https://doi.org/10.33472/AFJBS.6.05.2024. 5703-5712

African Journal of Biological Sciences

Determination Of Total Phenolic And Antioxidant Activity Of Wild Cherry Tomatoes - *Solanum Peruvianum:* Sample From Awang Sekmai, Manipur.

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Article History
Volume 6,Issue 5, 2024
Received: 27 mar 2024
Accepted : 05 apr 2024
Doi:-
10.33472/AFJBS.6.5.2024.
5703-5712

ABSTRACT

Objective: An investigation was carried out to assess the total phenolic, antioxidant characteristics, antioxidant components, and antioxidant enzyme activity in developed cherry tomato breeding lines at the point of harvest when the tomatoes are completely mature and have turned red in colour.

Methods: The tomato samples were successfully identified by the Department of Life Sciences at Manipur University (BOTANY) as *Solanum Peruvianum* (wild cherry tomatoes). Phenolic compound determination was carried out using Folin ciocalteu reagent (McDonald et al. 2001). DPPH Activity, Total Antioxidant Assay-FRAP and ABTS Assay was performed.

Results: The phenolic content was estimated in sample Tomato (111.9394 µg GAE/mg) i.e. 1 mg sample was found equivalent to 111.93 µg of the Gallic Acid. 50% inhibitory concentration and the antioxidant activity (DPPH Assay) of the tomato sample were determined based on the experimental study. The sample's ability to scavenge DPPH was discovered. Results showed that the tomato sample had imitated antioxidant activity (FRAP) and a 50% inhibitory concentration. Compared to ascorbic acid, the tomato sample had far reduced action. The standard ascorbic acid equalled 469.2 µg of the tomato sample. The ABTS Radical Scavenging Assay revealed that the tomato sample shown a marginal enhancement in activity when compared to standard ascorbic acid, as evidenced by a 50% inhibitory concentration. The results showed that 78.47 µg of tomato sample was equivalent to 2.311 µg of conventional ascorbic acid.

Conclusion: The study analysed cherry tomato breeding lines' antioxidant content, features, components, and enzyme activity, revealing significant antioxidant activity and bioactive ingredients, potentially contributing to health benefits.

Keywords: Antioxidant activity, ascorbic acid, DPHH, FRAP and ABTS

INTRODUCTION

The tomato (Solanum lycopersicum) is a globally cultivated and important staple vegetable. India's tomato production in 2016 amounted to 18.73 million metric tonnes, representing 10.44% of the global total (Anon., 2018). Tomatoes contain notable bioactive substances and qualities that

classify them as prominent members of the "functional foods" group. People consume tomatoes in both cooked and uncooked forms, including in vinaigrettes and various processed and preserved forms. The brilliant red colour of lycopene, a carotenoid pigment known for its antioxidant benefits, naturally attracts consumers. Campbell et al. (2004) have conclusively demonstrated the major phytochemical properties of tomato carotenoids and polyphenols in preventing prostate cancer. There are many protective chemicals in tomatoes, such as phenolics (phenolic acids and flavonoids), carotenoids (lycopene, β- and β-carotenes), vitamins (ascorbic acid and vitamin A), and glycoalkaloids (tomatine). These molecules aid in mitigating stressful conditions that may contribute to the development of malignancies, cardiovascular disorders, and neurological diseases. Furthermore, the bioavailability and concentrations of tomato phytochemicals remain mostly unaltered during typical cooking procedures (Chaudhary et al., 2018). Researchers place significant importance on studying the raw mode of intake because of its potential for advancement in terms of containing properties beyond simply providing nourishment. Aerobic metabolism possesses an inherent ability to counteract reactive oxygen species (ROS), which are perilous chemical molecules generated as a natural result. When things are exposed to harsh environments, they make too many reactive oxygen species (ROS), which are made up of superoxide radical anion, hydrogen peroxide, different types of peroxyl radicals, hydroxyl radicals, and singlet oxygen. The cell responds to the situation by recognising the difficulty and then adjusting the expression of certain genes. This, sets off biochemical pathways that make defense-related enzymes like superoxide dismutase (SOD), catalases (CAT), and a family of peroxidases (POD). Other important phytochemicals that are made are carotenoids, ascorbic acid, and phenolics. Transition entails a change from the typical maintenance mode to an elevated level of productivity. Despite the recognition of tomatoes' beneficial phytochemicals, there is currently a dearth of thorough quantitative data on these compounds and other protective substances, particularly in the Indian setting. Research by Chandra and Ramalingam (2011), George et al. (2004), and Kaur et al. (2013) has emphasised these findings. Due to a lack of sufficient information, the current researcher initiated a screening procedure involving eight advanced breeding lines of cherry tomatoes. The programme sought to identify the most effective lines based on their antioxidative capabilities. The goals were to identify significant lipophilic and hydrophilic antioxidant constituents, evaluate antioxidant activity using a variety of test techniques, and quantify the activity of essential enzymes that directly and indirectly influence antioxidative characteristics.

METHODS

The ripe fruit samples from selected cherry tomato breeding lines were collected in triplicate and stored in an ice box. The ice box was accessible from the field and maintained at a temperature below -80 degrees Celsius in the Food Processing Lab at the Department of Biochemistry, Manipur College. After the collection, the fruit samples from each replicate were cleaned using a soft tissue and immediately washed with tap water. After being pulverised, a uniform mixture was collected. The pulp is utilised to assess its antioxidant properties. The pulp was promptly tested for the presence of antioxidant components, including lycopene, ascorbic acid (vitamin C), and total phenol. The Folin-Ciocalteu reagent was used to identify phenolic compounds (McDonald et al. 2001). The enzymes associated with antioxidation properties were also examined, along with antioxidant activity using three distinct assay systems (DPPH, FRAP, and ABTS).

Total Phenolic Content Assay

Reagents Required: 1. Folin ciocalteu reagent (SRL- Cat no. 29520) -1:10 diluted in DM water
2. Na2CO3 (SRL- Cat no. 64079)- 1.0 M
3. Gallic Acid (SRL- Cat no. 13142 - Prepared in Methanol: Water (50:50 v/v)

The quantification of phenolic compounds was performed using the Folin ciocalteu reagent, following the methodology given by McDonald et al. (2001). The test samples were diluted and then mixed with 50 µl of diluted folin ciocalteu reagent and 40 µl of 1.0 M Na2CO3 solution. The reaction mixture was prepared in accordance with the directions outlined in the reaction mixture setup table. It remained uninterrupted for a period of 15 minutes. The mixture's absorbance was quantified at a wavelength of 760 nm using a twin beam JASCO V-630 spectrophotometer manufactured in Japan. A calibration curve for Gallic Acid was constructed using a solution containing Methanol and Water in a 50:50 ratio (v/v), with concentrations ranging from 25 µg/mL to 250 µg/mL.

DPHH Assay

Reagents Required: 1. 0.1 mM DPPH - (SRL Chem - Cat no.- SR-29128) in Methanol (SD fine- Cat no.- 10930IC250)
2. Ascorbic Acid - (SD Fine- F13A/0413/1106/62)

Five microliters of various test substance stock solutions were mixed to 0.1 milliliters of 0.1 millimolar DPPH in a 96-well plate. Triplicates of the reaction and blank solutions were created. The blank solution consisted of 0.2 ml DMSO/Methanol and 5µl of different chemical concentrations, as described in an excel file. Untreated wells were the control group. The plate was left in darkness for 30 minutes. A microplate reader (iMark, BioRad) recorded decolorization at 517 nm after incubation. A control was created by adding 20µl of deionized water to the reaction mixture. The inhibition percentage relative to the control measured scavenging activity. The IC50 was calculated using Graph Pad Prism 6. The X-axis (sample concentration) and Y-axis (percentage inhibition from control) were graphed.

DPPH scavenging activity = ((Abs Control - Abs Sample) / Abs Control) × 100.

FRAP Assay

The experiment involved adding 10µl of stock solutions of the test chemical and standard (Ascorbic Acid - SRL, Cat no. 23006) to 0.2 MsFe buffer (pH 6.6) and 0.05 ml of 1% K3Fe (CN)6] solution (SRL, Cat no. 15766). A vortex mixer vigorously swirled and heated the reaction mixture at 50°C for 20 minutes. Control wells were untreated. After incubation, 0.5 ml of SRL Cat no-92390 10% trichloroacetic acid was applied. Next, 50µl of deionized water and 0.1% ferric chloride solution (Fischer Scientific, Cat no. 23585) were added. The coloured solution was measured at 700 nm using a microplate reader (iMark, BioRad) and compared to a blank sample. The IC50 was determined in GraphPad Prism 6.

ABTS Radical Scavenging

The ABTS free radical reagent was prepared by mixing APS (2.45 mM) and ABTS (7mM) and diluting it 100-fold. On a 96-well plate, 200 microliters of ABTS free radical reagent were mixed with 10 microliters of different stock solutions of the standard (Ascorbic Acid -SD Fine-F13A/0413/1106/62, with concentrations given in the excel sheet) and samples. The solution was

then left at room temperature for 10 minutes without illumination. Untreated wells were the control group. After incubation, use a microplate reader (iMark, BioRad) to measure decolorization absorbance at 750nm. The results were compared to the negative control. The IC50 was calculated using Graph Pad Prism 9.5.1. A graph was created with the X-axis representing sample concentration and the Y-axis percentage inhibition relative to the control.

RESULTS AND DISCUSSION

Based on the results obtained from the study, total phenol content was estimated. The phenolic content was estimated in sample Tomato (111.9394 µg GAE/mg) i.e. 1 mg sample was found equivalent to 111.93 µg of the Gallic Acid.

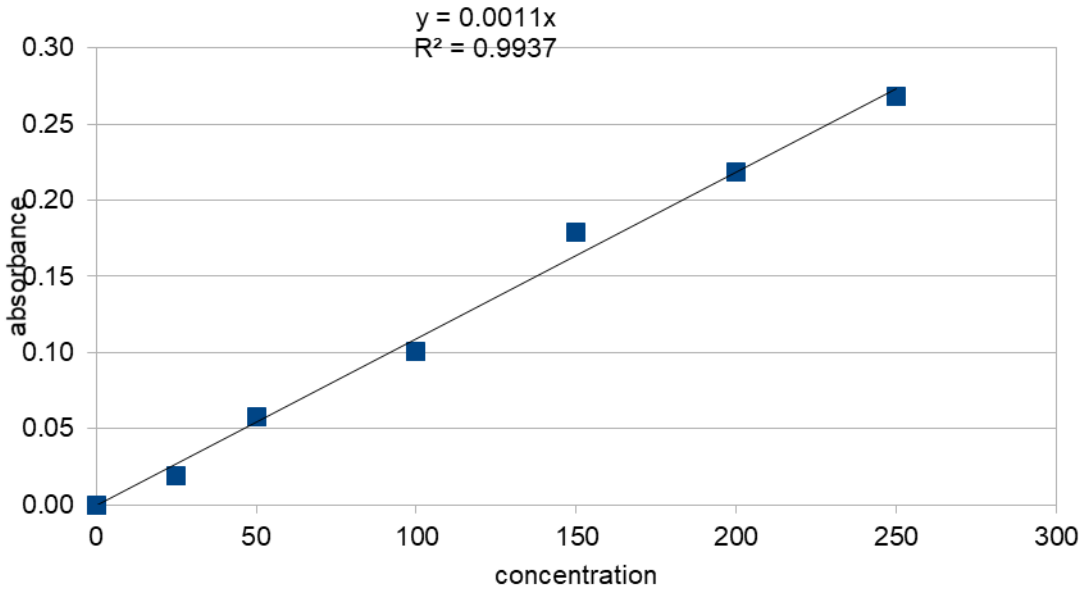

Figure 1Caliberation curve plot of total concentration against the absorbance

Table 1. Distribution of mean, Standard deviation of the tomato sample for calibration of total phenolic content

Sample	Mean	SD
D1 (1 mg/ml)	85.15	11.86
D2 (10 mg/ml)	122.73	9.49
D3 (100 mg/ml)	559.70	16.57

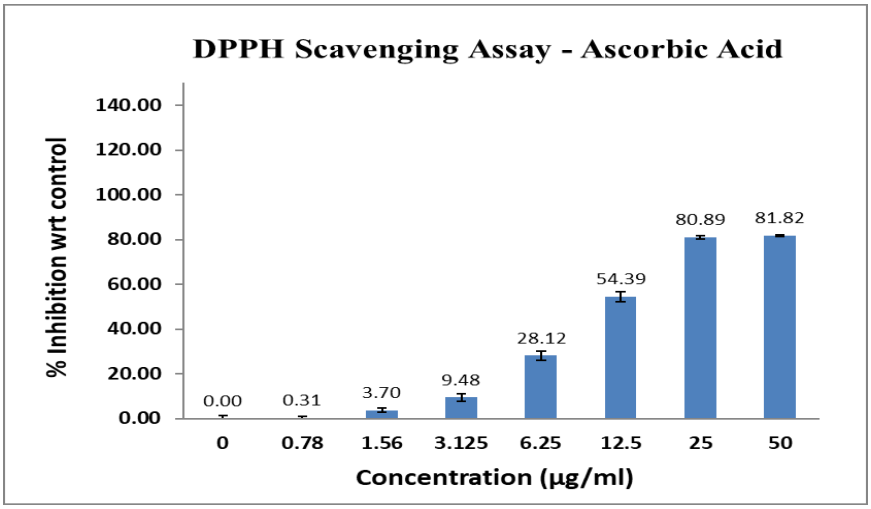

Figure 2 DPPH Scavenging assay plot for both the ascorbic acid

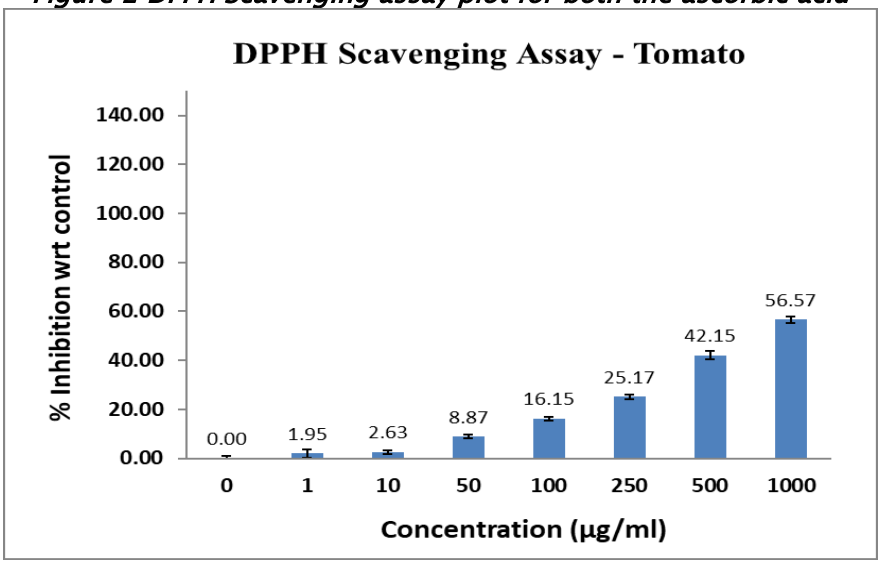

Figure 3DPHH Scavenging Assay for tomato

As a consequence of the experiment, data were generated, which enabled the DPPH test technique to be utilized in order to assess the level of antioxidant activity exhibited by the tomato sample. The data for the inhibitory concentration at fifty percent were supplied in table 2, which displayed the information. The material was able to successfully scavenge DPPH, as evidenced by its performance. An amount of substance that was equivalent to 11.51 micrograms (µg) of the standard ascorbic acid was present in the sample of tomato. The quantity of 749.5 micrograms (µg) was found in the sample.

Table 2. Distribution of Inhibitory concentration at 50 % (DPHH)

Sample code	IC50 value (µg/ml)
Ascorbic Acid	11.51 ± 0.03
Tomato	749.5 ± 0.02

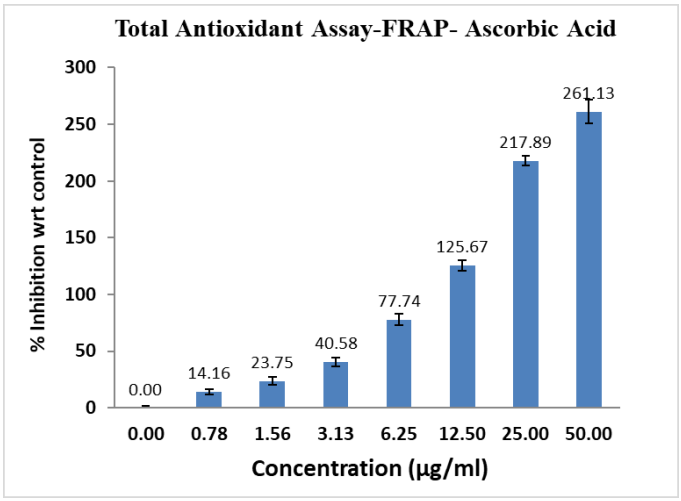

Figure 4 Antioxidant Assay of Ascorbic acid by FRAP

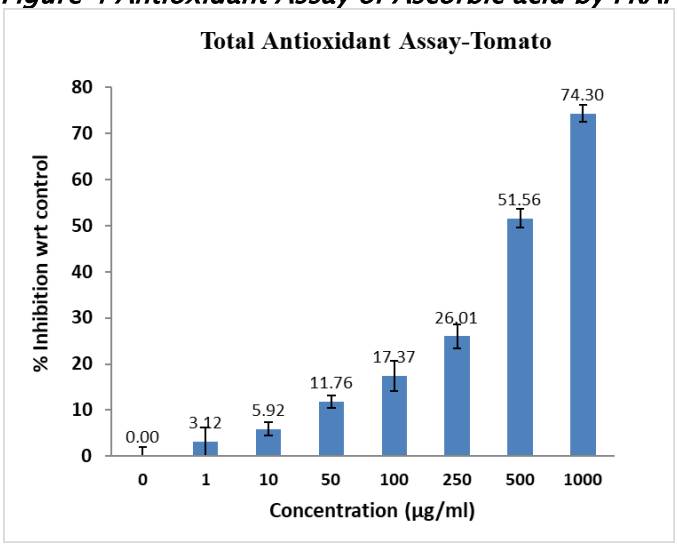

Figure 5 Total Antioxidant Assay for Tomato by FRAP

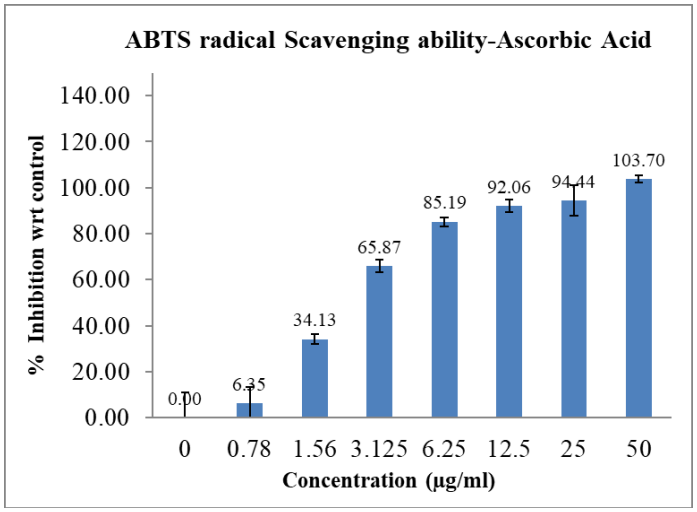

Figure 6 ABTS radical scavenging for ascorbic acid

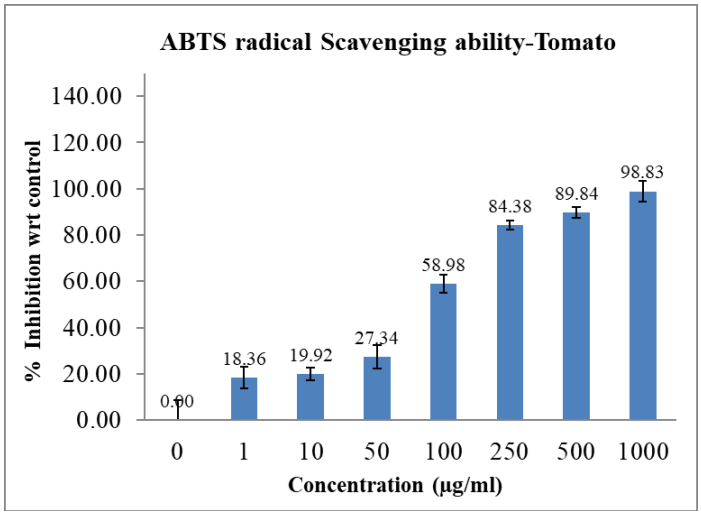

Figure 7 ABTS radical scavenging for tomato

Table 3. Distribution of Inhibitory concentration at 50 % (ABTS)

Sample code	***IC50* value (µg/ml)**
Ascorbic Acid	2.311 ± 0.27
Tomato	78.47 ± 0.10

The ABTS Radical Scavenging Assay was utilized in order to determine the antioxidant activity of the tomato sample. The results of the experiment were used to determine the antioxidant activity of the tomato sample. You may find the inhibitory concentration of fifty percent in table 3, which is shown here. When the sample tomato was compared to the standard ascorbic acid, it was discovered that the sample tomato had a mild influence on the aforementioned standard. After doing the analysis, it was shown that a sample of tomato containing 78.47 micrograms is equivalent to 2.311 micrograms of the standard ascorbic acid.

CONCLUSION

To sum up, the study examined cherry tomato breeding lines' total phenolic content, antioxidant features, antioxidant components, and antioxidant enzyme activity. The tomato samples' considerable antioxidant activity and bioactive ingredients, which may contribute to their potential health benefits, were demonstrated by the results.

Author contributions

Soibam Giri Singh: Visualizing; first draft, Project conception, administration, supervision, validation, and composition. **Maharabam Anandi Devi**: Conceptualization, validation, visualization, and writing, reviewing, and editing support. **L Bormani Singh**: First Initial support and wrote the first edition. Equal validity and content assessment and editing services by **Joykishan Sharma Hanjabam.**

Funding: Not Applicable

Conflict of interest: The authors declare that they have no competing interests.

Ethics approval: Not Applicable. The research work and the report were made in an ethical and responsible manner.

Consent to participate: Not Applicable.

Consent for publication: Not Applicable.

Availability of data and Material: The datasets used and analysed during the current study are available from the authors on reasonable request. The datasets generated and used in our present study are included in this article.

Acknowledgements: The authors would like to thank Dr Y. Sanatombi Devi, Department of Life Science, (Botany) Manipur University for identifying the species of Tomato.

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