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Biochemical assessment of CTC-black tea manufactured by bought leaf factories from leaves of small tea growers in Terai, West Bengal

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Abstract
In the past two decades, India's tea industry has undergone significant changes, marked by the rise of small tea growers, establishment of bought leaf factories, and increased competition from foreign markets. Small tea growers now contribute over 50% of total tea production, with their leaves processed by bought leaf factories. However, the quality of tea from bought leaf factories, particularly CTC-black tea, lacks thorough biochemical characterization compared to tea from larger gardens. A study was conducted to address this gap, revealing differences in chemical composition between tea from small gardens and large tea estates. The findings revealed lower phytochemical qualities in bought leaf factory-produced tea, indicating unorganized practices in small tea industry. These results are preliminary but alarming for the future of small tea growers. Thorough survey, training programs and corrective measures in both field management and factory processing are needed to resolve these quality issues from the root.

Keywords: Made tea, small tea growers, bought leaf factory, physicochemical, antioxidant

Introduction
A significant transformation in the tea industry of India was observed in the last twenty years with the emergence of small tea growers (STGs), establishment of bought leaf factories (BLFs), competition from foreign markets etc. The emergence of small tea growers (STGs) in Terai was based on commercial importance of tea and stability of income from this business as a profitable venture. Farmers of Terai and other tea growing regions of West Bengal, India converted a major portion of their agricultural land into tea gardens which were earlier used to cultivate grain crops, vegetables and fruits (mainly pineapple in Terai region) [1-3]. Among other factors, the menace created by wild animals in agricultural pockets influenced the expansion of small tea plantations which further succeeded through viability of sub-Himalayan foothill soil to yield tea; for example, elephant invasion in agricultural fields of Dooars region became as a major threat to the farmers who have shifted from vegetable (mainly potato) to tea cultivation [3]. There are about two-hundred and eighty-three tea estates located in the Dooars-Terai region of North Bengal who have their own in-garden tea manufacturing factories. While fifty thousand small tea growers of North Bengal reportedly do not have any manufacturing units of their own who earlier used to sell their leaves to set gardens [3, 4]. However, this process did not run long due to various conflicts which were bundled together and labeled as a new concern i.e., standard of the product or leaf quality. The emergence of bought leaf factories (BLFs) provided a comparatively profitable market for small tea growers, although their progress depended on the availability of raw leaves from middlemen or leaf agents who buy green leaves from STGs and sell those to BLFs. Consequently, tea estates and small tea growers were divided and separated them as two major sub-industries within the tea industry of India [4]. BLFs of Terai have successfully addressed the processing needs of small growers of this region and today they are playing crucial role in this value chain to be an integral part of this “small tea industry”. Overall, the rise of STGs and the establishment of BLFs reflect the changing dynamics of the tea industry in India, particularly in Terai, and its forward linkage effects (https://www.bizzbuzz.news/industry/agriculture/small-tea-growers-touch-5191-of-tea-production-bigger-players-crying-foul-1177932). Small tea growers (STGs) are now potential tea producers contributing more than 50% of the total produce. According to latest published reports on estimated tea production by Tea Board of India, STGs of Terai have been identified as more potential tea grower of this region compared to big gardens.

STGs contributed with a magnificent number i.e., 78.4% of total tea production of Terai region in the year of 2022-23 (https://www.teaboard.gov.in/pdf/Production_2022_and_2022_23_pdf7127.pdf) [3]. Therefore, its high time to evaluate the produce to judge the overall quality of the major share not only from consumers' perspective but also for the future economy and mankind. Sarkar *et al.* [5] already reported phytochemical quality of green leaves collected from STGs this region and its now essential to evaluate their manufactured tea to judge their suitability in domestic and foreign market, for comparative analysis from consumers' perspective, for recommendations with corrective measures, for training of STGs and BLFs and other future strategies.

Tea as a drink is an aromatic and most consumed brew in the world, which is commonly prepared as either infusion- pouring hot water in pot containing manufactured dried leaves of tea plant; or decoction- boiling manufactured tea leaves with water (additives- sugar, milk, spices, butter, honey etc. added sometimes on preference). Tea has been consumed socially and habitually by people since 3000 BC [5, 6]. The long and glorious history of tea has been celebrated in the scholarly world since long before it became an essential part of the economy in countries like India, China, Sri Lanka, Kenya etc. [5, 6] High-priced delicate made teas such as green tea, orthodox black tea, semifermented oolong and pu'erh tea have been recognized for high antioxidant, anticancer, antiaging, antimicrobial, cardioprotective, hepatoprotective, anti-inflammatory, neuroprotective activities [7-9]. Ongoing scientific exploration points out that certain potential health benefits derived from tea have important implications on human health and are useful in digestion, blood purification, lowering body temperature, strengthening teeth and bones, boosting immune system, enhancing heart function, suppressing aging, lowering the chance of food poisoning etc. [6] Tea is reported to contain nearly 4000 medicinally active components [5] which include, polyphenols, amino acids, proteins, chlorophylls, carbohydrates, volatile flavour compounds, tannin, terpenoids, steroids, alkaloids, vitamins, minerals and other trace elements [6]. Primary compounds belonging to green tea polyphenols are gallic acid, catechin, gallocatechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate [6]. In addition to catechins, tea contain polyphenolic compounds like flavonoids, flavanols, coumarins etc. [9] These polyphenols exhibit potent antioxidant properties and contribute to the overall health benefits associated with green tea consumption [10]. Takeo [11] defined the role of polyphenol oxidase that produce fermented or oxidized flavonoids such as derivatives of various theaflavins and thearubigins which carry the quality of black tea. During the process of manufacturing, oxidative polymerization into theaflavins and changes in catechins provide the characteristic flavor, color and aroma of each type of tea [6, 11, 12].

It has been reported that the number of polyphenols in tea varies depending on the variety, soil and climatic conditions, tenderness of shoot, the age of the plant and the processes applied during its production [6]. So, tea manufactured from different eco-geographical region has unique properties. CTC tea, which stands for Crush-Tear-Curl – tea, is a type of black tea that undergoes a specific processing method including withering, crushing, rolling/curling, fermentation/oxidation, drying and sorting while orthodox black tea leaves are left as whole-leaf or hardly cut but not crushed [6]. Das *et al.* [6] have studied bioactive potentials of comparatively less costly and bestselling marketed Indian black teas (CTC and orthodox black tea) collected from big tea gardens of major tea growing regions of India like Assam, Darjeeling, Terai, Dooars and Nilgiri. Their results showed that antioxidant activity of CTC-tea infusions from Terai and Dooars was low compared to other selected regions but for results obtained from orthodox tea infusions were quite good. According to their report, bioactive groups of metabolites such as cardiac glycosides, total phenol, flavonoids, sterols, amino acids were detected low in Terai's tea samples [6]. Moreover, CTC-tea and orthodox tea collected from Dooars and Terai region showed positive results in antibacterial assays, but inhibition zones produced by them were not very promising [6]. No such research has yet been reported on STGs or BLFs of this region. Therefore, this present study was designed to characterize the manufactured CTC-black tea in a comparative way (in comparison with big garden production as control) through various food-biochemical assessment standardized by our research team which has been conducted to meet the lacunae. The objective of the study was to carry out physical and biochemical characterization of manufactured CTC- tea collected from BLFs of Terai region work.

Materials and Methods

Study area

The study area comprised the west bank of Balason river located in plains of Darjeeling district that included Matigara, Phansidewa and Naxalbari blocks from Siliguri subdivision of Darjeeling District.

Collection of made tea samples

BOP (Broken Orange Pekoe), BOPSM (Broken Orange Pekoe Small), BP (Broken Pekoe), OF (Orange Fannings), D (Dust), and PD (Pekoe Dust) grades of CTC tea were bought from local market which from six local BLFs (F2-F6) and one set garden (F1) (as control for comparative analysis) located in the study area. These bought-leaf factories manufacture CTC-tea from the green leaves bought from all the small gardens of this study area.

Preparation of tea infusions

Different grades of CTC tea (2.5 g) were added in 100 ml of freshly boiled water after lowering its temperature by 100±5 °C and left undisturbed for 5 minutes to prepare the infusions which were evaluated for biochemical and physicochemical properties through the following experiments [6].

Physicochemical analysis

Determination of moisture content

The collected samples of CTC tea were placed in a pre-weighed beaker and dried in hot-air oven at 110 °C for 48 hours [6]. The final weights were then measured and the moisture content (%) was calculated from the given formula.

$$\text{\% of Moisture} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \text{ X } 100$$

Determination of crude fibre content

Determination of crude fibre content of tea leaves was done by following the protocol of Das *et al.* [6] Ghosh *et al.* [13]. Acid and alkali digestion process was followed to determine fibre percentage in a tea leaves. In this process, 2 g (W1) of CTC-tea was taken in a 250 ml beaker where 200 ml of 1.25% sulphuric acid was poured. The beaker was then heated to vigorously boil for 30 minutes. The acid extract was filtered through muslin cloth and repeatedly washed with double distilled water until its pH become neutral. The sediment parts of the acid washed tea leaves were then heated for 30 minutes in 200 ml of 1.25% sodium hydroxide solution for alkali wash. Extract was again filtered through muslin cloth and repeatedly washed with double distilled water until its pH become neutral. Remaining sediments of the sample was again washed with 1.25% of 25 ml sulphuric acid followed by washing with 50 ml double distilled water (three times) and 25 ml ethanol. Obtained fibre was then dried in a hot-air oven at 110°C for 2 hours and the dry weight of the fibre (W2) was measured. Finally, the crude fibre percentage was calculated from the recorded values. And results have been presented as mean of six BLF samples for each grade.

$$\%\text{ of Crude fibre} = \frac{W1 - W2}{W1} \text{ X } 100$$

Determination of Ash content

For determining ash content 5 g (W1) of CTC tea were kept in a pre-weighed crucible and placed inside muffle furnace for 6 hr at 600°C. Weight of the obtained ash was measured (W2) and final ash contained was calculated by using protocol of Das *et al.* [6] and Ghosh *et al.* [13].

$$\%\text{ of Ash} = \frac{W1 - W2}{W1} \text{ X } 100$$

Determination of pH: Then, pH of the infusions were recorded using a previously calibrated pH meter following protocols of Das *et al.* [6]; Majumder *et al.* [14, 15] standardized for tea infusion and beverages.

Qualitative biochemical tests

Following the protocols of Das *et al.* [6], Majumder *et al.* [15-17], presence of various bioactive constituents such as tannin, steroids, cardiac glycosides and coumarin were determined in the tea infusion samples through respective qualitative biochemical tests (Table 1). Table 1 delivers the protocols followed in qualitative tests. A heatmap of the results was prepared in Microsoft Excel (v.2016) for a comparative approach.

Table 1: Protocols of qualitative tests

Group of molecules	Qualitative detection method
Cardiac glycoside	0.5 mL of sample were evaporated and dissolved in 1 mL glacial acetic acid. 1 drop of 10% $FeCl_3$ solution followed by 1 mL of Conc. H_2SO_4 was added by the side of test tube. Appearance of brown colour rings at the interface would indicate the presence of glycosides.
Coumarin	Few drops of NaOH solution was added to 1 mL of sample. Yellow coloration would indicate the presence of coumarin
Tannin	To 0.5 mL sample and few drops of HNO_3 was added. The reddish to yellow colour of the solution would indicate the presence of tannins.
Steroid	For test of 0.5 mL samples were evaporated and dissolved in 2 mL chloroform. 2 mL of concentrated H_2SO_4 was introduced carefully by the sidewall of the test tube. Formation of red colour ring would confirm the presence of steroid.

Quantitative biochemical tests

Total phenol content

The determination of total phenolic content (TPC) was carried out following the procedure outlined by Labar *et al.* [18] and Majumder *et al.* [19]. In this approach, sample extracts (100 µl) were placed in clean test tubes and allowed to incubate at room temperature for a duration of five minutes after the addition of 400 µl of 10% Folin-Ciacalteu reagent. Subsequent to this incubation, 1 ml of 5% Na_2CO_3 was introduced, and the mixture was further incubated at room temperature for a period of 120 minutes. The quantification process involved measuring absorbance at 730 nm using a UV-Vis spectrophotometer (Agilent Technologies Cary 60 UV-Vis). For quantification, a standard curve prepared from gallic acid was employed ($R^2 = 0.998$; $y = 0.0043x - 0.1672$) and results were expressed as gallic acid equivalent (µg GAE/ml) [20] and also as per cup (mg/ 100ml). The data were expressed as means of three replicates ± Standard Deviation (SD).

Quantification of flavonoids

The determination of total flavonoid content was done following the protocol of Bhattacharya *et al.* [21] and Sarkar *et al.* [6]. For each tea clone, a sample extract of 250 µl was pipette-inserted into clean a test tube. Subsequently, 1.25 ml of double-distilled water was added, followed by the introduction of 75 µl of 5% $NaNO_2$. After an incubation period of five minutes, 150 µl of 10% $AlCl_3$ was added into the samples. The test tubes were then maintained at room temperature for a duration of six minutes. The subsequent steps involved the addition of 500 µl of 1 mM NaOH, followed by the introduction of 275 µl of double-distilled water. Following a final incubation period of 30 minutes, the absorbance was quantified using a UV-Vis spectrophotometer (Agilent Technologies Cary 60 UV-Vis) at a wavelength of 510 nm. A standard curve was established employing quercetin ($R^2 = 0.962$; $y = 0.207x - 0.204$). The resulting flavonoid concentrations were then denoted as milligrams of quercetin equivalent per milliliter of the sample (mg QE/ml) [15].

***In vitro* antioxidant activity (DPPH scavenging assay)**

The assessment of DPPH (2,2-Diphenyl-1-picrylhydrazyl) scavenging activity was carried out following the methodology outlined by Bhattacharya *et al.* [22] with a slight modification as detailed here. Each sample extract of 200 µl, was added to 2800 µl of freshly prepared methanolic DPPH solution already taken in test tubes. This mixture was then left at dark condition for half of an hour for letting the reaction/scavenging to complete. Subsequently, the absorbance (abs) was recorded at 517 nm in Agilent's Cary 60 UV-Vis spectrophotometer [14-17]. For calibration, ascorbic acid was employed to generate a standard curve ($R^2 = 0.993$; $y = 0.876x + 6.591$). To quantify the DPPH scavenging activity, the following formula was applied.

$$DPPH\ scavenging\ \% = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \text{X100}$$

Statistical analysis: Data obtained from the various experiments during this research of three consecutive years were analyzed statistically using Microsoft Excel (v.2016) and results have been expressed as mean ± SD (n = data of 3 years). Respective heatmaps and graphs of experimental results were also prepared in Microsoft Excel (v.2016).

Results and Discussion

Samples of manufactured tea from BLFs

Based on the quality and price, the grades (BOP, BOPSM, BP, OF, D and PD) considered in this research have been arranged in Table 2. Furthermore, these tea samples were collected from six BLFs where STGs of the study area (west Bank of Balason river, Terai region, West Bengal) sell their harvest. One set garden factory has been considered in this research as for control. In results, set garden's sample has been coded as F1 and F2-F7 samples for BLFs. Table 2 shows prices (bulk selling or wholesale price of CTC tea) of different grades of CTC tea which were recorded during sample collection. It shows tea produced by set gardens or tea estates are sold at higher prices compared to the ones by bought leaf companies.

Table 2: Different types of CTC grades

Grades	Full form	Made tea wholesale price (Rs./kg)	
		Bought leaf factory	Tea estate
BOP	Broken Orange Pekoe	100-110	255-260
BOPSM	Broken Orange Pekoe Small	115-118	260-270
BP	Broken Pekoe	120-125	270-280
OF	Orange Fannings	118-120	265-275
D	Dust	90-100	210-220
PD	Pekoe Dust	110-115	220-230

Moisture content

The moisture content of tea varies depending on various factors such as the temperature, processing timeframe, humidity, drying, packaging, effect of storage conditions and impact of grade quality. The considerable moisture percentage of CTC tea is around 3-4% [23]. In this research, the product of set garden revealed slightly less moisture % than that of BLFs (Table 3). The range of moisture contents of CTC tea produced from BLFs were in a range between 3.91% to 6.91% (Table 3) which is almost higher than the desired value. The moisture content in tea leaves varied among different factories and grades. Among the set tea garden samples (F1), orange fannings (OF) grade was found to contain the lowest moisture (2.27%), while sample F2 (BLF sample) showed the highest moisture percentage at 6.91% in the PD grade. Lower moisture levels are preferred in tea because a high level of moisture has negative impacts on tea quality i.e., causing microbial growth and decreasing shelf-life. Das *et al.* [6] recorded moisture in a range from 0.5% to 3.6% in CTC-tea samples collected from set gardens the same region. Overall, only three samples- specifically of OF grades from F1, F6 and F7 revealed acceptable moisture content. Previously, Shchegoleva *et al.* [24] reported higher moisture content in dust tea compared to manufactured black tea. According to them microparticles of tea dust have a large total surface area, which increases the rate of moisture exchange with the surrounding air which might affect the moisture content of the material. Table 3 revealed that grades such as D and PD had comparatively higher moisture content than quality grades which corresponds to the reports of Shchegoleva *et al.* [24]. Compared to BLFs, tea manufactured by set gardens contains less moisture (around 2-4%) which reflects the better quality as well.

Table 3: Moisture (%) content of different grades of CTC tea F1 (Set garden) and F2-F7 (Bought tea leaf factory)

Source	BOP	BOPSM	BP	OF	D	PD
F1	3.52±0.158	3.01±0.148	2.27±0.121	2.86±0.131	4.12±0.152	4.36±0.162
F2	5.83±0.21	4.95±0.18	5.88±0.20	5.69±0.20	6.74±0.26	6.91±0.21
F3	5.75±0.16	6.2±0.22	4.91±0.16	4.53±0.16	6.74±0.26	6.79±0.26
F4	6±0.24	6.36±0.23	4.81±0.16	5.66±0.20	6.5±0.25	6.79±0.28
F5	5.85±0.22	5.9±0.21	4.86±0.16	4.89±0.19	6.31±0.24	6.68±0.31
F6	6.03±0.23	6.52±0.23	5.37±0.18	3.96±0.17	6.26±0.23	6.57±0.39
F7	4.84±0.23	5.81±0.21	5.57±0.19	3.91±0.14	6.25±0.24	6.66±0.42

Crude fibre content

Fibre is one of the most important parameters or indicators that judges the quality of made tea. Tea with low fibre content is considered as of better quality. Being a plan-leave derived product, made tea usually contains fibres of different types such as lignin, cellulose, hemicellulose, pectin etc. that cannot be digested and assimilated by the human gastrointestinal tract [25]. In tea industry and for consumers, fibre in tea is considered adulterant which results in more tannin content in tea and it is undesirable. Crude fibre analysis is also the most frequent quality assessment test that is done to judge the product. Crude fibre of CTC tea is composed of mainly stalks, hard parts of the mature leaves and woody stems (cellular walls, sclerenchyma, collenchyma and transporting tissues) which are devoid of green leaf's phytochemicals that found high in tender leaves and buds. A certain amount of manufactured tea having crude fibre of more than 15% is considered as detrimental [6]. Śmiechowska and Dmowski [25] previously assessed crude fibre content in tea imported to Poland from China, India and Malawi. They also mentioned that 15% should be the margin for crude fibres to maintain the minimum quality considered as acceptable. Results suggests that only tea samples from set garden contained more than 15% crude fibre (that too in its cheapest grade- PD) while other grades passed the test. While in BLF samples, both D and PD showed significantly high crude fibre content (16.57-17.89%). Overall, crude fibre content was high in BLF samples compared to set garden ones and among different grades good results (low to high crude fibre content) were detected in this order BP < BOPSM < BOP < OF < D < PD (Fig 1).

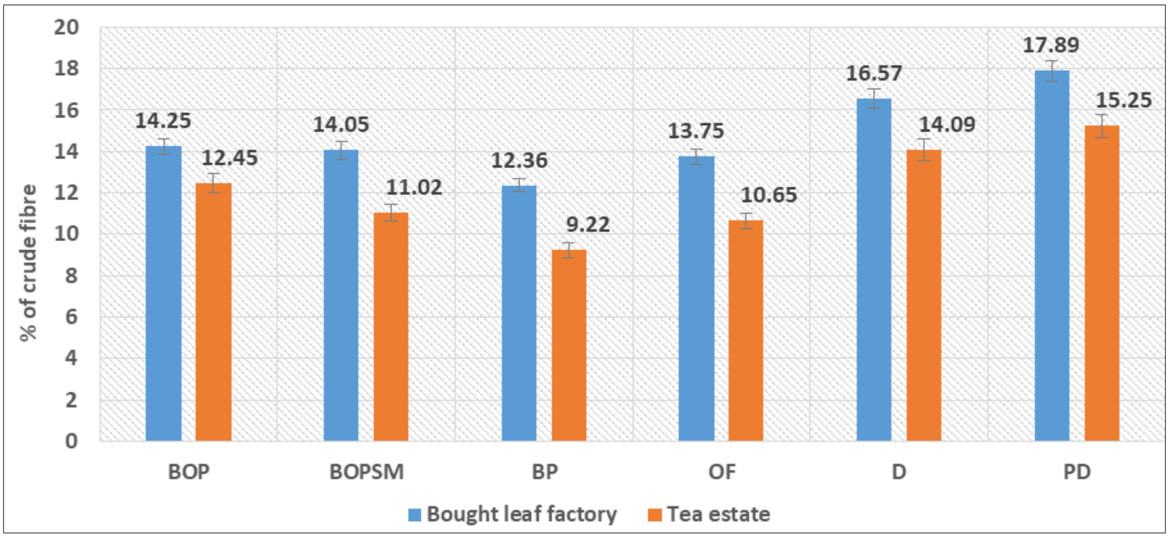

Fig 1: Crude fibre content of made tea collected from bought leaf factories compared to set garden

pH of CTC-tea infusion

Analysis of pH was considered as a physicochemical parameter as it is helpful to judge the quality and acceptability of manufactured tea for human consumption. The pH of tea can vary depending on various factors such as the type of tea, the brewing time, water quality, and additives. However, generally, the pH of tea falls within the acidic range [6]. In this research, pH of black tea infusions prepared from BLFs' CTC-tea were found to be ranged between 5.1±0.016 to 5.38±0.056, making it slightly acidic (Table 4) and comparable to previous reports [6]. Tea estate manufactures their tea (considered as control in this research) in the most organized way in their factories. That is why, pH obtained from tea infusions of set garden's CTC-tea were higher than the samples from BLF (Table 4). A grade-wise gradual change in pH was not observed in tea samples obtained from bought leaf tea factories (except F7) as found in set garden samples.

Table 4: pH of tea infusion prepared from different grades of CTC tea, F1 (Set garden) and F2-F7 (Bought tea leaf factory)

Source	BOP	BOPSM	BP	OF	D	PD
F1	5.4±0.086	5.33±0.091	5.3±0.097	5.22±0.090	5.19±0.072	5.18±0.071
F2	5.19±0.064	5.20±0.059	5.18±0.063	5.15±0.064	5.14±0.056	5.12±0.061
F3	5.17±0.055	5.19±0.059	5.20±0.055	5.15±0.042	5.12±0.031	5.1±0.016
F4	5.15±0.054	5.18±0.052	5.27±0.092	5.26±0.083	5.14±0.047	5.17±0.032
F5	5.19±0.057	5.21±0.052	5.26±0.057	5.25±0.051	5.18±0.064	5.14±0.058
F6	5.27±0.090	5.31±0.097	5.27±0.091	5.26±0.090	5.26±0.037	5.24±0.063
F7	5.29±0.053	5.38±0.056	5.32±0.060	5.22±0.053	5.2±0.058	5.19±0.059

Results of pH test revealed that the acidity of infusions prepared from all grades collected from all factories were in a considerable range i.e., pH 5.4 – pH 5.1. Experiment by Das *et al.* [6] revealed low pH- ranged between 5.29 – 5.54 in tea infusion prepared with CTC-tea from various tea growing regions of India including Terai region. CTC manufacturing involves the process of fermentation which leads towards production of organic acids by enzymatic reactions. It has already been reported that tea contains significant amount of oxalic acid [26] and malic acid [27]; citric, isocitric, and succinic acids [26]; shikimic acid, which is important for the biosynthesis of the polyphenols [28]. Therefore, an acidic condition in oxidized CTC-black tea is valid. Quality grades such as BOP, BOPSM and BP revealed a pH that was comparatively higher than pH of infusions of cheap grades like D and PD. A probable reason behind the lower pH of dust and PD grades' infusions could be presence of high tannin. High tannins or tannic acids are undesirable in a quality cup of tea. Generally, low quality grades and longer-steeped or re-steeped brews [29] are considered to contain more tannins or tannin acids. Tannins are wood products which are responsible for acidic pH [30] of woods of a plant. These low quality grades such as dust and PD contain more stalks and hard shoot parts than tender leaves where occurrence of wood product like tannins could play the role in lowering the pH of infusion.

Interestingly, for control sample or F1 (set garden), grade PD (followed by D) was found to exhibit the least quality while BP scored best results throughout the tests done so far (% moisture, % crude fibre and pH) which was corresponding to the class of price and quality. The pH values of set tea garden's made tea also revealed a gradual decrease of pH towards smaller size of grades from higher. While overall BLF samples did not reveal such organized results might reflecting unorganized practices from field (uneven plucking, pruning and other plant managements) to factory.

Qualitative detection of tannin, steroid, cardiac glycoside and coumarin

Tannins are a class of polyphenolic compounds [31] found in various plants including tea. CTC tea predominantly contains condensed tannins which contribute to the astringency and color of the tea infusion of which most pekoe dust grade CTC teas generally have high tannin content, which can contribute to a pronounced bitter taste [32]. The sample from bought leaf factor- F6 appeared to be an exception, as its tea exhibited very low tannin contents in all grades. This suggests that tea from this factory, specifically in the CTC form, are less bitter in comparison to their pekoe dust counterparts. Sample F2's orange fanning grade stood out with a very high amount of tannin. This grade is likely to have an intense bitter taste due to the elevated tannin levels and the pH was also found to be low (Table 4). In the majority of quality CTC grades (BOP, BOPSM, BP and OF), tannin content fell within the medium to low range. This suggests that these grades are likely to have a less bitter, but astringent taste compared to pekoe dust grades. A very high amount of steroids in infusions prepared with CTC tea of the grades from the bought leaf factory- F6 has been recorded (Fig 2). Cardiac glycosides were found low in the tested samples. Highest cardiac glycoside was observed in the pekoe dust grade of sample F2 (Fig 2).

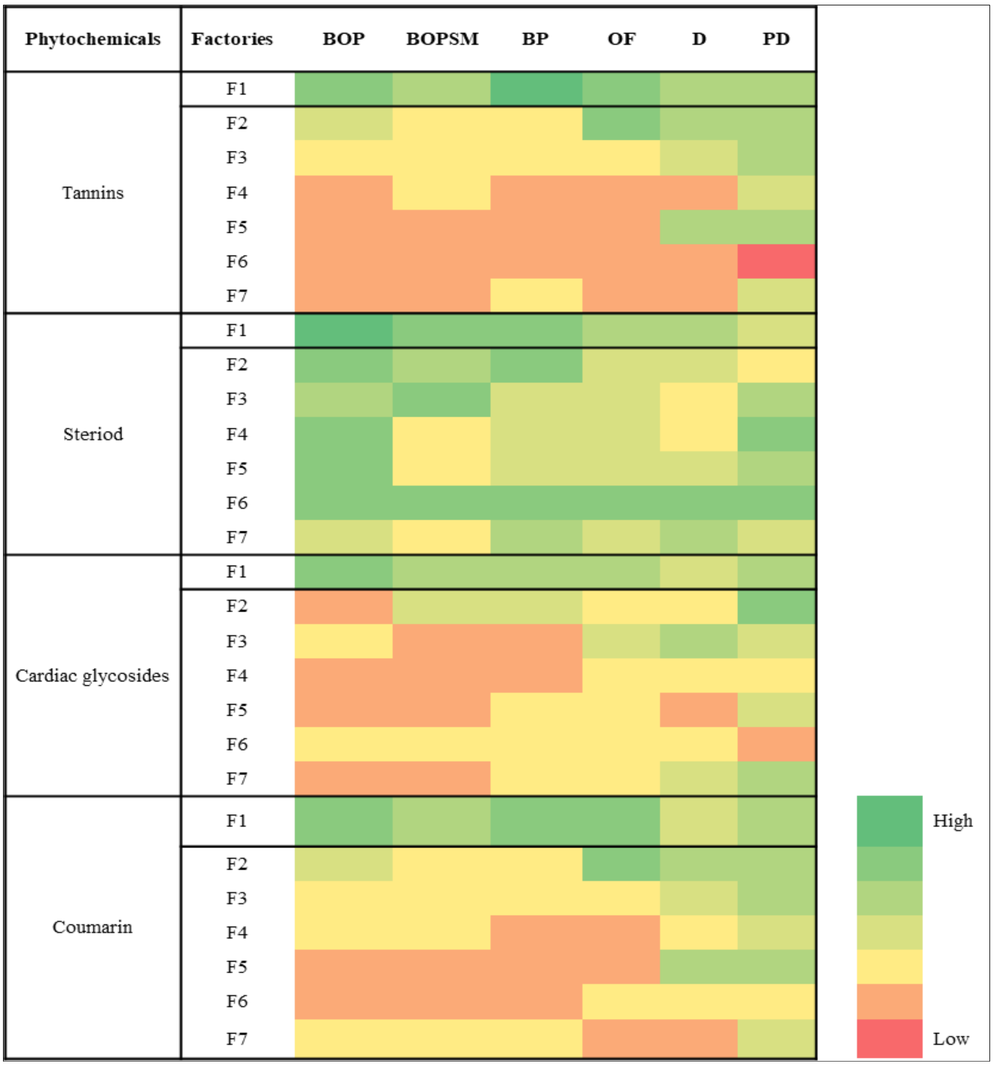

Fig 2: Qualitative assessment of CTC tea F1 (Set garden) and F2-F7 (Bought tea leaf factory)

Total phenol content

Bhuyan *et al.* [33] reported that CTC-black tea contains high phenolic compounds including tannin and coumarin. The phenolic content in the different grades of CTC tea collected from various factories varied significantly, ranging from 642.18±8.414 µg GAE/ml [around 65 mg/ cup] to 947.6±4.267 µg GAE/ml [around 95 mg/ cup] (Table 5). This indicates that the phenolic content can be quite different depending on the type of grade and where it was produced. The highest phenolic content among the BLF samples- was found in BP (the highest quality grade) from F5, with a value of 947.6±4.267 µg GAE/ml [around 95 mg/ cup]. In set garden- F1 samples, highest total phenol has also been recorded in BP infusion (also highest overall). On the other hand, the lowest phenolic compound content was found in the bought leaf factory- F3 orange fannings grade, with a value of 642.18±7.225 µg GAE/ml [around 65 mg/ cup]. Other notable BLF samples with high phenolic contents were BOP, OF and PD of sample F5; OF from F2; D from F4, F5 and F7. Overall, set gardens samples were better than BLFs.

Table 5: Quantification of total phenol (µg gallic acid equivalent or GAE/ml of CTC made tea), F1 (Set garden) and F2-F7 (Bought tea leaf factory)

Source	BOP	BOPSM	BP	OF	D	PD
F1	954.26±6.668	1012.12±6.942	1385.14±5.568	1296.54±5.462	989.58±5.862	978.85±3.265
F2	825.93±4.998	796.79±5.431	685.81±6.5978	877.69±6.254	748.12±6.355	751.88±7.158
F3	815.09±5.083	822.02±6.256	704.51±4.520	642.18±7.225	801.74±7.552	763.11±6.058
F4	751.74±4.386	707.25±4.374	645.3±4.474	703.18±4.528	855.91±5.582	699.16±8.071
F5	860.11±6.304	801.32±4.672	947.6±4.267	860.88±6.353	829.21±6.584	865.18±5.028
F6	818.09±5.120	793.25±6.667	821.30±6.258	832.22±8.758	779.97±5.214	722.48±7.746
F7	800.81±3.815	650.49±5.962	728.09±5.639	681.81±6.325	883.44±8.143	761.72±6.751

Total flavonoid content

Flavonoids are a class of naturally occurring compounds found in various plants, including tea leaves, and they have antioxidant properties and potential health benefits. The flavonoid content in CTC grades of tea varied within a range of 2.03 to 3.95 mg QE/ml of sample. This range indicates that the amount of flavonoids can differ significantly among different grades of CTC tea. CTC tea has been reported to have higher levels of theaflavins and thearubigins (signature polyphenols of black tea) compared to other types of tea due to the processing method employed [34]. The flavonoid content in CTC tea can be influenced by multiple factors. The variety of tea plant, growing conditions, processing techniques, and brewing parameters such as temperature and steeping time can all impact the flavonoid composition and content [35]. For example, studies have shown that higher temperatures and longer steeping times tend to result in greater extraction of flavonoids from tea leaves. Table 6 quantification of total flavonoid expressed as mg quercetin equivalent (QE)/ml of sample used for extraction. In control sample again BP resulted best followed by other quality grades (OF, BOPSM and BOP). BLF samples revealed low flavonoid content compared to set garden samples.

Table 6: Quantification of flavonoid content (quercetin equivalent of CTC made tea) (mg QE/ml), F1 (Set garden) and F2-F7 (Bought tea leaf factory)

Source	BOP	BOPSM	BP	OF	D	PD
F1	3.28±0.022	3.62±0.038	3.95±0.044	3.78±0.052	2.89±0.044	3.05±0.038
F2	2.61±0.056	2.81±0.060	2.71±0.054	2.11±0.084	2.52±0.063	2.71±0.047
F3	2.05±0.076	2.79±0.055	2.88±0.030	2.4±0.039	2.03±0.051	2.52±0.067
F4	2.33±0.061	2.61±0.064	2.64±0.039	2.04±0.067	2.67±0.038	2.11±0.060
F5	2.19±0.043	2.14±0.084	2.65±0.063	2.78±0.075	2.54±0.067	2.59±0.069
F6	2.48±0.055	2.44±0.048	2.60±0.070	2.41±0.054	2.58±0.050	2.5±0.054
F7	2.81±0.043	2.78±0.080	2.67±0.051	2.44±0.051	2.57±0.051	2.44±0.075

***In vitro* DPPH free radical scavenging assay**

Antioxidants have diverse roles to play in the life of organisms. Several diseases and disorders are mainly due to the imbalance between pro-oxidation and anti-oxidation homeostatic phenomenon in the body [36]. Tea contains good amount of antioxidants and may provide a number of health benefits by removing free radicals [5, 6, 18]. CTC tea, a type of tea popular for its strong flavor, has gained helpfulness for its potential health benefits, particularly its antioxidant properties. In terms of DPPH free radical scavenging activity, different grades of CTC tea explored in this study showed variable scavenging activity (Table 7). CTC tea grades such as broken orange pekoe (BOP of F6: 77.48±0.427) and broken orange pekoe small (BOPSM of F2: 76.96±0.453) revealed comparatively higher DPPH free radical scavenging activity among all BLF samples. On the other hand, DPPH free radical scavenging activity by pekoe dust (PD of F2: 70.04±0.556) grade of CTC tea were lowest followed by dust (D of F5: 70.44±0.422) and. Based on this test, it can be inferred that as the grade size of tea decreases, its DPPH free radical scavenging activity also declines. The main reason behind this decreasing antioxidant activity is occurrence of fibre content in low quality CTC grades like dust and pekoe dust tea. This fibre contains less polyphenol (catechins and theaflavins) and others bioactive compounds but high tannin content (as wood product in hard stem and stalks) as revealed in the qualitative test. Therefore, the resulting low DPPH free radical scavenging activity of dust and pekoe dust tea is completely valid. DPPH free radical scavenging activity was found highest in broken orange pekoe of bought leaf factory-F6 and it was lowest in pekoe dust grade collected from bought leaf factory- F2. Control or set garden samples were again remarkable incomparable with the BLF samples. In a similar research, Das *et al.* [6] reported maximum free radical scavenging activity to be observed in the tea manufactured at Nilgiri (86.256%) and minimum result was obtained in tea manufactured at Terai region (the study area of this research) (83.29168%) which suggests that this quality issue in Terai's tea is very deep rooted which can be corrected by organized and proper management practices straight from the field.

Table 7: DPPH scavenging percentage (%) of made tea samples in water extracts, F1 (Set garden) and F2-F7 (Bought tea leaf factory)

Source	BOP	BPOSM	BP	OF	D	PD
F1	86.99±0.556	84.12±0.579	89.45±0.634	81.36±0.445	79.9±0.354	79.07±0.425
F2	75.38±0.417	76.96±0.453	75.72±0.254	74.53±0.199	73.59±0.524	70.04±0.556
F3	75.95±0.424	76.26±0.685	75.41±0.236	74.28±0.254	75.02±0.625	74.01±0.656
F4	75.72±0.366	75.98±0.365	74.75±0.332	75.79±0.422	71.01±0.450	74.55±0.452
F5	74.05±0.552	75.93±0.389	74.05±0.402	75.41±0.535	70.44±0.442	72.5±0.472
F6	77.48±0.427	76.52±0.556	75.21±0.236	76.48±0.458	74.84±0.338	74.66±0.559
F7	75.14±0.318	73.59±0.345	74.87±0.503	74.22±0.453	75.76±0.458	72.43±0.591

Comparative analysis revealed DPPH antioxidant activity values were significantly higher in the tea infusions prepared from CTC-tea of set gardens (>78%) than the highest resulting BLF sample. All the qualitative and quantitative biochemical characters were detected high in made tea samples of set gardens (F1 samples) compared to BLF ones (F2-F7) which reflects that set gardens' products contain more quality in terms of both physicochemical and phytochemical qualities. OF grade of sample F2 resulted best in the coumarin test which also showed highest tannin content (Fig 2). Therefore, polyphenols other than flavonoids (determined low as given in Table 6) were recorded high in F2's OF grade. Results suggest that this particular grade of sample F2 has a significant concentration of total phenol content, which includes mainly coumarins and tannins, naturally occurring compounds known for their bitter taste and dark liquor. Another correlation has been observed for sample F6 whose all grades resulted low in biochemical tests for tannin, coumarin and flavonoids while the steroid content and antioxidant activity were very high. Therefore, the high antioxidant properties in all grades of F6 were probably due to high phytosterol content in all grades of this sample. F6 low moisture content and comparatively high pH might also have role in these results that can also be correlated but further experiments and volatile profiling is needed.

Conclusion
Overall, compared to set garden samples, small tea growers' product- i.e., manufactured tea from bought leaf factories did not reveal such organized results reflecting unorganized practices from field (uneven plucking, pruning and other plant managements) to factory. Components tested in tea and tea infusions were variable. The differences in chemical nature between manufactured tea from set garden and bought leaf factories were reflected in our results. Tea plant clone and soil conditions have a profound role to play in imparting taste to the beverage. Clone specific quality characterization can provide a new direction in producing tea of better quality. Moreover, further chromatography and metabolite profiling can be supportive of these findings or can call attention to other issues (like pesticides). These results are preliminary but alarming for the future of small tea growers. Thorough survey, training programs and corrective measures at the field level is needed to resolve these quality issues from the root. Last but not least, reputation of small tea growers is intertwined with bought leaf factories who manufacture their leaves and sell their products to the consumers. Therefore, liabilities should be equal on both sides, even more on STGs, as they are an integral part of this industry.

Conflict of interest: Authors declare no conflict of interest.

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Authors' contribution
Malay Bhattacharya conceptualized this research and designed the protocols. Sahadeb Sarkar did sample collection and performed all the biochemical tests and statistical analysis. Soumya Majumder analyzed the data and wrote the draft manuscript. Arindam Ghosh helped in biochemical tests. Sukanya Acharyya and Malay Bhattacharya revised the manuscript.

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