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Antioxidant and antimicrobial activity of different varieties of Bangladesh tea and Tocklai vegetative tea (*Camellia sinensis*) clones

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Abstract

Green tea's health-promoting properties are primarily attributed to its phytochemical content and antioxidant properties. The study was carried out to evaluate the antioxidant and antibacterial activities of green teas manufactured from three Bangladesh tea (BT) and three Tocklai vegetative (TV) teas. Antioxidant activity was estimated by the determination of DPPH free radical scavenging activity (DRSA), ferric reducing antioxidant power (FRAP), total phenolic content (TPC), and total antioxidant capacity (TAC). BT2 exhibited the maximum DRSA of 89.17%, FRAP of 122.15 mg GAE/g, TPC of 95.75 mg GAE/g and TAC of 46.71 mg GAE/g among all clones. The antimicrobial activities of these teas were analyzed against Gram-positive bacteria *Bacillus* spp. and Gram-negative bacteria *Escherichia coli*. The extracts of TV13 and BT2 showed a better zone of inhibition (ZOI) of 14.33 and 16.33 mm against *Escherichia coli* and *Bacillus* spp. The methanolic extracts of all samples exhibited potential antioxidant and antibacterial activity. These tea extracts can be used as an alternative to synthetic antioxidants and antimicrobial agents in therapeutics and the food industry.

1. Introduction

Tea is an aromatic non-alcoholic beverage that has been established in Bangladesh since 1854. However, the cultivation of tea was first started in Chittagong in the 1840s with China plants from the Calcutta botanical garden and a few Assam plants (Aziz *et al.,* 2011). Tea is an evergreen plant that originated in China, spread to India and Japan, and then to Europe and Russia before arriving in the United States in the late seventeenth century (Sharangi, 2009). In Bangladesh, good-quality black tea is commonly produced. Black tea is a fermented tea that has been oxidized in the open air to turn the leaf black through the development of important chemical compounds. Theaflavin and thearubigin are produced during this period those are responsible for the flavor and color of tea liquor (Pou, 2016). The black tea is produced by the CTC (Crush, Tear, and Curl) technique which can ensure the formation of granules and can also provide the best possibility for oxidation (Gohain *et al.,* 2012). However, green tea is not subjected to the same withering and oxidation processes as oolong and black teas. Green tea is one of the least processed teas as it is prepared from unoxidized leaves. As a result, it has the highest amount of antioxidants and polyphenols that are beneficial to health.

Tea is known as the "Master of Chemical Diversity" since its full chemical composition has yet to be found. Fresh leaves contain 30-40% polyphenol, 6% amino acid, 11% carbohydrate, 5% methylxanthine (bitter taste), minerals (aluminium, arsenic, fluorine, iodine, manganese, nickel, potassium, and selenium), 0.01% volatiles (aroma and flavor), and color pigments such as chlorophyll and carotenoids that give the beverage its distinct characteristics (Dufresne and Farnworth, 2001). The Bangladesh Tea Research Institute (BTRI) contributes to the development of new varieties by investigating, advancing, and institutionalizing quality, and by sharing its exploratory discoveries with the tea industry (Aziz *et al.,* 2011). By manipulating the current population, it has already released 20 clones (Islam *et al.,* 2005). The emerging and established plant gardeners both are interested in taking those clones that satisfy the yielding as well as the quality attributes of tea (Islam *et al.,* 2013). Since 1949, 31 Tocklai vegetative (TV) tea

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clones have been developed for commercial purposes at the Tocklai Experimental Station in Jorhat, Assam, India (Thakur *et al.,* 2011).

Natural antioxidants can protect the human body against free radical damage and chronic illnesses, as well as lipid oxidative rancidity in food (Sarkar, Rahman, Sarkar *et al.,* 2020). Synthetic antioxidants are harmful to health because of their toxicity and cancer-causing nature (Alam *et al.*, 2020; Hossain *et al.,* 2021; Roy, Imran, Alam *et al.,* 2021). As a result, there is an urgent need to replace synthetic antioxidant and antibacterial compounds with natural products (Roy, Ullah, Alam *et al.,* 2021; Sarkar, Ahmed, Alam *et al.,* 2020; Sarkar *et al.,* 2021). The most basic requirement of life is safe and secure health; nevertheless, as a growing number of bacterial species are developing resistance to antibiotics and re-emerging infectious pathogens, it is necessary to develop alternative substances with novel modes of action and chemical structures (Dever and Dermody, 1991). Nowadays, there is a lot of research continuing to find novel products with multifunctional properties like antioxidants and antimicrobials. To protect themselves from outside influences, the plants produce secondary metabolites. As a result, plant extracts are the major natural source for discovering new chemicals with unique antibacterial mechanisms as well as antioxidant activities (Angiolella *et al.,* 2018).

Tea has many medicinal properties as well as health benefits. Medicinal properties of tea include anticancer, anti-inflammatory effect, antioxidant, antiviral, antihelminthic and antimicrobial (Benzie and Wachtel-Galor, 2011). Utilization of natural products such as teas as an antioxidant or antimicrobial agent is safe and beneficial to health (Gyawali and Ibrahim, 2014); but, its applications are limited because of just little study to date. The objectives of the present study were to determine and compare the antioxidant and antibacterial properties of three Bangladesh Tea (BT) and three Tocklai Vegetative (TV) tea clones.

2. Materials and methods

2.1 Sampling

Leaf samples of three BT and three TV tea clones were collected from the experimental tea garden of the Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology, Sylhet. The clones used in this study are BT1, BT2, BT6, TV9, TV13 and TV23. Finally, green teas were made by following the standard procedure of Qin *et al.* (2022).

2.2 Preparation of sample extract

The method of Saikia *et al.* (2015) was used for the extraction of the samples. Briefly, 10 g samples were extracted with 100 mL 70% methanol at a 1:10 ratio (sample: solvent) in a shaking incubator (SI-200, Korea) at 20°C for 90 mins. The extract was then centrifuged at 3,000 rpm for 15 mins. The supernatants were carefully collected and stored at -20°C for further investigation.

2.2.1 Total phenolic content

The total phenolic content (TPC) was determined by following the method of Turkmen *et al.* (2007). Briefly, 1 mL extract was introduced into test tubes followed by 1.0 mL of Folin Ciocalteu's reagent (diluted 3 times with deionized water) and 2.0 mL of sodium carbonate (35%, w/v). The mixture was shaken thoroughly and diluted to 6 mL with deionized water. The mixture was kept for 30 mins (at room temperature), after that, the absorbance was measured at 700 nm using a UV-Vis spectrophotometer (Model-UV-1800, Shimadzu Scientific Instruments, Japan).

2.2.2 DPPH radical scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was estimated by the method of Turkmen *et al.* (2007). Antioxidant activity was calculated as percentage inhibition (% I) of the DPPH radical and was determined by the following equation:

% I = [1 − absorbance of the sample/absorbance of the control] × 100.

2.2.3 Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) of tea extracts was estimated using the procedure of Turkmen *et al.* (2007). Briefly, 0.5 mL extracts were added to 1.25 mL phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of potassium ferric cyanide (1%, w/v). The solution was incubated at 50°C for 20 mins and after that trichloroacetic acid solution (1.25 mL, 10%, w/v) was added. The mixture was then separated into aliquots of 1.25 mL and diluted with 1.25 mL of water. To each diluted aliquot, 0.25 mL of ferric chloride solution (0.1%, w/v) was mixed. After 10 mins, absorbance was taken at 700 nm.

2.2.4 Total antioxidant capacity

Total antioxidant capacity (TAC) was estimated by using the method of Banerjee *et al.* (2005). The assay is based on the reduction of molybdenum (VI)-molybdenum (V) [Mo (VI)–Mo (V)] by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. Briefly, 0.1 mL extracts were mixed with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was incubated at 95°C for 90

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mins and then cooled to ambient temperature. Finally, the absorbance was taken at 695 nm.

2.3 Antimicrobial activity test

Approximately 10 g of each of the powdered samples were extracted with 100 mL 70% methanol at room temperature (20°C) in a shaker for 2 days to produce crude extracts containing active compounds.

2.3.1 Preparation of culture media

Nutrient agar is used where a solid culturing media is needed. Nutrient agar was freshly prepared. For 1000 mL of solution, 28 g of nutrient broth was needed. It was autoclaved before use.

The test organisms were collected from the Dept. of Genetic Engineering and Biotechnology, SUST to investigate the potential activity of the extracts. They include Gram-positive bacteria *Bacillus* spp. and Gram-negative *Escherichia coli*. Each bacterium was first sub-cultured at 37°C for 24 hrs in nutrient agar media. A standardized inoculum of each bacterium was spread with the help of a sterilized cotton bar onto a nutrient agar plate to achieve a confluent growth.

2.3.2 Inoculation of test plates

A sterile cotton swab was dipped into the suspension, excess fluid was removed by pushing and rotating the swab firmly against the wall. The entire dried surface of the Nutrient agar plate was streaked by the swab 2-3 times; which results in an even distribution of the inoculum over the entire surface. This process was run in the aseptic condition (Goyal *et al.,* 2008; Pandey *et al.,* 2011).

2.3.3 Antimicrobial activity of different tea extracts

The antibacterial activity of different tea extracts was determined by the disc diffusion method of Bonev *et al.,* (2008). Blank discs were soaked into the extracts in a Petri plate for about 2 hrs, then the discs were ready to use. Discs were placed onto the inoculated nutrient agar media. The plates were kept standing for 1 hr or more for diffusion to occur and after that incubated at 37°C for 24 hrs. Ciprofloxacin (5 µg/disc) was used as a positive control test. This antibiotic (ciprofloxacin) is effective only for bacterial infections. After 24 hrs of incubation, each plate was examined. There was a uniformly circular zone of inhibition on the media surface. The diameters of the zones were measured using a millimeter scale.

2.4 Statistical analysis

Data were analyzed using SPSS software (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were used to analyze the statistical difference. Differences with p-values < 0.05 were considered statistically significant.

3. Result and discussion

3.1 Antioxidant activities of Bangladesh tea and three Tocklai vegetative clones

Phenolics, which are secondary metabolites of plants, have been shown to help people's health by regulating cellular processes and acting as antioxidants. Due to their multiple health benefits, such as free-radical scavenging, coronary heart disease prevention, anticancer, and antiviral properties, researchers are becoming increasingly interested in phenolics found in diverse sources of dietary supplements. Tea contains polyphenols, which act as antioxidants in the body, aiding in the detoxification of free radicals and the protection of cells (Azadi Gonbad *et al.*, 2015). BT2 showed a significantly higher TPC of 95.75 mg GAE/g than those of other brands of tea. The second highest TPC was found in the BT1, while the third highest content was found in the TV23. The TV13, TV9, and BT6 tea clones had TPC in the range of 56.89 to 75.05 mg GAE/g. All the tea clones contained total polyphenols in the range of 56.89 to 95.75 mg GAE/g (Figure 1). TPC is also positively correlated with other antioxidant activities. Variations in clones, genetic contents, geographic location, and cultural influences could all contribute to these disparities. Lower TPC levels could be due to chemical compound mobility inside the plant and changes in leaf morphology as the plant matures (Kc *et al.*, 2020). Tea's TPC might vary according to how it is harvested, handled, processed, and brewed (Yasin *et al.*, 2020). These findings comply with the findings of Nibir *et al.* (2017). Tea polyphenols have strong antioxidant properties and DPPH activity due to the possession of a phenolic hydroxyl group linked to the flavan-3-ol structure (Izzreen and Fadzelly, 2013; Kaur *et al.,* 2015).

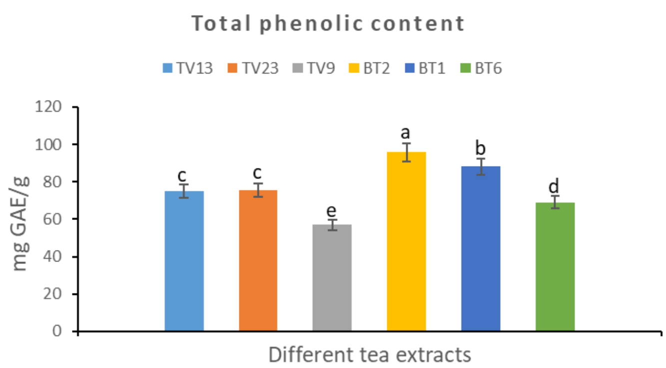

Figure 1. Total phenolic content of different tea clones. Error bars indicate mean±SD of three replicates. Bars with different notations are statistically significantly different (P<0.05).

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A fast, straightforward, and reasonable strategy to quantify the antioxidant capacity of tea involves the utilization of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH was generally used to evaluate the capacity of compounds to react as free radical scavengers or hydrogen donors and to estimate antioxidants. Free radicals are directly or indirectly responsible for several diseases in humans such as cancer, neurodegenerative diseases, angina pectoris and atherosclerosis (Lobo *et al.*, 2010). Antioxidants possessing free radical scavenging activity are beneficial for reducing or preventing diseases. BT2 showed the maximum DPPH-radical scavenging activity of 89.17%. The next one was TV13, which contained 88.72%. For the others, values ranged from 85.13 to 87.75% (Figure 2). This finding complies with the finding of Nibir *et al.* (2017).

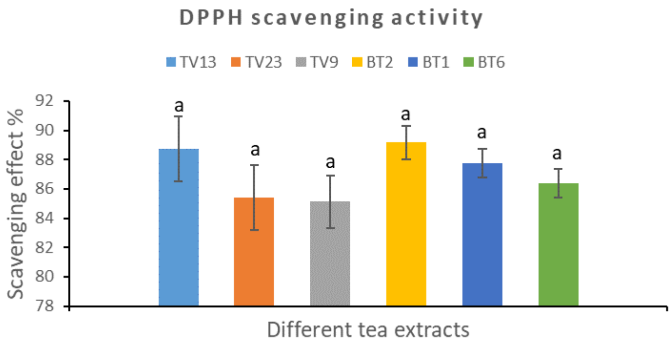

Figure 2. DPPH scavenging activity of different tea clones. Error bars indicate mean±SD of three replicates. Bars with different notations are statistically significantly different (P<0.05).

The range of total antioxidant capacity of the tea clones was found 32.28 mg GAE/g to 46.71 mg GAE/g where maximum TAC was found in BT2, and it was 46.71 mg GAE/g. The lowest amount of TAC was found in BT6 which was 32.28 mg GAE/g (Figure 3). These differences could be attributed to variations in clones, genetic constituents, geographic location, and cultural factors.

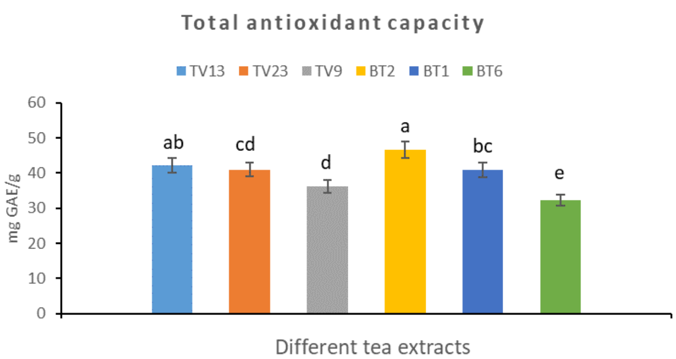

Figure 3. Total antioxidant capacity of different tea clones. Error bars indicate mean±SD of three replicates. Bars with different notations are statistically significantly different (P<0.05).

BT2 exhibited the highest 122.15 mg GAE/g FRAP. The FRAP of other clones ranged from 109.43 to 122.15 mg GAE/g (Figure 4). The phosphomolybdenum assay normally quantifies antioxidants such as phenolics, carotenoids, α-tocopherol, and ascorbic acid. Total antioxidant capacity (TAC) and ferric reducing antioxidant power (FRAP) of BT2 were also comparatively higher than all other varieties. Our study showed that there is a strong correlation between total phenolic content and total antioxidant capacity which indicates that polyphenols have antioxidant properties to protect against oxidative damage (Izzreen and Fadzelly, 2013).

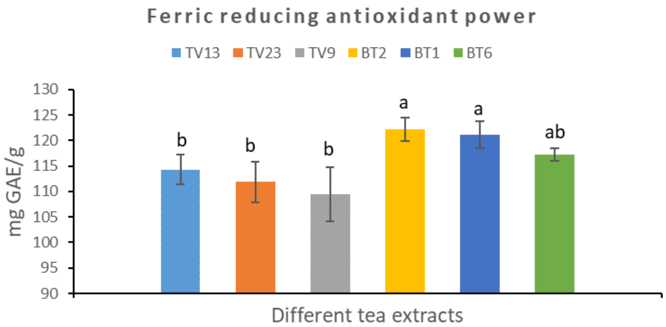

Figure 4. Ferric reducing antioxidant power of different tea clones. Error bars indicate mean±SD of three replicates. Bars with different notations are statistically significantly different (P<0.05).

3.2 Antimicrobial activity

Six different types of tea clone extracts of 100 mg/mL were prepared. The extracts of TV13 and BT2 showed a better zone of inhibition 14.33 and 13.67 mm respectively at 100 mg/mL against *Escherichia coli,* and extracts of BT2 and TV23 showed better zones of inhibition of 16.33 and 13.00 mm respectively against *Bacillus* spp. A study on the disc diffusion method showed that the potential activity of TV13 tea clones against *E. coli* is highest among other examined tea clones and the zone of inhibition (ZOI) is 14.33 mm. On the other hand, the potential activity of the BT2 tea clone against *Bacillus* spp. is highest and the zone of inhibition (ZOI) is 16.33 mm (Table 1). All six clones inhibited microbial growth. The extracts of TV13 and BT2 showed a better zone of inhibition against *Escherichia coli.* The extracts of BT2 and TV23 exhibited a better zone of inhibition against *Bacillus species.* These variations might be due to the different clones, geographical location, and cultural variables. Our results comply with the findings of several earlier research demonstrating the antimicrobial activities of green tea (Almajano *et al.,* 2008; Bancirova, 2010). It was mentioned earlier that all six samples were green tea, and the green tea extract was more effective in inhibiting microbial pathogens. Green tea extracts show

Table 1. Antimicrobial activity of different tea extracts.

Tea Clones	Concentrations (mg/mL)	ZOI (mm) for *E. coli*	ZOI (mm) for *Bacillus* spp.
TV13	100	14.33±0.05[a]	12.33±0.10[b]
TV23	100	12.67±0.03[ab]	13.00±1.05[b]
TV9	100	9.00±1.20[d]	9.67±1.15[c]
BT2	100	13.67±0.09[a]	16.33±0.85[a]
BT1	100	11.00±0.75[bc]	12.00±0.95[b]
BT6	100	10.67±0.69[cd]	11.33±0.07[bc]

Values are presented as mean±SD of three replicates. Values with different letter in a column differs significantly at P < 0.05.

antimicrobial activity due to the presence of catechins, especially epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) (Tahir and Moeen, 2011; Akter *et al.,* 2015).

4. Conclusion

The BT2 tea clone outperformed the other tea clones in terms of antioxidant and antibacterial activities. TV13 also performed better antibacterial activity against *Escherichia coli* and showed better antioxidant properties. Most antioxidant activity tests revealed a substantial connection with TPC. Considering the importance of antioxidants in the treatment of a variety of degenerative diseases, it can be concluded that Bangladesh teas (BT) are a good source of antioxidants and may be used as an important dietetic. According to the findings, these tea clones could be a very promising natural antioxidant as well as an alternative to synthetic antibacterial agents in therapeutics and the food industry.

Conflict of interests

The authors declare no conflict of interests

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