.37	53.26
400 µg/ml	0.35	0.34	0.35	0.35	56.84

Table 3.8: Absorbance of extract of germplasm No. 6 (BARI tomato – 8)

Concentration	1st reading	2nd reading	3rd reading	Average	% inhibition
1.57 µg/ml	0.73	0.72	0.72	0.72	10.35
3.13µg/ml	0.71	0.72	0.71	0.71	11.34
6.25 µg/ml	0.70	0.71	0.70	0.70	12.82
12.5 µg/ml	0.68	0.67	0.68	0.68	16.15
25 µg/ml	0.65	0.65	0.66	0.65	18.74
50 µg/ml	0.52	0.52	0.52	0.52	35.51
100 µg/ml	0.41	0.42	0.42	0.41	48.33
200 µg/ml	0.34	0.34	0.34	0.34	57.33
400 µg/ml	0.30	0.31	0.31	0.31	61.65

Table 3.9: Absorbance of extract of germplasm No. 7 (Local tomato – 1, Mintu Super)

Concentration	1st reading	2nd reading	3rd reading	Average	% inhibition
1.57 µg/ml	0.71	0.72	0.72	0.71	11.34
3.13µg/ml	0.63	0.65	0.65	0.64	20.09
6.25 µg/ml	0.54	0.56	0.56	0.55	31.31
12.5 µg/ml	0.53	0.54	0.54	0.54	33.41
25 µg/ml	0.43	0.45	0.44	0.44	45.25
50 µg/ml	0.29	0.30	0.30	0.30	63.00
100 µg/ml	0.24	0.25	0.24	0.25	69.17
200 µg/ml	0.18	0.18	0.18	0.18	77.10
400 µg/ml	0.09	0.10	0.09	0.09	87.79

Table 3.10: Absorbance of extract of germplasm No. 8 (BARI Hybrid tomato – 6)

Concentration	1st reading	2nd reading	3rd reading	Average	% inhibition
1.57 µg/ml	0.79	0.79	0.79	0.79	2.21
3.13µg/ml	0.78	0.78	0.78	0.7	3.20
6.25 µg/ml	0.75	0.75	0.75	0.75	7.15
12.5 µg/ml	0.64	0.64	0.64	0.64	20.34
25 µg/ml	0.63	0.62	0.62	0.62	22.56
50 µg/ml	0.58	0.57	0.58	0.58	28.23
100 µg/ml	0.55	0.54	0.54	0.55	32.18
200 µg/ml	0.41	0.41	0.41	0.41	49.07
400 µg/ml	0.34	0.32	0.34	0.33	58.44

Table 3.11: Absorbance of extract of germplasm No. 9 (Local tomato – 2, Sathi)

Concentration	1st reading	2nd reading	3rd reading	Average	% inhibition
1.57 µg/ml	0.79	0.79	0.79	0.79	2.21
3.13µg/ml	0.77	0.77	0.78	0.77	4.06
6.25 µg/ml	0.74	0.74	0.75	0.74	7.76
12.5 µg/ml	0.72	0.72	0.72	0.72	10.72
25 µg/ml	0.69	0.69	0.69	0.69	14.42
50 µg/ml	0.68	0.68	0.68	0.68	15.78
100 µg/ml	0.67	0.66	0.67	0.66	17.50
200 µg/ml	0.59	0.59	0.59	0.59	26.51
400 µg/ml	0.38	0.38	0.38	0.38	52.28

Table 3.12: Absorbance of extract of germplasm No. 10 (BARI tomato – 12)

Concentration	1st reading	2nd reading	3rd reading	Average	% inhibition
1.57 µg/ml	0.74	0.73	0.74	0.74	8.26
3.13µg/ml	0.72	0.72	0.72	0.72	10.60
6.25 µg/ml	0.70	0.70	0.70	0.70	12.94
12.5 µg/ml	0.69	0.69	0.69	0.69	14.42
25 µg/ml	0.68	0.67	0.68	0.68	15.90
50 µg/ml	0.57	0.56	0.57	0.57	25.59
100 µg/ml	0.43	0.43	0.43	0.43	46.36
200 µg/ml	0.38	0.37	0.38	0.38	53.02
400 µg/ml	0.36	0.35	0.36	0.35	55.73

Table 3.13: Absorbance of extract of germplasm No. 11 (BARI tomato – 11)

Concentration	1st reading	2nd reading	3rd reading	Average	% inhibition
1.57 µg/ml	0.74	0.75	0.75	0.75	7.52
3.13µg/ml	0.73	0.73	0.7	0.73	9.24
6.25 µg/ml	0.71	0.72	0.72	0.72	11.22
12.5 µg/ml	0.71	0.70	0.70	0.70	12.57
25 µg/ml	0.67	0.66	0.66	0.66	17.50
50 µg/ml	0.45	0.45	0.46	0.45	43.36
100 µg/ml	0.44	0.45	0.44	0.45	44.51
200 µg/ml	0.33	0.33	0.33	0.33	58.93
400 µg/ml	0.20	0.20	0.20	0.20	75.09

Table 3.14: Absorbance of extract of germplasm No. 12 (BARI Hybrid tomato – 3 summer)

Concentration	1st reading	2nd reading	3rd reading	Average	% inhibition
1.57 µg/ml	0.74	0.75	0.75	0.74	7.64
3.13µg/ml	0.69	0.68	0.69	0.68	15.28
6.25 µg/ml	0.66	0.67	0.65	0.66	18.61
12.5 µg/ml	0.63	0.62	0.63	0.62	22.68
25 µg/ml	0.57	0.58	0.57	0.57	29.34
50 µg/ml	0.51	0.52	0.51	0.51	36.74
100 µg/ml	0.43	0.43	0.44	0.43	46.60
200 µg/ml	0.36	0.37	0.39	0.37	54.00
400 µg/ml	0.31	0.32	0.31	0.31	61.40

Table 3.15: Absorbance of extract of germplasm No. 13 (BARI tomato – 3)

Concentration	1st reading	2nd reading	3rd reading	Average	% inhibition
1.57 µg/ml	0.73	0.73	0.74	0.73	9.37
3.13µg/ml	0.67	0.67	0.67	0.67	17.01
6.25 µg/ml	0.63	0.63	0.62	0.62	22.44
12.5 µg/ml	0.58	0.59	0.59	0.58	27.62
25 µg/ml	0.52	0.53	0.53	0.52	34.89
50 µg/ml	0.47	0.47	0.48	0.47	41.43
100 µg/ml	0.41	0.41	0.42	0.41	48.58
200 µg/ml	0.35	0.35	0.36	0.35	56.10
400 µg/ml	0.28	0.28	0.29	0.28	64.85

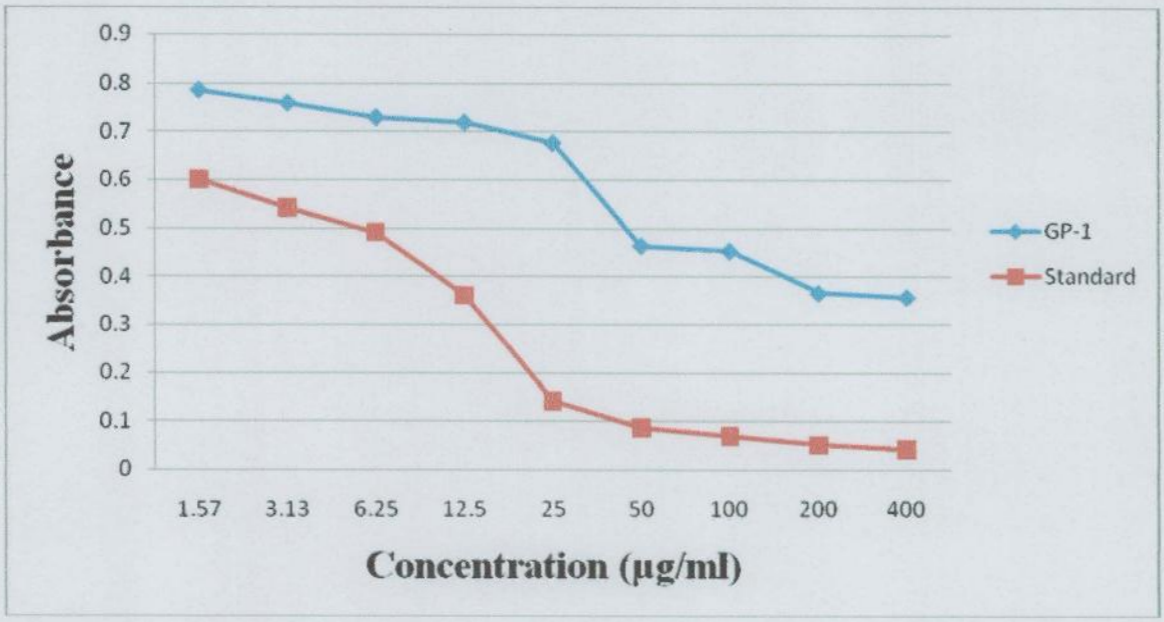


Figure 2. Absorbance against concentration of BARI Tomato-1 (GP. No. 1)

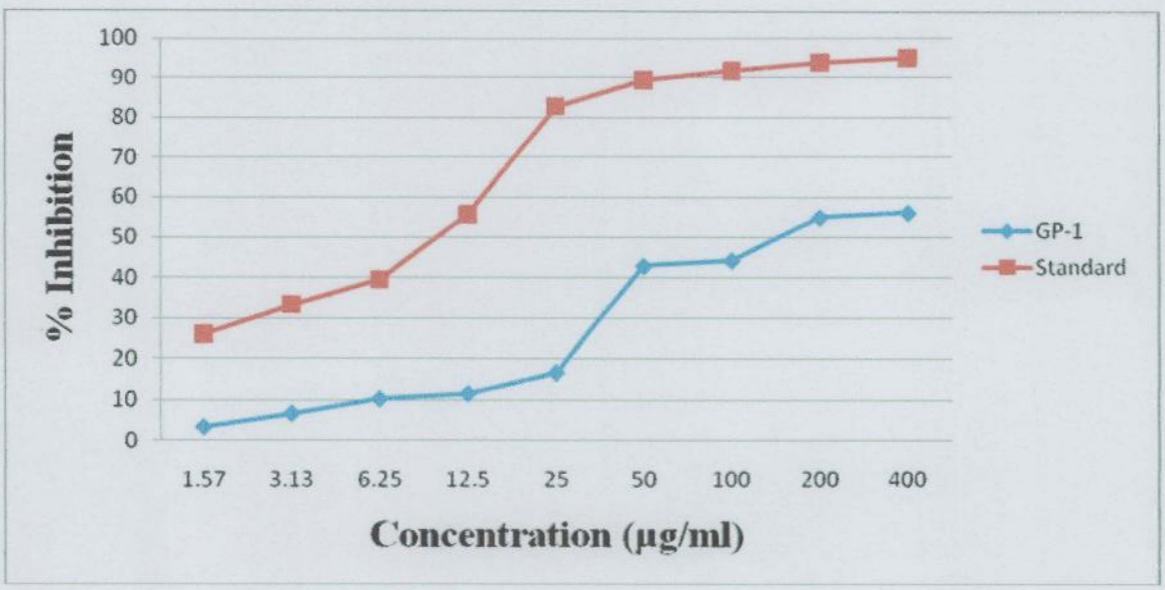


Figure 3. % inhibition against concentration of BARI Tomato-1 (GP. No. 1)

In the quantitative assay, tomato germplasm-1 displayed a free radical scavenging activity in the DPPH assay and the IC_{50} value was 160 µg/mL (approx) where the IC_{50} value of ascorbic acid (standard antioxidant), was 11 µg/mL (approx).

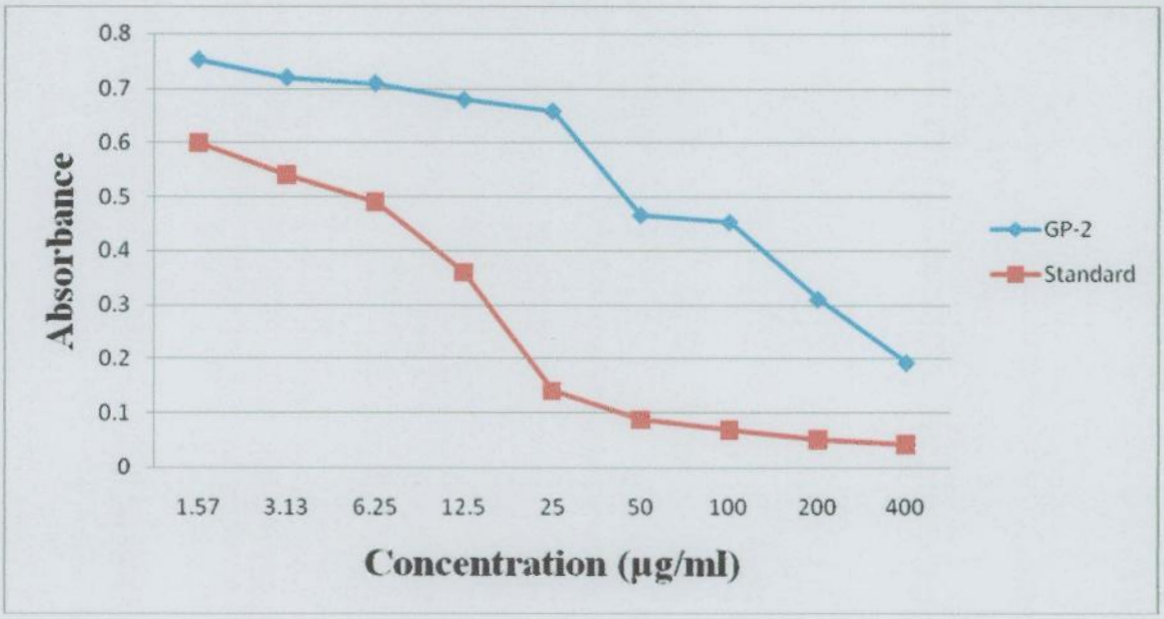


Figure 4. Absorbance against concentration of BARI Tomato-7 (GP. No. 2)

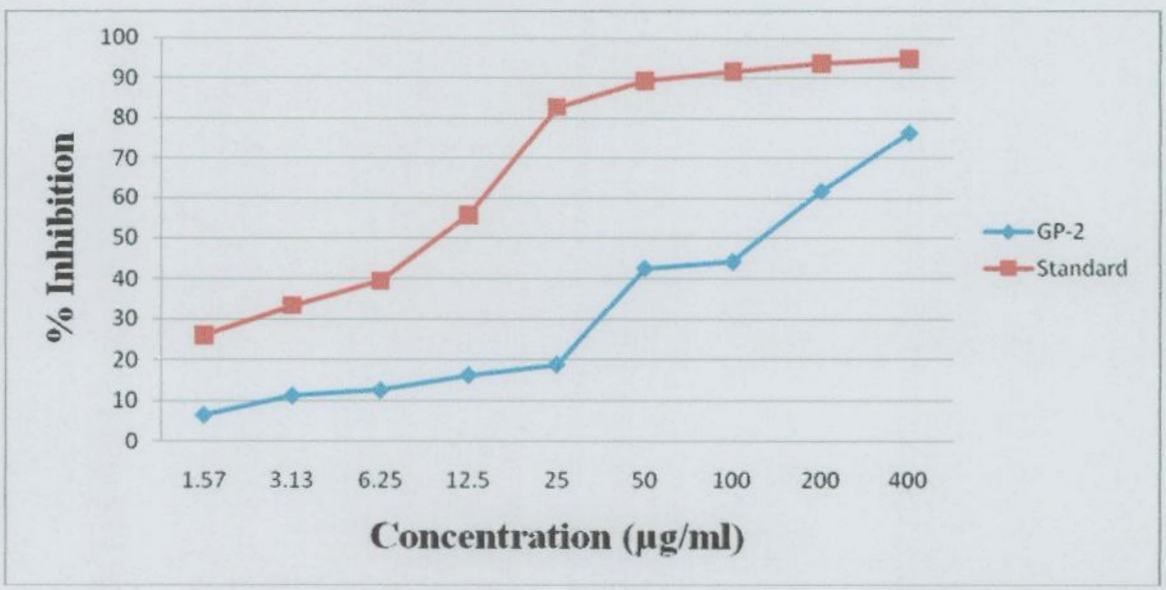


Figure 5. % inhibition against concentration of BARI Tomato-7 (GP. No. 2)

In the quantitative assay, tomato germplasm-2 displayed a free radical scavenging activity in the DPPH assay and the IC_{50} value was 130 µg/mL (approx) where the IC_{50} value of ascorbic acid (standard antioxidant), was 11 µg/mL (approx).

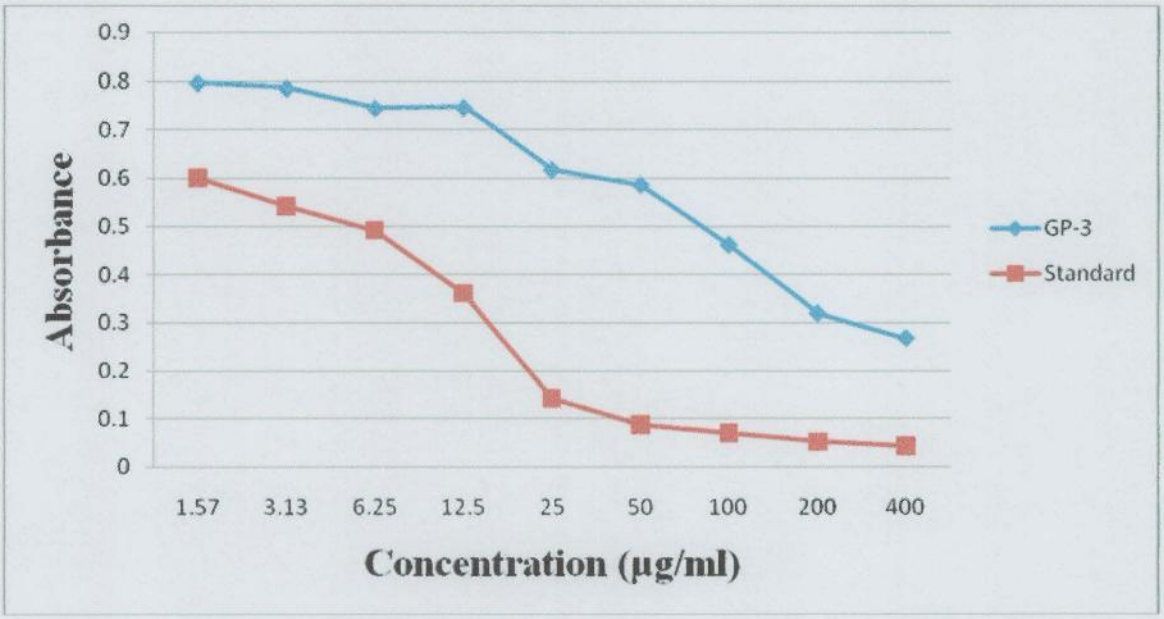


Figure 6. Absorbance against concentration of BARI Tomato-2 (GP. No. 3)

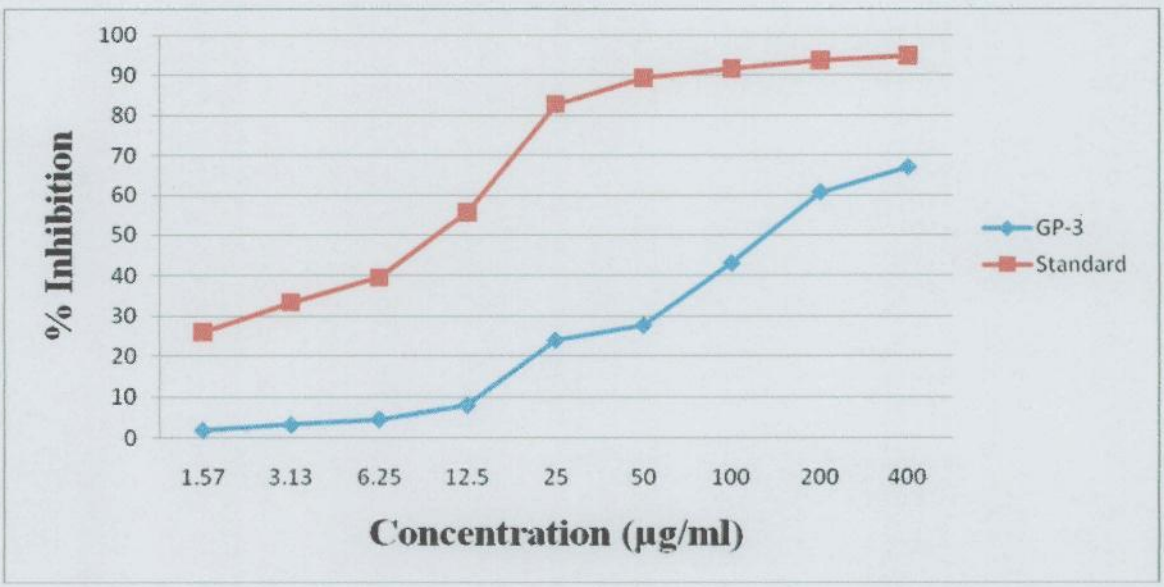


Figure 7. % inhibition against concentration of BARI Tomato-2 (GP. No. 3)

In the quantitative assay, tomato germplasm-3 displayed a free radical scavenging activity in the DPPH assay and the IC_{50} value was 140 µg/mL (approx) where the IC_{50} value of ascorbic acid (standard antioxidant), was 10 µg/mL (approx).

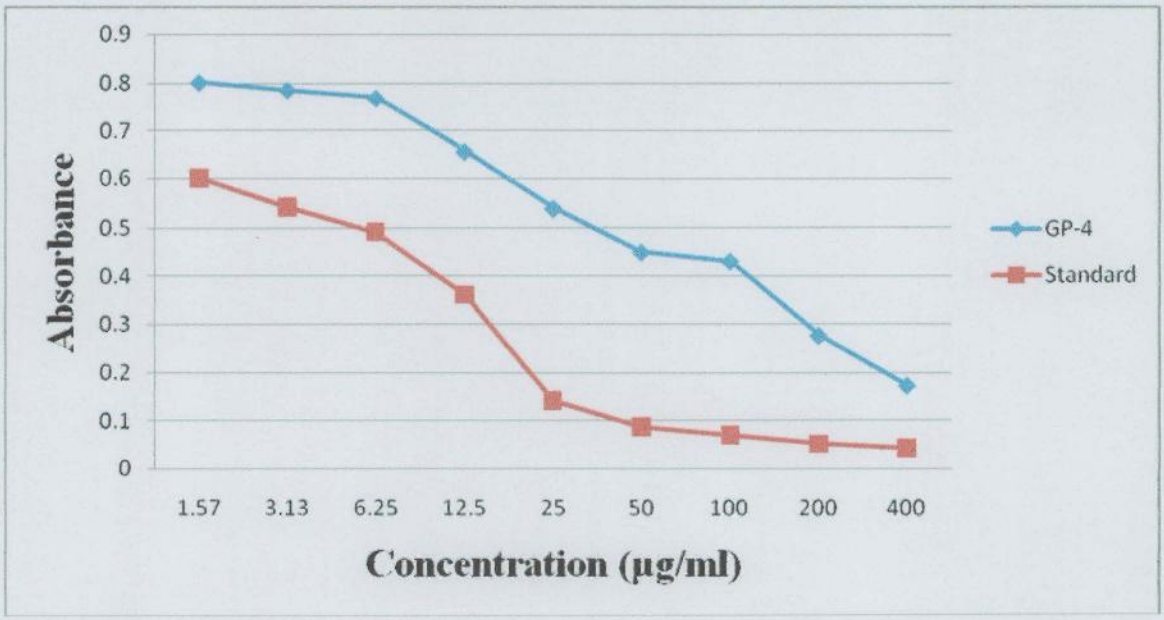


Figure 8. Absorbance against concentration of BARI Tomato-14 (GP. No. 4)

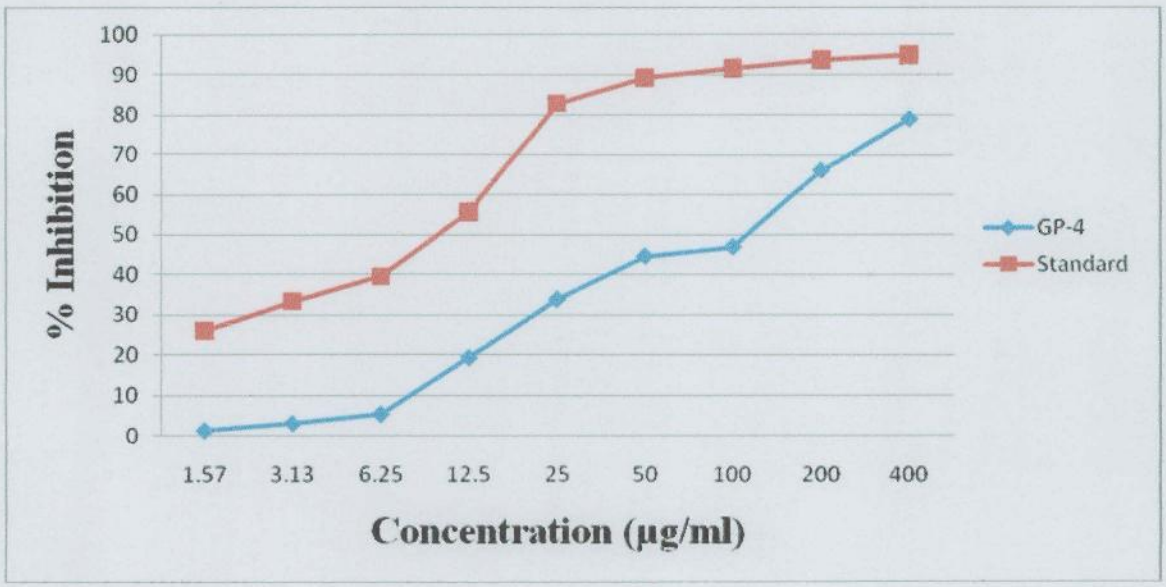


Figure 9. % inhibition against concentration of BARI Tomato-14 (GP. No. 4)

In the quantitative assay, tomato germplasm-4 displayed a free radical scavenging activity in the DPPH assay and the IC_{50} value was 120 µg/mL (approx) where the IC_{50} value of ascorbic acid (standard antioxidant), was 10.5 µg/mL (approx).

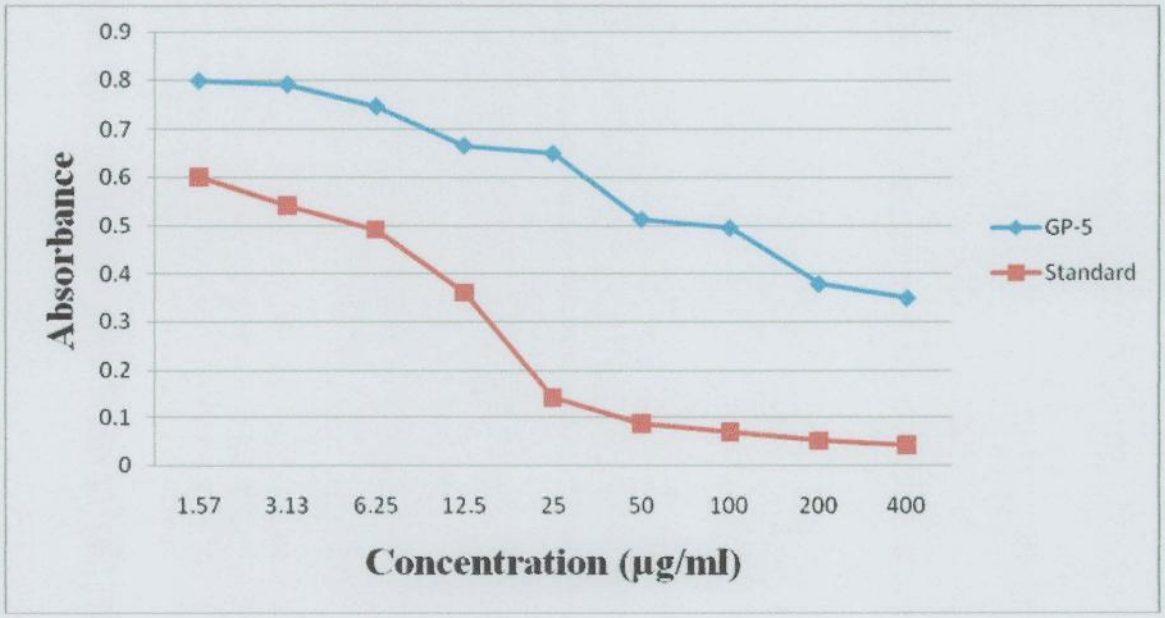


Figure 10. Absorbance against concentration of BARI Tomato-9 (GP. No. 5)

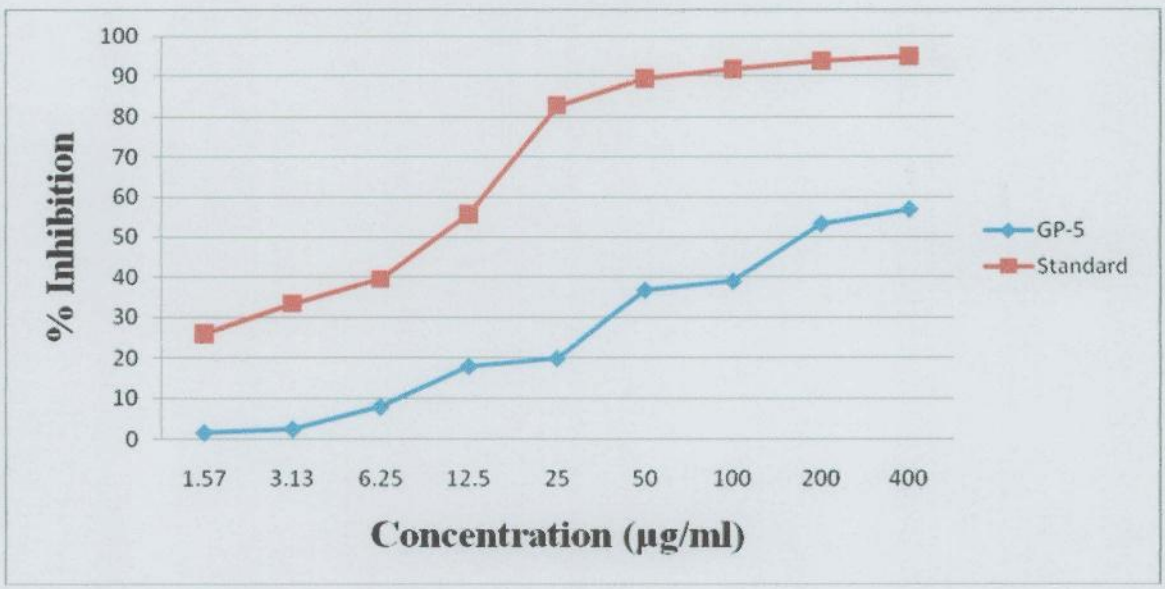


Figure 11. % inhibition against concentration of BARI Tomato-9 (GP. No. 5)

In the quantitative assay, tomato germplasm-5 displayed a free radical scavenging activity in the DPPH assay and the IC_{50} value was 180 µg/mL (approx) where the IC_{50} value of ascorbic acid (standard antioxidant), was 10 µg/mL (approx).

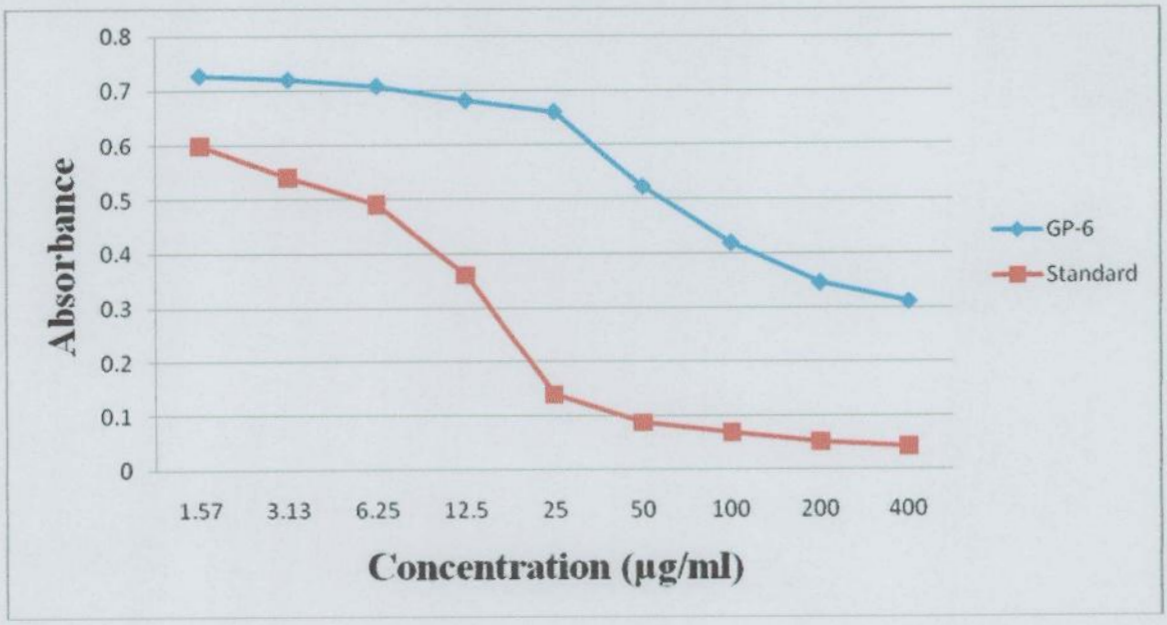


Figure 12. Absorbance against concentration of BARI Tomato-8 (GP. No. 6)

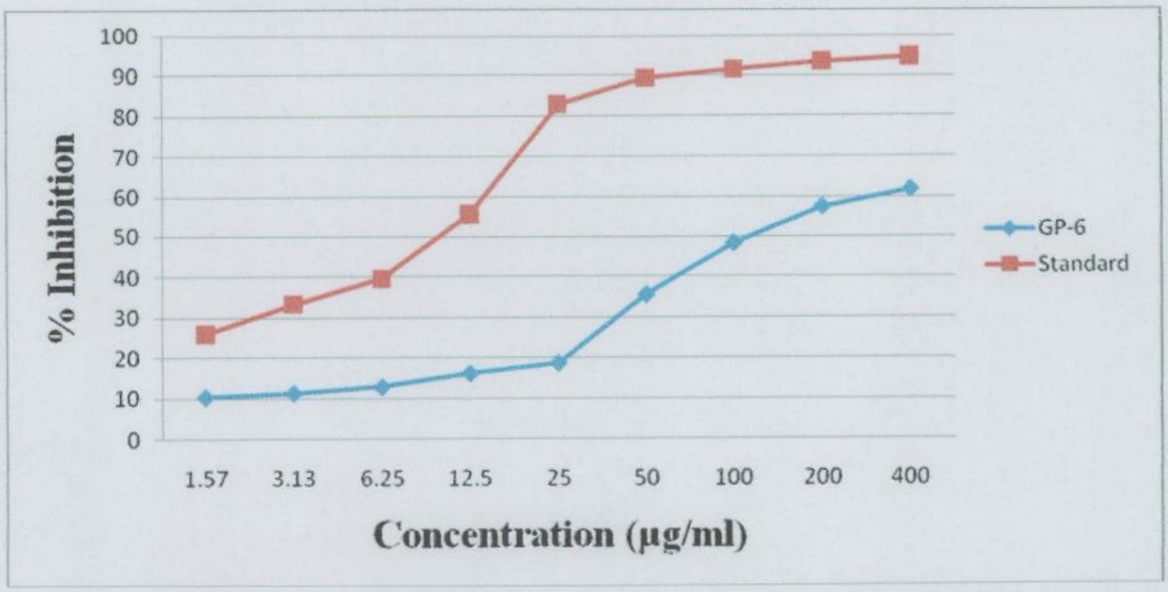


Figure 13. % inhibition against concentration of BARI Tomato-8 (GP. No. 6)

In the quantitative assay, tomato germplasm-6 displayed a free radical scavenging activity in the DPPH assay and the IC_{50} value was 100 µg/mL (approx) where the IC_{50} value of ascorbic acid (standard antioxidant), was 10.2 µg/mL (approx).

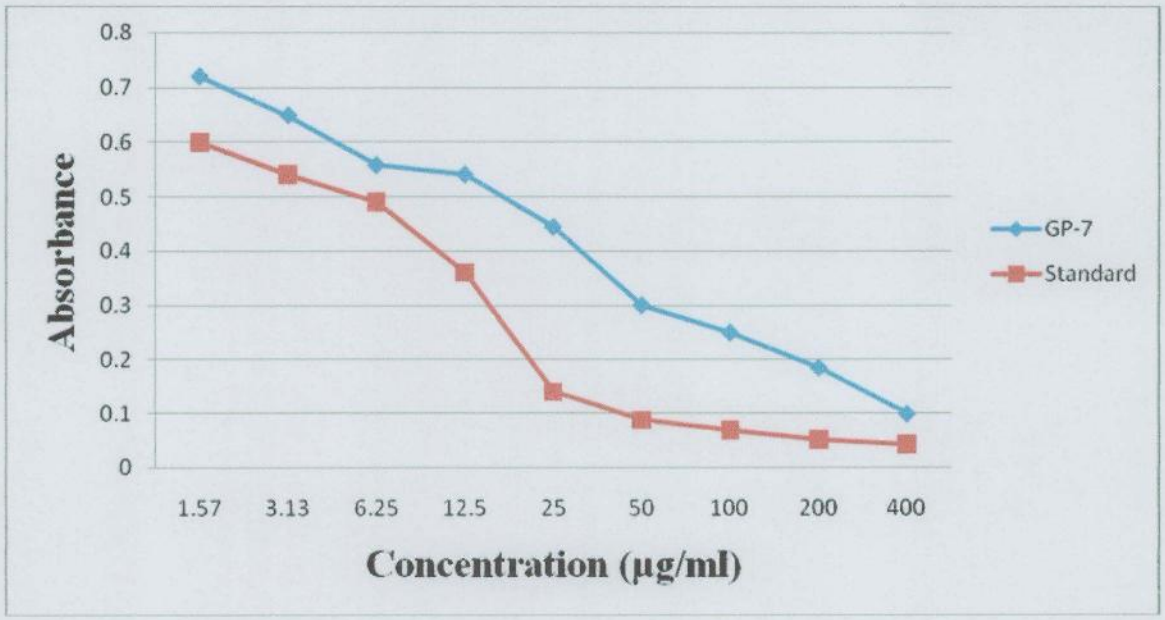


Figure 14. Absorbance against concentration of Local tomato-1 (GP. No. 7)

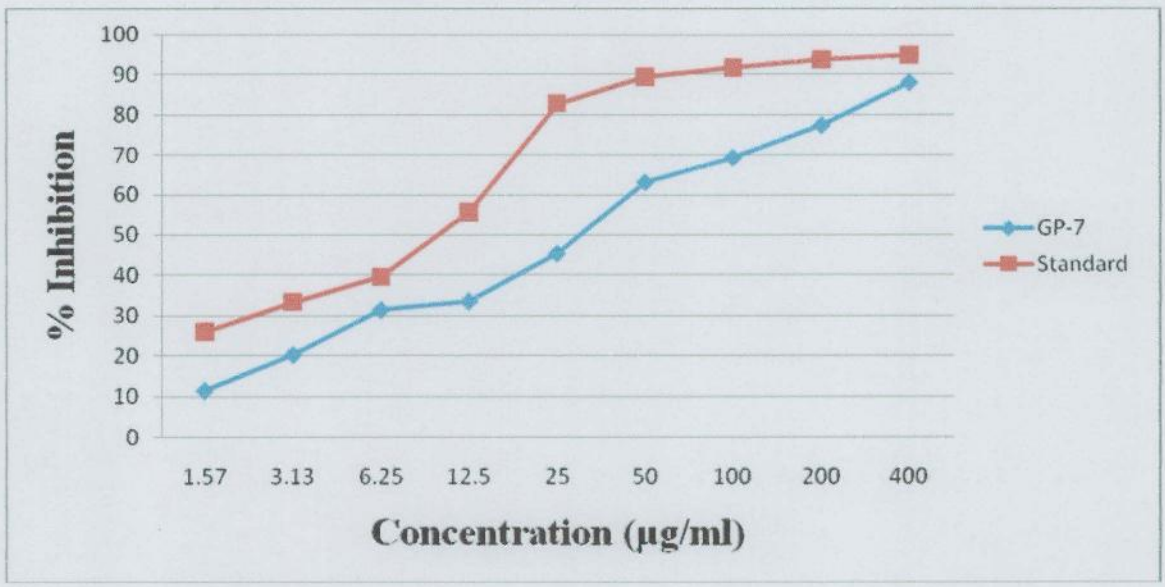


Figure 15. % inhibition against concentration of Local tomato-1 (GP. No. 7)

In the quantitative assay, tomato germplasm-7 displayed a free radical scavenging activity in the DPPH assay and the IC_{50} value was 30 µg/mL (approx) where the IC_{50} value of ascorbic acid (standard antioxidant), was 10.5 µg/mL (approx).

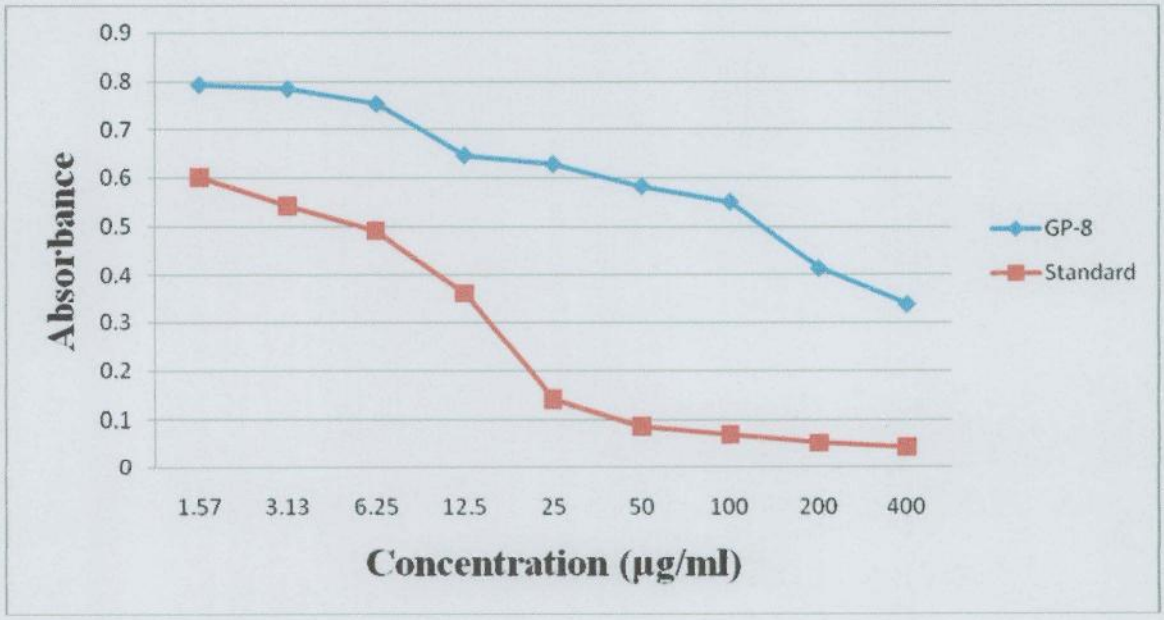


Figure 16. Absorbance against concentration of BARI Hybrid Tomato-6 (GP. No. 8)

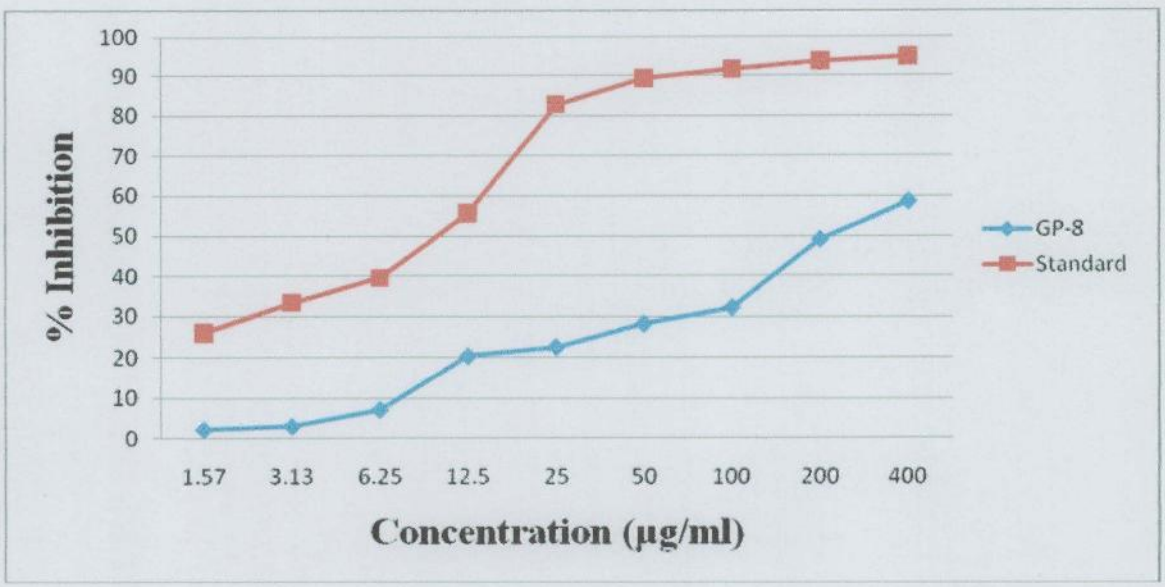


Figure 17. % inhibition against concentration of BARI Hybrid tomato-6 (GP. No. 8)

In the quantitative assay, tomato germplasm-8 displayed a free radical scavenging activity in the DPPH assay and the IC_{50} value was 200 µg/mL (approx) where the IC_{50} value of ascorbic acid (standard antioxidant), was 11 µg/mL (approx).

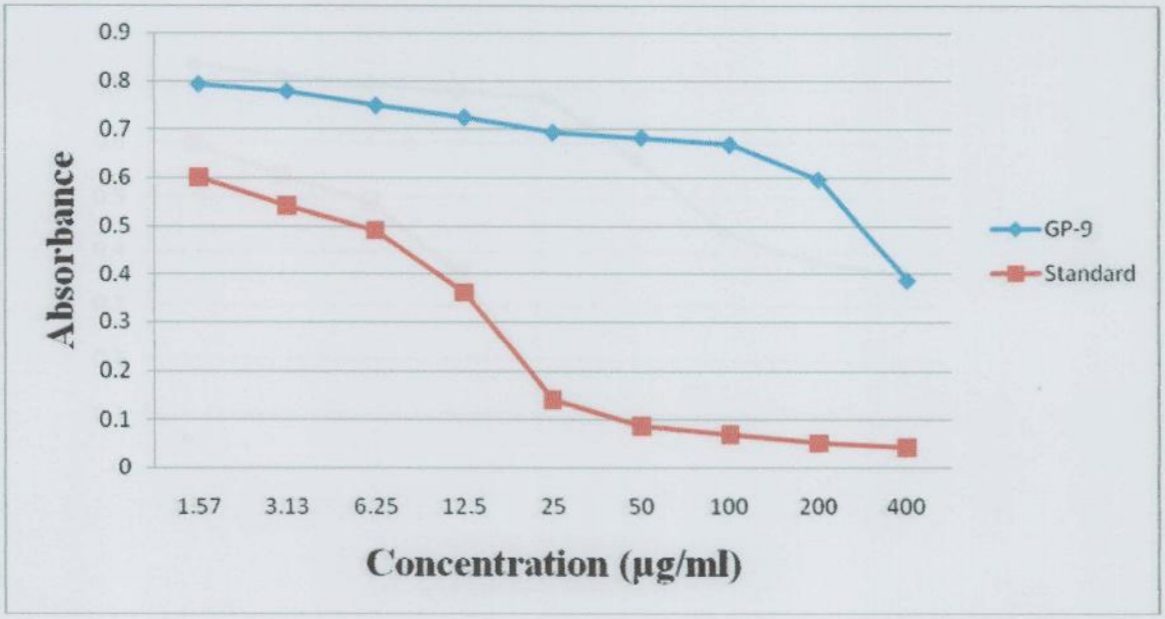


Figure 18. Absorbance against concentration of Local Tomato-2 (GP. No. 9)

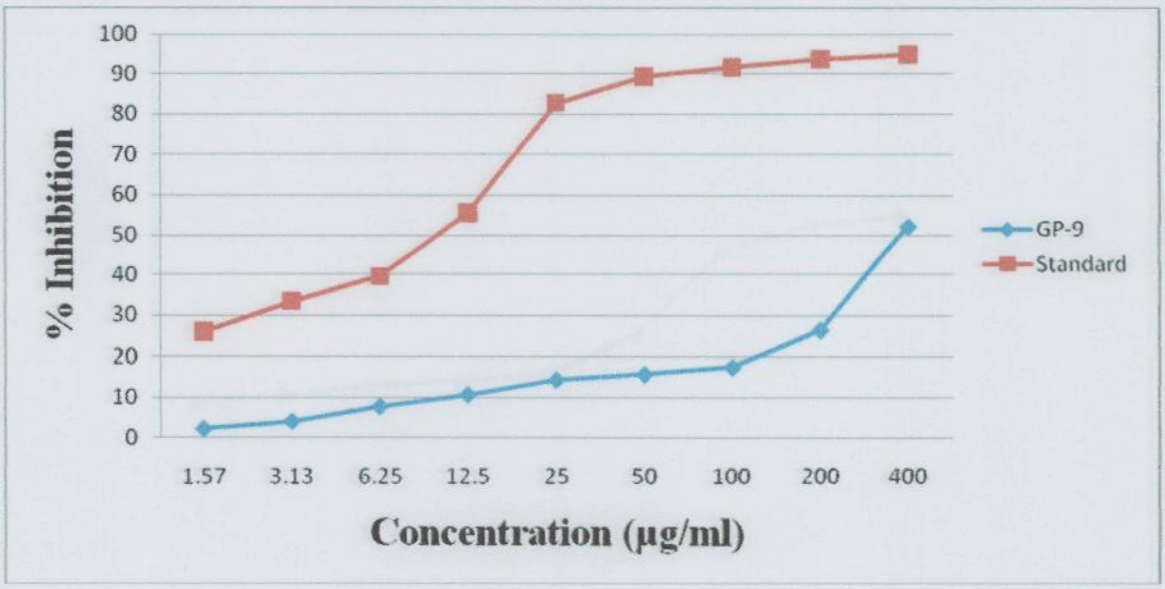


Figure 19. % inhibition against concentration of Local Tomato-2 (GP. No. 9)

In the quantitative assay, tomato germplasm-9 displayed a free radical scavenging activity in the DPPH assay and the IC_{50} value was 400 µg/mL (approx) where the IC_{50} value of ascorbic acid (standard antioxidant), was 10.7 µg/mL (approx).

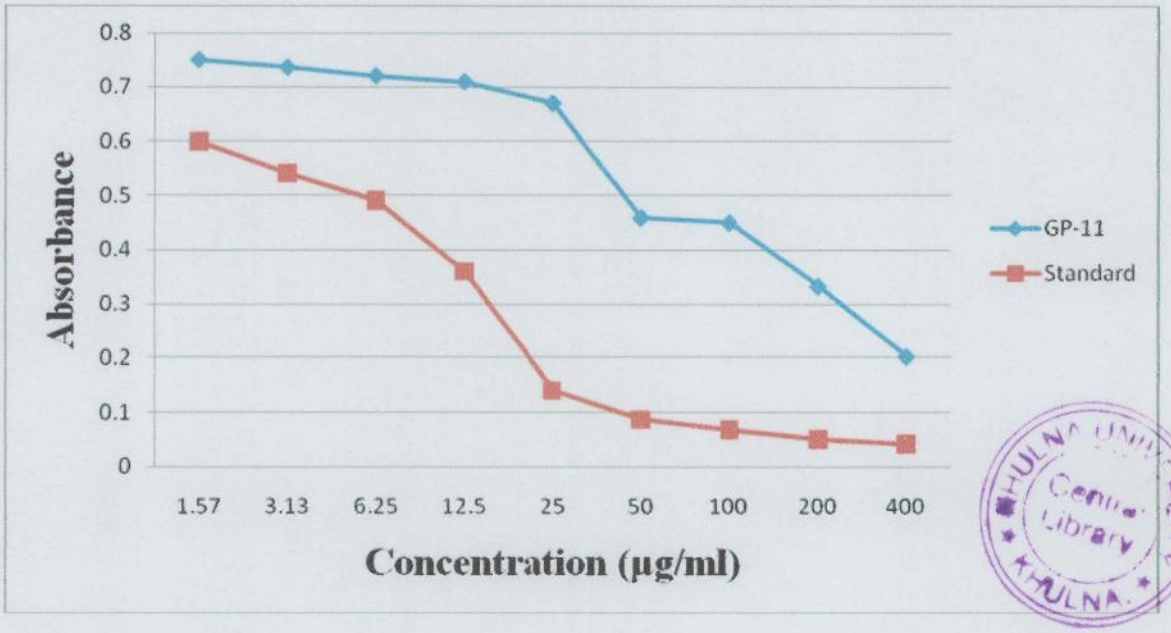


Figure 22. Absorbance against concentration of BARI Tomato-11 (GP. No. 11)

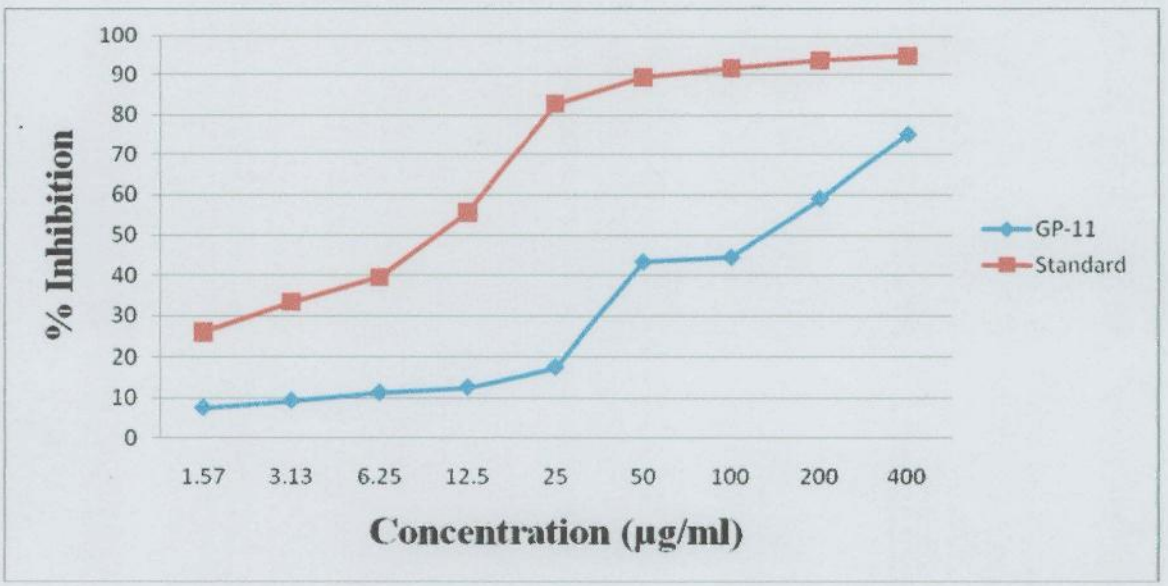


Figure 23. % inhibition against concentration of BARI Tomato-11 (GP. No. 11)

In the quantitative assay, tomato germplasm-11 displayed a free radical scavenging activity in the DPPH assay and the IC_{50} value was 125.5 µg/mL (approx) where the IC_{50} value of ascorbic acid (standard antioxidant), was 10.9 µg/mL (approx).

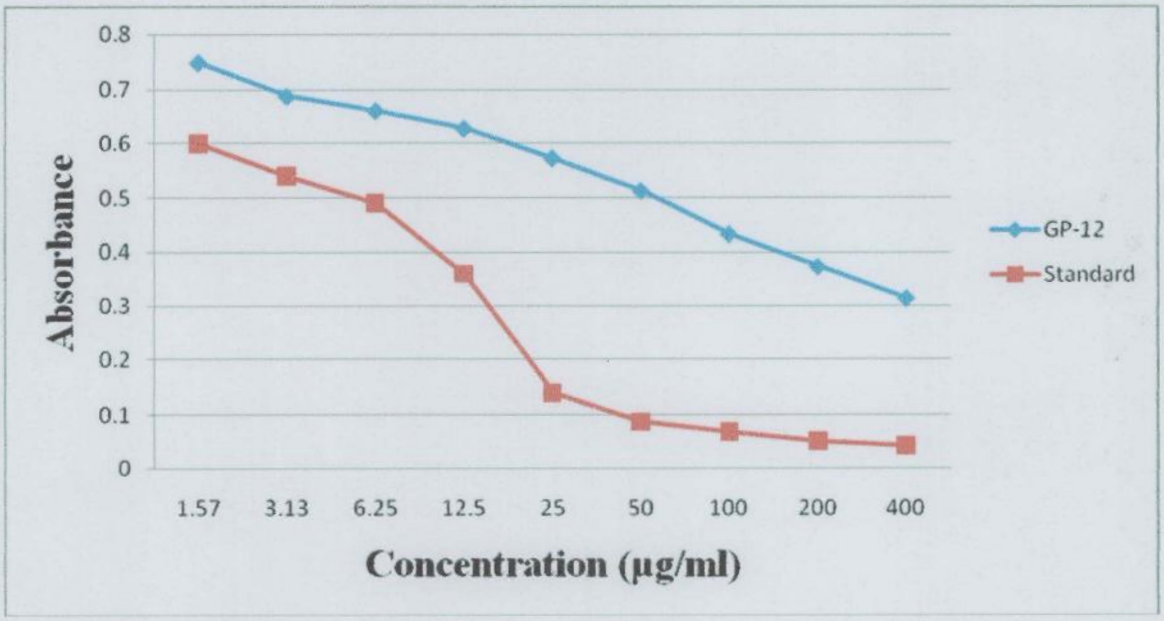


Figure 24. Absorbance against concentration of BARI Hybrid tomato-3(GP. No. 12)

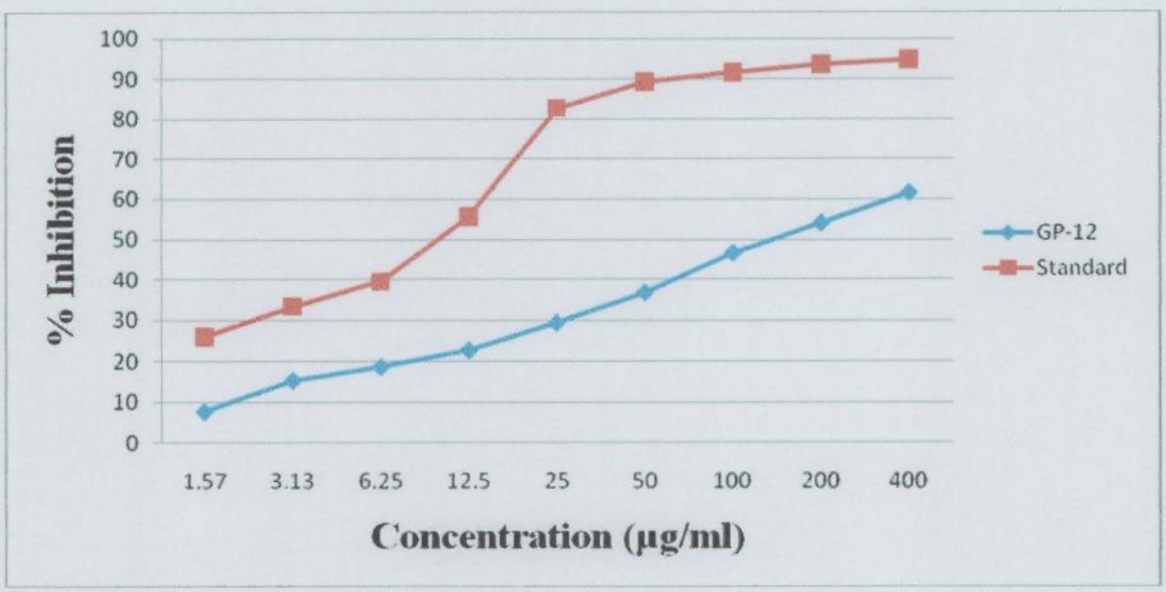


Figure 25. % inhibition against concentration of BARI Hybrid tomato-3(GP. No. 12)

In the quantitative assay, tomato germplasm-12 displayed a free radical scavenging activity in the DPPH assay and the IC_{50} value was 150 µg/mL (approx) where the IC_{50} value of ascorbic acid (standard antioxidant), was 10.8 µg/mL (approx).

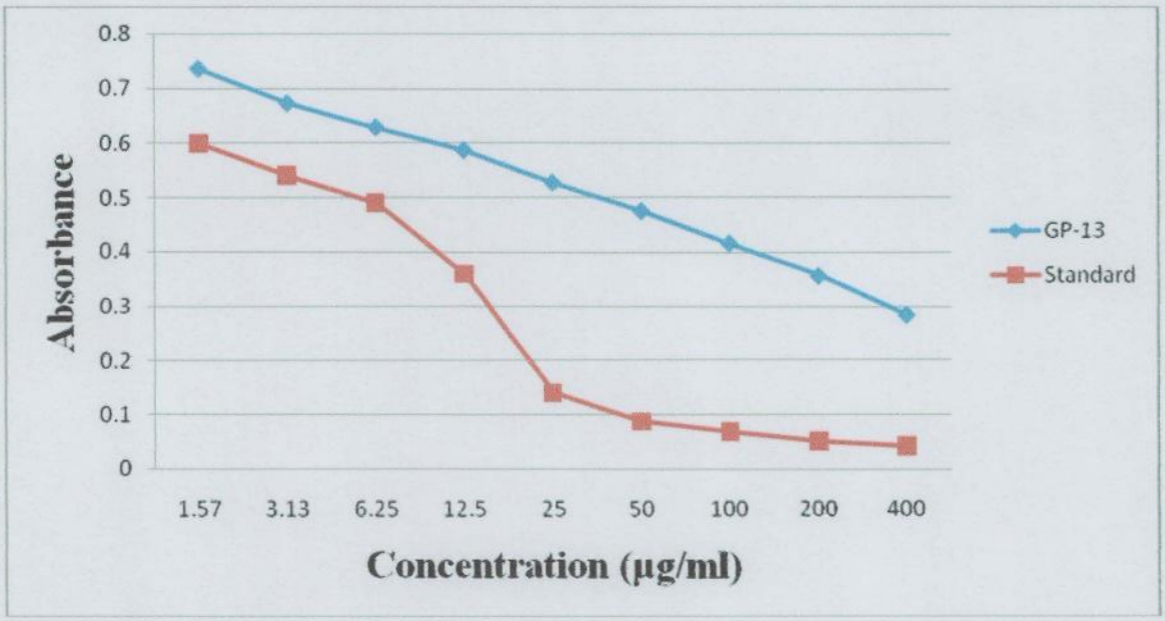


Figure 26. Absorbance against concentration of BARI Tomato-3 (GP. No. 13)

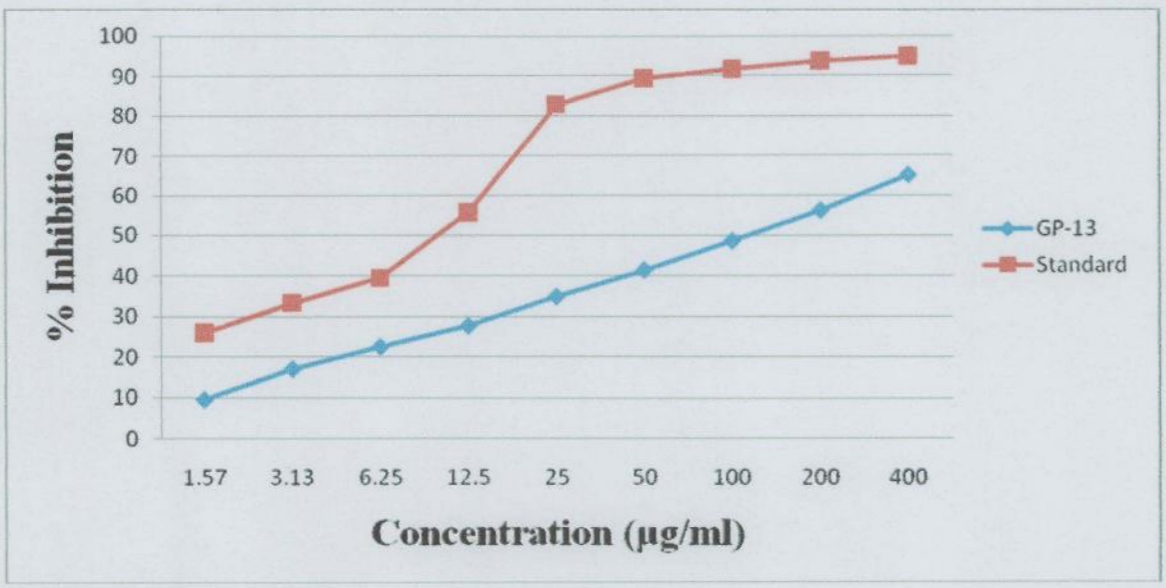


Figure 27. % inhibition against concentration of BARI Tomato-3 (GP. No. 13)

In the quantitative assay, tomato germplasm-13 displayed a free radical scavenging activity in the DPPH assay and the IC_{50} value was 100 µg/mL (approx) where the IC_{50} value of ascorbic acid (standard antioxidant), was 10.8 µg/mL (approx).

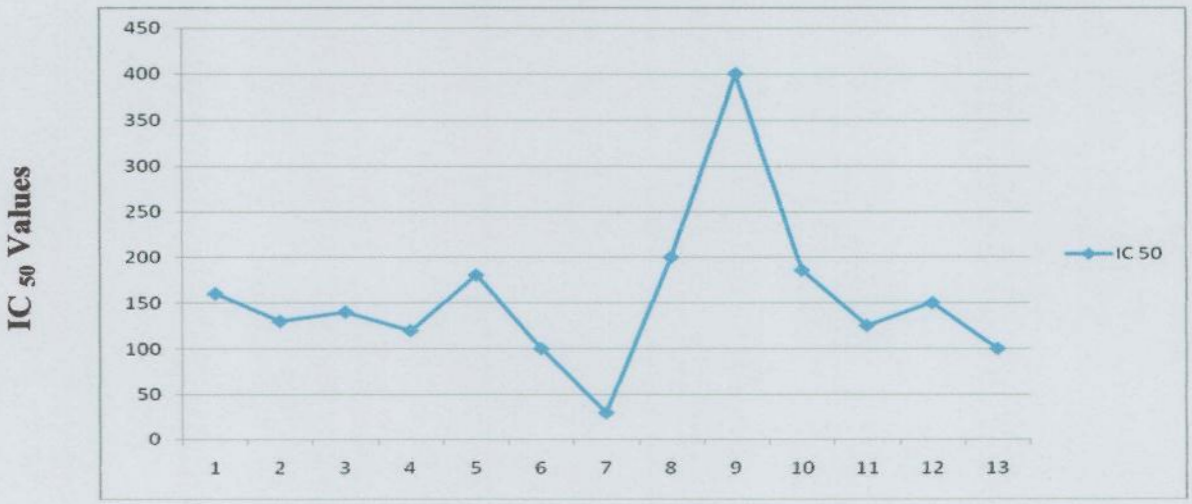


Figure 28. IC_{50} values of 13 Tomato germplasm

IC_{50} values varies significantly among the 13 tomato germplasm. The highest IC_{50} value (400 µg/ml) was found in germplasm No. 9 followed by germplasm No. 8 (200 µg/ml) and No. 10 (185.5 µg/ml) and that was the lowest in germplasm No. 7 (30 µg/ml) preceded by germplasm No. 6 (100 µg/ml), No. 13 (100 µg/ml) and No. 4 (120 µg/ml). The IC_{50} value determines the inhibition concentration standard. Low IC_{50} value indicates the highest antioxidant content where as the highest IC_{50} value determines the lowest antioxidant content. Germplasm No. 7 showed highest antioxidant and lowest was observed in germplasm No. 9.

SUMMARY

A study on the physico-chemical characteristics of 13 germplasm of tomato was carried out during the period from December 2012 to April 2013. The tomato germplasm were selected from the South Western region of Bangladesh. The collected tomato germplasm were brought to the Molecular Horticulture Laboratory of the Agrotechnology Discipline of Khulna University, Khulnn and kept in ambient temperature to determine their physico-chemical characteristics.

The study was laid out in a Completely Randomized Design (CRD) with three replications. Thirteen germplasm were selected as the experimental materials for the investigation. Data were recorded and statistically analyzed to study the variabilities among these selected tomato germplasm.

Morphological characteristics of tomato germplasm were determined under quantitative characters such as weight of tomato, length of tomato, width of tomato, and depth of tomato and seed weight of tomato. Chemical characteristics of selected tomato germplasm were estimated based on some parameters, such as pH, total soluble solids, T-acidity, vitamin C, carotenoids, anthocyanin and anti oxidant content.

The highest weight of tomato fruits (262.017 g) was observed from germplasm No. 6 followed by germplasm No. 5 (104.470 g) and No. 8 (131.677 g) and the minimum weight was recorded from germplasm No. 11 (40.940 g) preceded by germplasm No. 12 (47.937 g) and germplasm No. 13(59.237g), while average observed fruit weight was 105.958g. The highest length of tomato (7.047 cm) was found in germplasm No. 6 and the lowest length of tomato (4.625 cm) was found in germplasm No. 2 preceded by germplasm No. 13 (4.681cm) and germplasm No. 7 (4.722 cm) and average length was found 5.478 cm per fruit.

The highest width of tomato (7.564 cm) was recorded from germplasm No. 6 and the lowest width of tomato (3.343 cm) was recorded from germplasm No. 11 while average width of tomato was recorded 5.429 cm per fruit.

The highest percentage of seed weight (6.857 g) was found in germplasm No. 2 and the lowest percentage of seed weight (2.850 g) was found in germplasm No. 5 while average percentage of seed weight was found 4.034 g per fruit.

The highest pH of tomato was found in germplasm No. 13 (4.833) followed by germplasm No. 7 (4.750) and the lowest pH of tomato pulp (3.500) was found in germplasm No. 5 while average pH of tomato pulp was about 4.138.

The highest percentage of TSS was observed in germplasm No. 3 (6.033%) followed by germplasm No. 2 (6.000%), germplasm No. 11 (5.567%), germplasm No. 4 (5.367), germplasm No. 5 (5.367%) and lowest percentage of TSS was observed in germplasm No. 8 (5.100%) preceded by germplasm No. 12 (5.100%), No. 9 (5.133%), No. 7(5.200%), No. 6 (5.233%), No. 10 (5.233%), No. 13 (5.233%) and No. 1 (5.300%) and average observed percentage of TSS was 8.69%.

The highest percentage of T-acidity was found in germplasm No. 12 (0.953%) followed by germplasm No. 13 (0.930%) and lowest percentage of T-acidity was found in germplasm No. 5 (0.218%) preceded by germplasm No. 6 (0.254%) and average percentage of T-acidity was found 0.656% per fruit.

The maximum vitamin C content of tomato pulp (24.33 mg /100 g) was recorded from germplasm No. 3 and the minimum vitamin C content of tomato pulp (8.16 mg /100 g) was recorded from germplasm No. 7 while average recorded vitamin C content of tomato pulp was 16.49 mg /100 g.

The maximum carotenoids content of tomato pulp (0.848 mg /100 g) was found in germplasm No. 5 and the minimum carotenoids content of tomato pulp (0.127 mg /100 g) was found in germplasm No. 8 while average carotenoids content was found 0.320 mg /100 g.

The maximum anthocyanin content of tomato pulp (0.125 mg /100 g) was found in germplasm No. 2 and the minimum carotenoids content of tomato pulp (0.003 mg /100 g) was found in germplasm No. 4 while average carotenoids content was found 0.036 mg /100 g.

The maximum flavonoids content of tomato pulp (5.270 g /100 g) was found in germplasm No. 11 and the minimum flavonoids content of tomato pulp (1.720 g /100 g) was found in germplasm No. 5 while average carotenoids content was found 2.751 g /100g.

The highest IC_{50} value (400 µg/ml) was found in germplasm No. 9 and that was the lowest in germplasm No. 7 (30 µg/ml). The IC50 value determines the inhibition concentration standard. Low IC_{50} value indicates the highest antioxidant content where as the highest IC_{50} value determines the lowest. Germplasm No. 7 showed highest antioxidant and lowest was observed in germplasm No. 9.

CONCLUSION AND RECOMMENDATION

The best performance in respect of morphological characters was showed by germplasm No. 6 in respect of total weight, length, width and depth of tomato among the 13 selected germplasm. On the basis of seed weight of tomato better performance was observed from germplasm No. 2.

It was observed from the data of chemical analysis of tomato fruits that germplasm No. 13 showed best performance in respect of pH. On the basis of TSS content of fruit pulp better performance was observed from germplasm No. 3. The better performance was recorded from germplasm No. 12 in respect of T- acidity and germplasm No. 3 in respect of vitamin C content of tomato pulp among the 13 selected germplasm. In respect of carotenoids, anthocyanin and flavonoids content of tomato pulp germplasm No. 5, 2 and 11 gave better performance, respectively.

At the concluding point it can be cited that the criteria for the selection of the tomato germplasm may be weight, length, width and depth of the tomato fruit. Most of the characters were superior in germplasm No. 6. By considering T-acidity and vitamin C content of tomato pulp of germplasm No. 12 and germplasm No. 3 was mostly superior. Besides the characteristics which were experimented in this investigation some other characteristics such as reducing and non-reducing sugar, riboflavin, phenol content of the fruit pulp of the above mentioned germplasm is needed for the selection, consumption as well as cultivation.

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APPENDIX I

Analysis of variance of the data in respect of morphological characteristics of tomato

Source of Variation	Degrees of Freedom	Mean Sum Square				
		Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Fruit depth(cm)	Seed weight (g)
Germplasm	12	9460.799*	1.375*	3.632*	3.732*	3.686*
Error	26	163.621	0.187	0.151	0.134	0.005
Total	38	-	-	-	-	-

* Significant at 1% level of probability.

APPENDIX II

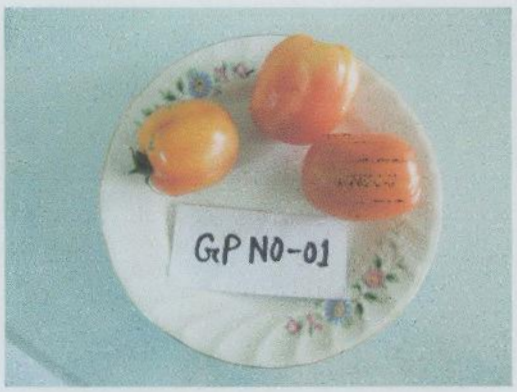
Analysis of variance of the data in respect of chemical characteristics of tomato

Source of Variation	Degrees of Freedom	Mean Sum Square						
		pH	TSS (%)	T- acidity (%)	Vitamin C (mg/100 g)	Carotenoids (mg/100 g)	Anthocyanin (mg/100 g)	Flavonoids (g/100 g)
Germplasm	12	0.546*	0.292*	0.139*	55.582*	0.115 *	0.003 *	2.661 *
Error	26	0.008	0.076	0.001	0.283	0.000	0.000	0.004
Total	38	-	-	-	-	-	-	-

* Significant at 1% level of probability.

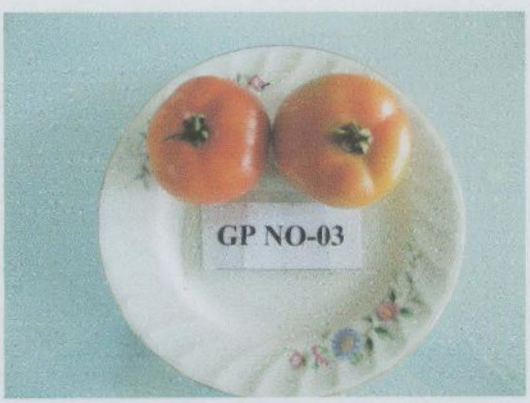
APPENDIX III

Images of Tomato of Some Selected Germplasm

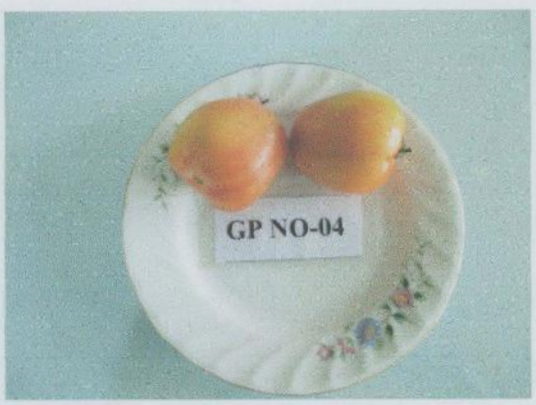


Germplasm No. 1

Germplasm No. 2

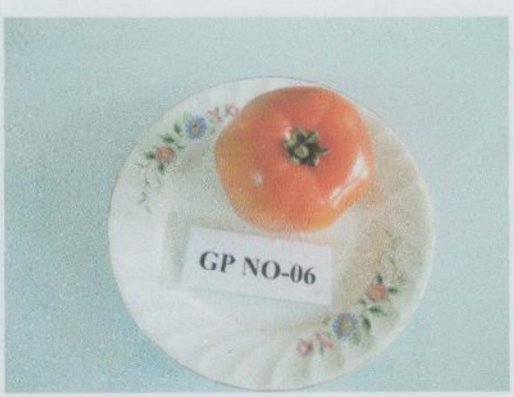


Germplasm No. 3



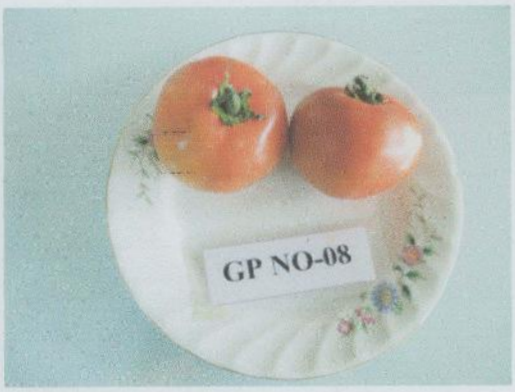
Germplasm No. 4

Germplasm No. 5



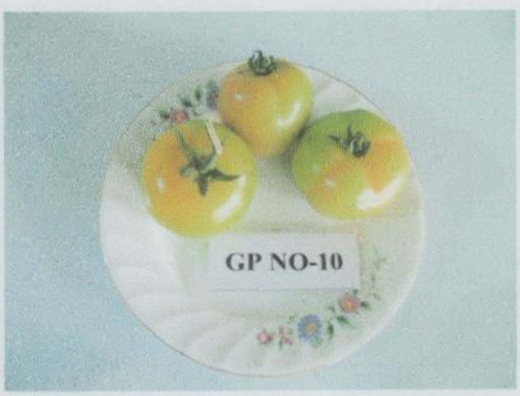
Germplasm No. 6

Germplasm No. 7

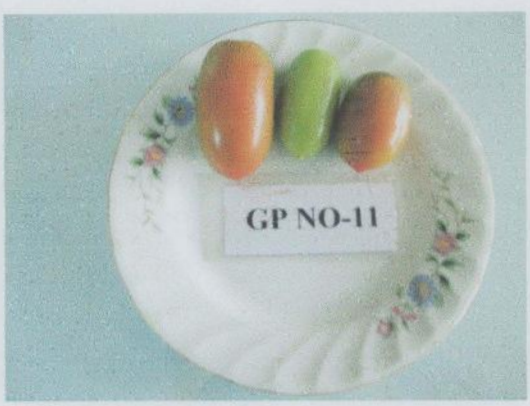


Germplasm No. 8

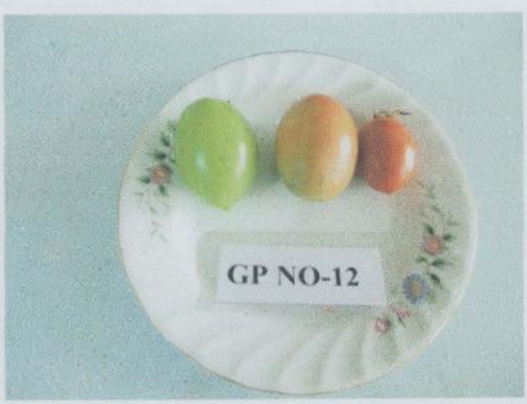
Germplasm No. 9



Germplasm No. 10



Germplasm No. 11



Germplasm No. 12

Germplasm No. 13