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Ayush Kumar Das
Molecular Biology and Tissue Culture Laboratory, Department of Tea Science, University of North Bengal, Siliguri, Darjeeling, West Bengal, India

Arindam Ghosh
Molecular Biology and Tissue Culture Laboratory, Department of Tea Science, University of North Bengal, Siliguri, Darjeeling, West Bengal, India

Soumya Majumder
Molecular Biology and Tissue Culture Laboratory, Department of Tea Science, University of North Bengal, Siliguri, Darjeeling, West Bengal, India

Sumedha Saha
Molecular Biology and Tissue Culture Laboratory, Department of Tea Science, University of North Bengal, Siliguri, Darjeeling, West Bengal, India

Sukanya Acharyya
Molecular Biology and Tissue Culture Laboratory, Department of Tea Science, University of North Bengal, Siliguri, Darjeeling, West Bengal, India

Sahadeb Sarkar
Molecular Biology and Tissue Culture Laboratory, Department of Tea Science, University of North Bengal, Siliguri, Darjeeling, West Bengal, India

Sourav Chakraborty
Molecular Biology and Tissue Culture Laboratory, Department of Tea Science, University of North Bengal, Siliguri, Darjeeling, West Bengal, India

Mainak Mukherjee
Soil Analysis Laboratory, Department of Tea Science, University of North Bengal, Siliguri, 734013, Darjeeling, West Bengal, India

Malay Bhattacharya
Molecular Biology and Tissue Culture Laboratory, Department of Tea Science, University of North Bengal, Siliguri, Darjeeling, West Bengal, India

Corresponding Author:
Malay Bhattacharya
Molecular Biology and Tissue Culture Laboratory, Department of Tea Science, University of North Bengal, Siliguri, Darjeeling, West Bengal, India

Characterization of tea and tea infusion: A study of marketed black tea samples from some tea growing regions of India

Ayush Kumar Das, Arindam Ghosh, Soumya Majumder, Sumedha Saha, Sukanya Acharyya, Sahadeb Sarkar, Sourav Chakraborty, Mainak Mukherjee and Malay Bhattacharya

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Abstract
Tea as a refreshing beverage is consumed socially and habitually. Tea contains several bioactive compounds. The present research work has been designed to work on characterization of tea and tea infusion collected from major tea growing regions of India as climate influences aroma and taste of tea. CTC-tea and Orthodox-tea (O-tea) samples were collected from different tea growing regions of India. In both the type of tea samples maximum and minimum percentage of moisture were recorded in Nilgiri and Dooars tea. Amount of Crude fibres in manufactured CTC-tea was more than O-tea. Ash content of CTC-tea and O-tea ranged between 1.308%-1.66% and 0.233-0.315% respectively. The pH of infusions was found to be acidic. Optical density of O-tea infusions was found to be less than CTC-tea infusions. Insoluble and soluble matter in tea infusions was extremely variable. Theaflavin of tea infusions prepared from CTC-tea were more than that of O-tea of the same region. Thearubigins was recorded more in all CTC-tea samples over O-tea except for the sample manufactured at Nilgiri. Caffeine content was high in CTC-tea samples of Nilgiri, Terai and Shillong whereas for Assam and Dooars it was high in O-tea. Free radical scavenging activity was more in CTC-tea than O-tea of the same region. O-tea showed better antibacterial activity than CTC-tea.

Keywords: Manufactured tea, physical, chemical, microbial, India

Introduction
Tea, an aromatic beverage only second to water with regard to consumption, is commonly prepared by pouring hot or boiling water over cured leaves of tea plant (*Camellia sinensis* L. Kuntze). It has been consumed socially and habitually by people since 3000 BC [1]. The pleasing astringent taste and refreshing nature are so attractive that its potential health benefits with medicinal properties are often overlooked. Ongoing scientific exploration points 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. [2].
Tea is reported to contain nearly 4000 bioactive compounds. These include alkaloids, polyphenols, amino acids, proteins, chlorophylls, carbohydrates, volatile organic compounds, fluoride, aluminum, minerals and trace elements [3]. Primary compounds belonging to tea polyphenols are gallic acid, catechin, gallocatechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate [4]. 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 [5]. During the process of manufacturing, oxidative polymerization into theaflavins [6] and changes in catechins provide the characteristic flavor, color and aroma of each type of tea [5]. So, tea manufactured from different eco-geographical region has unique properties. Research on the cultivation, management, manufacturing and value-added products, medicinal and beverage properties of tea have been explored for centuries. But, a comparative study on infusion made from tea of different locations of India is lacking. So, the present research work has been designed to characterize tea and tea infusions collected from major tea growing regions of India.

Materials and Method

Collection of manufactured tea

Tea samples manufactured by crush tear curl (CTC) and Orthodox (OT) processes were collected from different tea growing regions of India (Assam, Darjeeling, Terai, Dooars and Nilgiri). The samples which were sealed packets were procured from local market or purchased through online trading portals. These were preserved in air tight conditions.

Characterization of tea

Moisture content

Percentage of moisture loss was determined by drying 10gm of manufactured tea samples at 110 ºC for one hour. The difference in mass was expressed as percentage of moisture present in tea samples.

Crude fibre

Crude fibre content present in manufactured tea was determined by acid and alkali digestion. Tea samples (2g) were boiled in 200ml of sulphuric acid for 30minutes. The extracts were filtered and washed several times with distilled water until the pH of fibrous residue became neutral. The residue was boiled in 200ml of sodium hydroxide solution for 30minutes and was filtered again. It was washed several times with distilled water to neutralize its pH. The residue was washed with 25ml of boiling 1.25% H_2SO_4 and was washed with 50ml distilled water and 25ml of ethyl alcohol. The residue was taken in a crucible (W_1) and dried in a hot air oven for 2hours at 130±2 °C. Finally, the crucibles containing the residue (W_2) were placed in muffle furnace for 30minutes at 600±15 °C. The residues were cooled down and their masses (W_3) were recorded.

Percentage of crude fibre content was calculated by the formula-

Crude fibre (%) =100-(moisture + fat) X weight of fibre / weight of the sample taken (moisture and fat free) (W1); where, weight of the sample = W_1 g, weight of the crucible + sample before heating at 600ºC=W_3 g and weight of the crude fibre= ($W_2 - W_3$) g.

Ash content

Tea samples (5g) were taken in crucibles and kept in a muffle furnace at 600°C for 6hours. Percentage of ash content was calculated by the formula-Ash (%) = (weight of residue/ weight of sample)*100.

Qualitative characterization of tea infusion

Preparation of tea infusion

Manufactured tea (2.5g) was added in 100ml of boiled water after lowering its temperature by 10-15°C and keeping undisturbed for 5minutes to prepare the infusion.

Qualitative analysis

Tests for flavonoids, tannin, cardiac glycosides, protein, coumarin, terpenoid, steroid and total phenol was conducted following protocol of Labar *et al*. 2019 [7] (Supplementary 1). Reducing sugar in tea infusion was tested following Moore's test. To about 2 ml of sample an equal volume of 5% NaOH solution was added followed by boiling for 2-5 minutes in a water bath. Change of colouration from yellow to reddish-brown due to the formation of a condensation product of the sugar indicates presence of reducing sugar.

pH of tea infusion

The pH of the infusions was tested by a pre-calibrated pH meter by dipping electrodes and temperature sensor in 50ml of tea infusions brought down to room temperature. The results were recorded as average of three replicates.

Optical density (OD) and transmittance (T)

OD and T of tea samples were measured in a colorimeter at 420nm. The results were recorded as average of three replicates.

Insoluble (IM) and Soluble (SM) matter

Insoluble matter in tea infusions was determined by centrifugation. One ml of each tea infusion was centrifuged at 10000RPM for 10minutes. The supernatants were discarded and the pellet were dried to record their mass. For soluble matter, 1ml of each tea infusion was left for evaporation at room temperature. The left off residue was considered as soluble matter and its mass was recorded. Insoluble and soluble matters were recorded as percentage.

Correlation studies

Correlation studies were conducted in between moisture, crude fibre and ash content of manufactured tea samples of different regions using Microsoft Office Excel Worksheet.

Theaflavin (TF), thearubigins (TR), total colour (TC) and total brightness (TB)

Amount of TF, TR, TC and TB in tea infusions made from tea collected from different regions was calculated following Akuli *et al*. 2012 [8] with slight modifications. 2.5gm of each sample was taken into a thermo-flask and 100ml of boiling water was added to it for preparing infusions. After filtration, 6ml of extract from each infusion was taken into a glass vessel and 10ml of ethyl acetate was added to it and shaken vigorously. The upper layer was collected and again 5ml of ethyl acetate was added to the separating funnel. 10ml of each extract was collected in a 25ml volumetric flask. Diluted mixtures (E1 to E3) were prepared, where E1=Extract (10ml) + Methanol (15ml), E2= Methanol (15ml) + Water (9ml) + Extract (1ml) and E3=Methanol (15ml) + Water (8ml) + 10% Oxalic acid (1ml) and Extract (1ml). Absorbance was measured at 380nm. Calculation of TF (%) and TR (%) was done by the following formula: - % of TF = 2.25*E1; **%** of TR=7.06 (4E3-E1). The ratio of TF:TR was calculated by the results obtained from the experiment of quantifying TF and TR. Total color was calculated by following: - 6.25*4E2.

Total brightness was calculated by the following formula: - Total Brightness= (E1/4E2) *100.

Caffeine

Tea samples were used as the source of caffeine for this experiment. 5gm of each tea sample was taken and 200ml of distilled water was added to it. The content was boiled to decoction, brought down to room temperature and kept in an ice box to reduce the temperature below 20 °C. Extraction was carried out thrice by using 30ml of dichloromethane. Removal of the solvents produced a residue of yellowish green - white crystalline caffeine. Percentage of extractable caffeine was determined from the result.

Free radical scavenging activity of tea infusion

DPPH scavenging activity

Free radical scavenging activity by DPPH assay was conducted following the protocol of Labar *et al*. 2019 [7]; Bhattacharya *et al*. 2009 [9]. To 2ml of the methanol solution of 0.2mM DPPH, 0.2ml of tea infusion was added. The mixture was vortexed vigorously and incubated at room temperature for 30minutes in the dark at room temperature. Absorbance was measured at 517nm by UV-Vis spectrophotometer. DPPH scavenging percentage was calculated as -Free radical scavenging % = [{(Control – Sample)/ Control} X 100]

Change of DPPH scavenging activity of tea infusions upon storage and further boiling

Tea infusions prepared for DPPH assay were kept at room temperature for two hours. One part of the tea infusions was tested for its free radical scavenging activity while the other part was further boiled for one minute followed by its test of free radical scavenging activity. The same protocol for DPPH assay as described earlier in this report was followed.

Free radical scavenging activity of herbal additives mixed CTC tea infusions

To test the effect of herbal additives on free radical scavenging activity, additives like lemon juice with black pepper, lemon juice, ginger and cardamom were used for 100ml of each tea infusion. The infusions were brought down to room temperature and DPPH assays were performed. The same protocol for DPPH assay as described earlier in this report was followed.

Antibacterial activity of tea infusions

Antibacterial activity of each tea infusion was tested by well diffusion method [10]. Overnight grown broth culture of two gram positive (*Staphylococcus aureus* and *Bacillus cereus*) and two gram negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria were used for the present study to assess the antibacterial activities of synthesized samples. Mueller-Hinton (MH) agar media was used for susceptibility tests. The media was sterilized by autoclaving and plated under sterile condition of Laminar air flow cabinet. 100µl of bacterial strains were added separately to each plate containing media. Circular wells were dug out by sterilized steel cork-borer. 100µl of each tea infusion was poured into the well by using sterilized pipettes. The plates were incubated overnight at 37 ºC. Reading and photographs were taken after 24h of incubation.

Result and Discussion

Collection of manufactured tea

Tea samples- CTC-tea and Orthodox-tea (CTC-T and O-T) were collected from different tea growing regions of India (Supplementary 2A and 2B1) either from local market or purchased through online trading portals. Moisture deteriorates tea samples when kept open, so, the samples were preserved in airtight packets in desiccators.

Characterization of tea

Moisture

Moisture is undesirable as it degrades the quality of infusion and reduces the shelf life. High amount of moisture invites microbes due to which they start deteriorating very fast. In both CTC-tea and O-tea samples maximum and minimum percentage of moisture were recorded in Nilgiri and Dooars tea samples (table 1). In CTC-tea samples maximum percentage of moisture was 3.6% while the minimum was 0.5%. In O-tea samples maximum percentage of moisture was 6.2% while the minimum was 0.2%.

Table 1. Moisture, crude fibre and ash content of tea samples collected from tea growing regions of India.

(%)	Nilgiri		Assam		Terai		Dooars		Shillong	
	CTC	OT	CTC	OT	CTC	OT	CTC	OT	CTC	OT
Moisture	3.6	6.2	2.4	4	1.7	2.8	0.5	0.2	2	4.5
Crude fibre	19.24	7.72	13.35	9.4	14.6	13.84	13.96	8.52	19.25	9.68
Ash content	1.366	0.279	1.66	0.315	1.308	0.287	1.005	0.292	1.66	0.233

Crude fibre

Crude fibre in plants originates from cell wall, sclerenchyma, collenchyma and conducting tissues. Amount of Crude fibres present in manufactured tea was determined by acid and alkali digestion. Percentage of crude fibers in manufactured CTC-tea was more than that of O-tea (table 1). In CTC-tea samples, maximum and minimum percentages of crude fibres were observed in Shillong (19.25%) and Assam (13.35%) respectively. While in O-tea infusions maximum and minimum crude fibre was observed in Assam (9.4%) and Terai (13.84%) respectively. High percentage of crude fibres is undesirable. The content of crude fibre in young tea leaves is much less than in older ones. This could be because young cells have thin cellular walls and lesser quantities of mechanical and conducting tissues. Plant tissues become harder as the plant grows and as they provide the plant with protection from wind, excess transpiration and influence of other undesirable factors [11].

Ash content

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter. Ash content of tea samples are depicted in table 1. Ash content of CTC-T and O-T ranged between 1.308%-1.66% and 0.233-0.315% respectively. Ash content was more in CTC tea over orthodox tea. As expected, the amount and composition of ash remaining after combustion of manufactured tea varied considerably according to the harvested leaf, age, cultural treatment etc. As O-tea is manufactured from finer leaf harvest compared to CTC-tea, it is obvious that ash content of CTC-tea will be higher than O-tea.

Preparation of tea infusion

Tea infusion, as most people think, is not a coloured solution containing boiled water and tea. In all our experiments, freshly prepared infusions were used. Standard infusions were prepared in all the experiments keeping the tea leaves and water at a constant ratio. Time and temperature were kept same during preparation of all the infusions.

Qualitative analysis of tea infusion

Qualitative presence of flavonoid, tannin, cardiac glycosides, protein, coumarin, terpenoid, steroid, phenol and reducing sugar in tea infusions was calculated and a heat-map was generated based on results as depicted in Fig. 1 and its descending order of quantity given in supplementary 3. The

quantitative presences of biomolecular groups were highly variable and beyond any predictable order.

Quantitative characterization of tea infusion

Physical characters like color and flavor are mostly considered as prime quality of tea infusions. But, scientifically pH, optical density (OD), percentage of transmittance (T), insoluble (IM) and soluble (SM) matter are of much more importance. Considering these parameters like pH, OD, T%, IM and SM were tested for the tea infusions.

PLACE	TYPE	F	T	CG	P	C	T	S	TP	RS
Nilgiri	CTC	2	2	3	2	6	2	4	6	6
	OT	6	4	5	6	2	2	4	4	4
Assam	CTC	2	3	4	2	6	1	4	5	6
	OT	6	6	5	4	4	3	3	4	2
Dooars	CTC	4	4	2	2	4	6	4	6	4
	OT	6	6	7	6	4	4	2	2	4
Terai	CTC	2	2	3	2	6	2	1	4	2
	OT	4	5	4	4	6	5	2	2	2
Shillong	CTC	4	6	2	4	2	1	6	6	6
	OT	6	7	8	6	4	1	7	2	4

Flavonoid (F), Tannin (T), Cardiac glycoside (CG), Protein (P), Coumarime (C), Tepenoids (T), Steroids (S), Total phenol (TP) and Reducing sugar (RS)

Fig 1: Qualitative characters of tea infusion

pH

pH of the infusions was tested to determine the quantity of acidity or alkalinity of the infusions. The pH of infusions was found to be acidic (Table 2). pH of CTC-tea and O-tea infusions ranged between 5.29-5.54 and 5.8-6.14 respectively. Maximum pH was recorded in the infusion prepared from O-tea manufactured in Terai tea gardens while the minimum pH was recorded in CTC- tea manufactured in tea garden of Assam. In general pH of O-tea infusions were greater than the CTC-tea infusions. This variability of pH may be related to the manufacturing processes. CTC-tea manufacturing involves the process of fermentation. During the process of fermentation production of organic acids by some fermenting microorganisms are common. It has already been reported that tea contains a significant amount of oxalic acid [12] and malic acid [13], along with citric, isocitric, and succinic acids [12]. Tea also contains shikimic acid, which is important for the biosynthesis of the polyphenols [14]. Vitamin C (ascorbic acid) has also been detected in green tea and black tea [15-16].

Optical density (OD) and transmittance (T)

Optical density and transmittance are determinants of sensory quality of tea infusions. OD and T% are shown in table:2. OD of O-tea infusions was found to be less than CTC-tea infusions. In CTC-tea infusions maximum and minimum OD were observed in Terai (0.23) and Assam (0.19) respectively. While in O-tea infusions maximum and minimum OD were observed in Assam (1.14) and Dooars (0.09) respectively. In CTC-tea infusions maximum and minimum percentage of T was observed in Assam (64%) and Terai (58%) respectively. While in O-tea infusions maximum and minimum percentage of T was observed in Dooars (81%) and Assam (65%) respectively. These two parameters of tea infusions were inversely related in all the observations.

Insoluble (IM) and Soluble (SM) matter

Insoluble and soluble matter present in tea infusions determines taste of tea. In our experiment they showed varied results (Table 2). There was no definite pattern in between CTC-tea and O-tea with regard to IM and SM. The quantity of IM depends on manufacturing process, age and clone of harvested leaf while SM depends on the phytochemistry of the clones. So, it is quite difficult to give a conclusive opinion on these two test parameters. However, it can be said that insoluble matter present in tea infusions is undesirable.

Table 2: pH, optical density, transmission, insoluble and soluble matter of tea infusion

	Nilgiri		Assam		Terai		Dooars		Shillong	
	CTC	OT	CTC	OT	CTC	OT	CTC	OT	CTC	OT
pH	5.51	5.8	5.29	5.53	5.46	6.14	5.54	5.97	5.42	5.85
OD	0.2	0.14	0.19	0.18	0.23	1.14	0.22	0.09	0.21	0.15
T (%)	63	72	64	65	58	71	59	81	62	70
Insoluble matter%	0.089	0.908	0.09	0.091	0.444	0.269	0.087	0.088	0.092	0.262
Soluble matter %	0.681	0.32	0.399	0.157	0.317	0.296	0.365	0.308	0.504	0.302

Correlation studies

Correlation studies were conducted in between moisture, crude fibre and ash content of manufactured tea samples of different regions. Negative correlation existed between moisture-crude fibre (-0.2969) and moisture-ash content (-0.2969), but positive correlation existed between crude fibre-ash content (0.802601). Correlation between pH, OD, T%, IM and SM of tea infusions showed a positive correlation between pH-OD (0.53289), pH-T% (0.779286), pH-IM (0.270919), OD-T% (0.089595), OD-IM (0.010113) and T%-IM (0.173414) but negative correlation between pH-SM (-0.36234), OD-SM (-0.12522), T%-SM (-0.30278) and IM-SM (-0.22953).

Theaflavin (TF), thearubigins (TR), total colour (TC) and total brightness (TB)

The values obtained for Theaflavin (TF), thearubigins (TR), total colour (TC) and total brightness (TB) are depicted in figure 2. Theaflavin (TF) and its derivatives, are polyphenols that are formed during the enzymatic oxidation of tea. Upon disruption of the intracellular compartments, polyphenols present in the cell vacuoles are oxidized by the tea oxidative enzymes, leading to the formation of theaflavin and thearubigin pigments which is the characteristic of black tea [17]. TF content of tea infusions prepared from CTC-tea was more than that of O-tea of the same region. Highest quantity of TF was recorded in tea infusion made from Nilgiri and Assam for CTC-tea and O-tea respectively. Thearubigins (TR) are polymeric polyphenols that are formed during enzymatic oxidation and condensation of epigallocatechin and epigallocatechin gallate by polyphenol oxidase during manufacture of black tea [18]. TR was recorded more in all CTC-tea samples over O-tea except for the sample manufactured at Nilgiri. Highest quantity of TR was recorded in tea infusion made from Shillong and Assam for CTC-tea and O-tea respectively. Ratio of TF and TR is one of the greatest determinants of medicinal property of tea. It is recommended that the ratio of TF and TR should be in between 1:11-12. In none of tea infusions the recommended ratio was noticed. In all the infusions except Dooars O-tea, the ratio of TF: TR in CTC tea infusions was less than 11 and the ratio of TF: TR in orthodox tea infusions was more than 12. The theaflavins impart color, brightness, and astringency to black tea liquors [19]. Thearubigins are red in colour and are responsible for much of the staining effect of tea. Therefore, a black (fully oxidized) tea often appears red while a green or white tea has a much clearer appearance. The colour of a black tea, however, is affected by many other factors as well, such as the amount of theaflavins, another oxidized form of polyphenols.

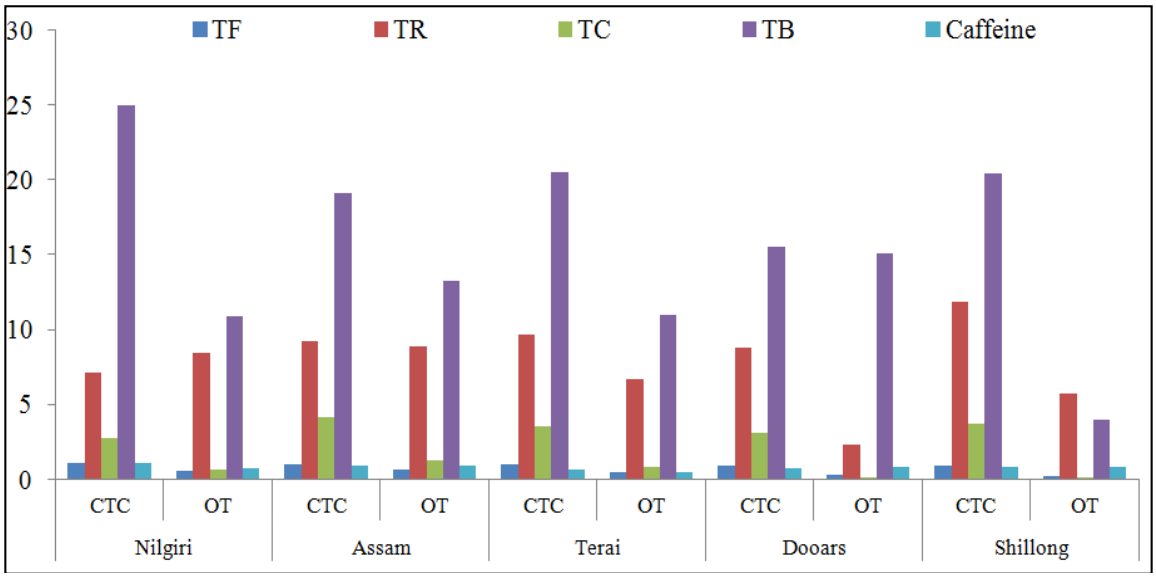

Fig 2: TF, TR, TC, TB and caffeine of different samples

Caffeine

Caffeine is viewed as an important constituent of tea, bestowing mood and cognitive-enhancing properties [20]. Caffeine content was recorded to be high in CTC-tea samples of Nilgiri, Terai and Shillong while for Assam and Dooars it was high in O-tea (Figure 2). In CTC-tea samples highest amount of caffeine was detected in Nilgiri (1.06 %) while lowest was recorded Terai (0.68%). In O-tea samples highest amount of caffeine was detected in Assam (0.94%) while lowest was detected Terai (0.5%).

Free radical scavenging activity of tea infusions

Antioxidants and reactive oxygen species have diverse roles to play in the life of organisms as it has been realized that a majority of the diseases and disorders are mainly due to the imbalance between pro-oxidation and anti-oxidation homeostatic phenomenon in the body [21]. Tea is known to provide a number of health benefits and consuming it can help to remove free radicals. This ultimately may help decrease the risk of chronic diseases [22]. Tea infusions made from tea samples collected from different region showed high free radical scavenging activity (Fig: 3). In general, the free radical scavenging activity was more in CTC-tea than O-tea of the same region. In CTC-tea, maximum free radical scavenging activity was observed in the tea manufactured at Nilgiri, while minimum in tea manufactured at Terai region. The order of tea samples on the basis of decrease in free radical activity is Nilgiri (86.256%) < Shillong (85.0048%) < Assam (84.8123%) < Dooars (83.5874%) < Terai (83.29168%). Similarly, in O-tea, maximum free radical scavenging activity was observed in the tea manufactured at Assam, while minimum in tea manufactured at Nilgiri region. The order of tea samples on the basis of decrease in free radical activity is Assam (83.3879%) < Dooars (83.1569%) < Shillong (82.0982%) < Terai (81.5874%) < Nilgiri (68.5082%). It is known that tea polyphenols (catechins) have

strong antioxidant activity, antimutagenic and anticarcinogenic effects. Among catechins, (-)-epigallocatechin gallate has the highest antioxidant activity; this is followed by (-)-epicatechingallate,(-)-epigallocatechin, (-)-epicatechin and (+)-catechin [23]. Antioxidant property revealed by tea infusions may be due to presence of these compounds.

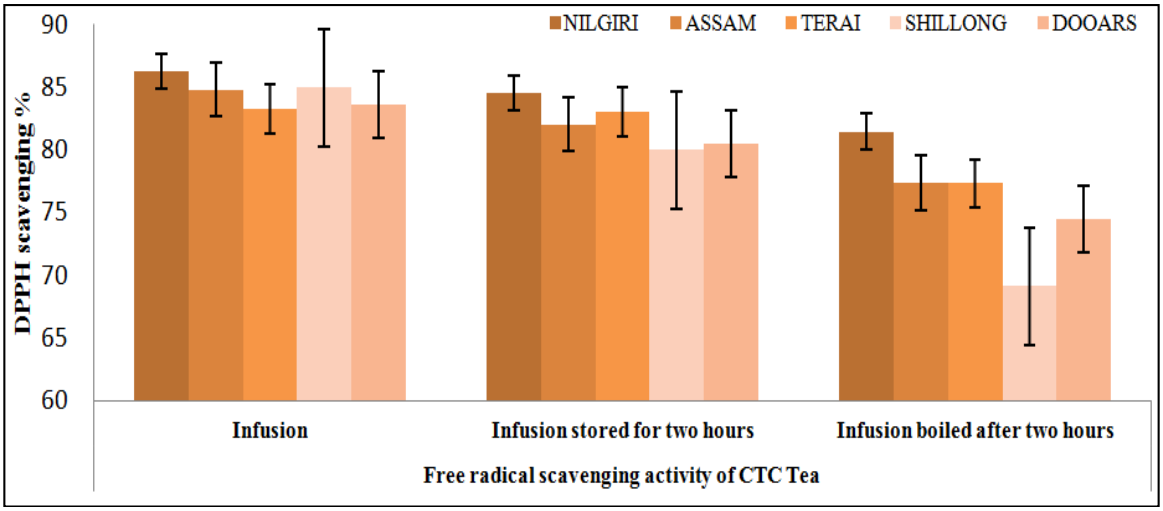

Fig 3: Free radical scavenging activity of CTC tea infusions upon storage and further boiling

Change of Free radical scavenging activity of tea infusions upon storage and further boiling

A downstream experiment was conducted to assess the free radical scavenging activity of CTC-tea and O-tea upon storage (2 hours) and boiling (after two hours). The results are depicted in fig 4 and 5. In case of CTC-tea there was a slight decrease in free radical scavenging activity upon storage but leaving the tea collected from Shillong, all the O-tea samples showed increase in free radical activity upon storage for one hour. This may be due to the process of manufacturing. In case of CTC tea, as the fresh leaves are crushed during manufacturing, all the cellular compounds become readily available to water. But, in case of O-tea, as the leaves are fully or partially intact the cellular materials take more time to diffuse into water.

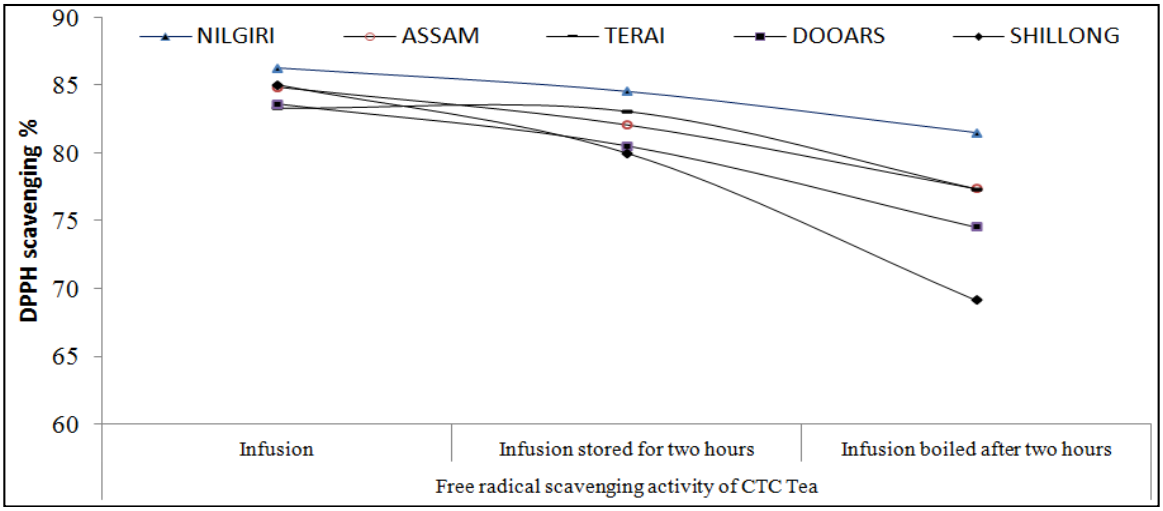

Fig 4: Free radical scavenging activity of CTC tea infusions

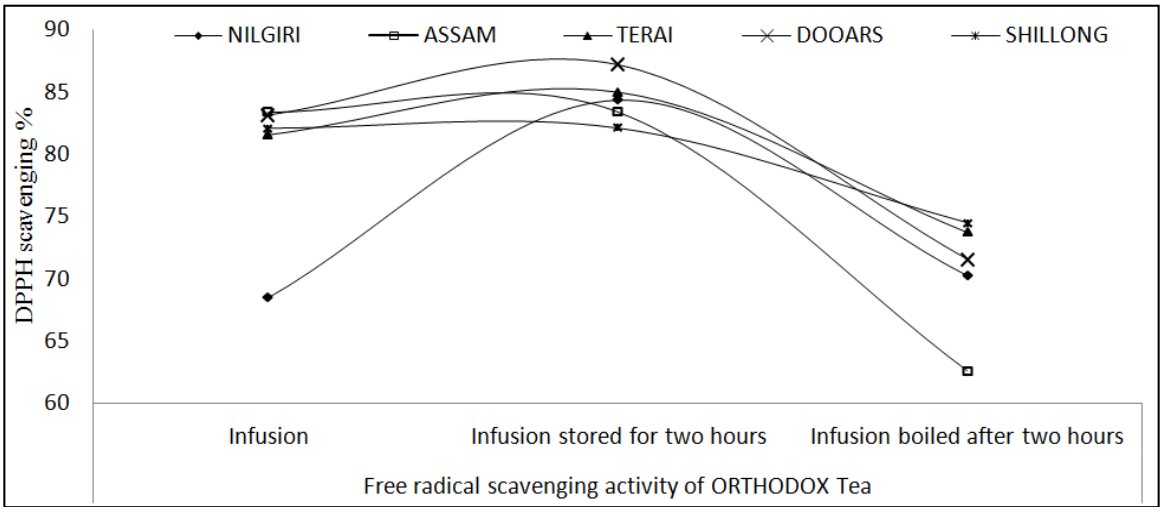

Fig 5: Free radical scavenging activity of ORTHODOX tea infusions

Effect of additives on Free radical scavenging activity of CTC-tea infusions

We tested the change in free radical scavenging activity of CTC-tea upon addition of some herbals like Lemon with black pepper (LBP), Lemon (L), Ginger (G) and Cardamom (C). The result obtained was quite variable (Figure 6). In case of CTC-tea additives like LBP and L, there was significant decrease in free radical scavenging activity. Additives like G and C resulted in slight decrease of free radical scavenging activity in all the samples except the sample collected from Dooars region. All the additives like L, BP, G and C are rich sources of antioxidants. But their use with CTC-tea reduces the free radical scavenging activity of the tea. So, there must be some phytochemicals within CTC and/or the additives that interact to produce some products that reduce the free radical scavenging activity. In our findings, there must be such products being produced more in the tea manufactured at Dooars region. Detection and characterization of these antagonistic products may be explored by further research.

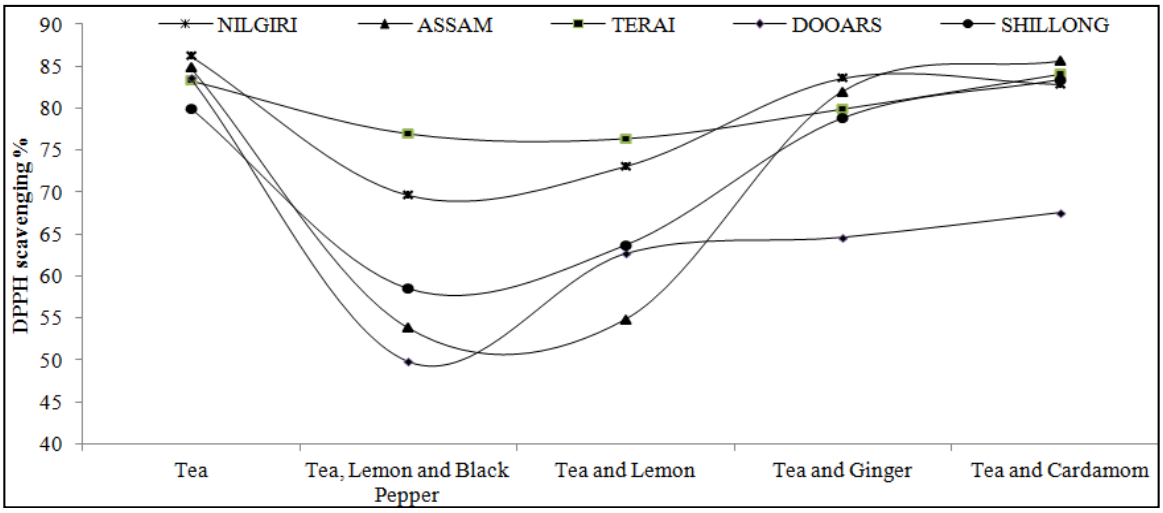

Fig 6: Effect of additives on Free radical scavenging activity of CTC-tea infusions

Antibacterial activity of tea infusions

Inhibition of bacterial growth by tea samples was observed and the result is depicted in Table 3. All the infusions responded to both gram- positive and gram-negative bacterial samples. In tea samples of all the areas, O-tea showed better antibacterial activity than CTC-tea. O-tea sample collected from Nilgiri region, showed the best result and the maximum inhibition zones were recorded in all the four bacterial samples. CTC-tea infusions of Nilgiri tea showed some good results in inhibiting *E coli*. In general, both CTC-tea and O-tea from Nilgiri region showed very promising results in inhibiting bacterial growth. CTC-tea and O-tea collected from Dooars and Terai region showed positive results but their inhibition zones were not very promising. As antibacterial property in manufactured tea depends on the phytochemistry of green leaf and manufacturing and post manufacturing changes, tea clones play a crucial role. As we are unaware of the clonal properties of manufactured tea, it is very difficult to develop a hypothesis on the inhibition of bacterial growth by our collected tea samples.

Table 3: Antibacterial activity of tea infusions

Sample	Type	Gram (-)		Gram (+)	
		EC	KP	BS	SA
Nilgiri	CTC	15	8	12	12
	OT	16	15	16	15
Assam	CTC	12	12	12	12

	OT	14	8	14	11
Terai	CTC	9	8	10	9
	OT	10	9	13	13
Dooars	CTC	10	8	11	11
	OT	13	12	16	8
Shillong	CTC	10	10	12	10
	OT	14	10	12	12
E.coli (EC), *K. pneumoniae* (KP), *B. subtilis* (BS) and *S.aureus* (SA)					

Supplementary materials

Supplementary 1

Group	Test
Flavonoids	Few drops of 10% $FeCl_3$ solution was added to 1ml of tea infusion. A green or blue colour indicated the presence of Flavonoids.
Tannins	To 0.5ml tea infusion and few drops of HNO_3 was added. The reddish to yellow colour of the solution indicated presence of tannins.
Cardiac glycosides	0.5ml of sample were evaporated and dissolved in 1ml glacial acetic acid. 1 drop of 10% $FeCl_3$ solution followed by 1ml of Conc. H_2SO_4 was added by the side of test tube. Appearance of brown colour ring at the interface indicated the presence of cardiac glycosides.
Protein	1ml of 4% NaOH solution and a few drops of 1% CuSO4 solution were added to 3ml of sample solution. A violet or pink colour is produced indicates presence of protein.
Coumarin	Few drops of NaOH solution was added to 1ml of tea infusion. Yellow colouration indicates the presence of Coumarin. To test terpenoid, 250 µl extract was evaporated. The remaining was dissolved in chloroform and concentrated H_2SO_4 was added from the sidewall of test tubes. Formation of red to reddish brown coloration at the base confirmed the presence of terpenoids.
Steroid	For test of 0.5 ml tea infusion were evaporated and dissolved in 2 ml chloroform. 2 ml of Conc. H_2SO_4 was introduced carefully by the side wall of the test tube. Formation of red colour ring confirmed the presence of Steroid.
Total phenol	$¼^{th}$ tea spoon $FeCl_3$ was added to 1 ml of tea infusion, followed by vigorous shaking. Green colouration indicates the presence of Phenol.

Supplementary 2A

Region	**State**	**Altitude (m)**	**Rainfall**	**Temperature**
Nilgiri	Tamil Nadu, Karnataka and Kerala	2,637	1920 mm	Temperate and most Equable, Summer: 25 °C – 10 °C, Winter: 21 °C – 2 °C
Assam	Assam	45-1,960	1927 mm	23.2 °C
Terai	West Bengal	67 -300	1600 mm	Summer temperatures exceed 37 °C, winter temperatures range from 7 °C to 23 °C
Dooars	West Bengal and some parts of Assam	90 - 1,750	3429 mm	The temperature ranges between 21 to 31 °C
Shillong	Meghalaya	1,495-1,965	3385 mm	The average annual temperature in Shillong is 17.1 °C

Supplementary 2B

Sample name	**Collected from**	**Price (Rs)**
Assam CTC	Local market	1250
Assam Orthodox	Local market	500
Dooars CTC	Local market	400
Dooars Orthodox	Local market	2200
Terai CTC	Naxalbari tea garden	400
Terai Orthodox	Naxalbari tea garden	600
Shillong CTC	Local market	280
Shillong Orthodox	Local market	1000
Nilgiri CTC	Amazon India	430
Nilgiri Orthodox	Amazon India	1500

Supplementary 3

Flavonoids	CTC-tea	Dooars and Shillong > Nilgiri, Assam and Terai
	O-tea	Nilgiri, Assam, Dooars and Shillong > Terai.
Tannin	CTC-tea	Shillong > Dooars > Assam > Terai and Nilgiri
	O-tea	Shillong > Assam, Dooars > Terai > Nilgiri.
Cardiac glycosides	CTC-tea	Assam > Nilgiri, Terai > Dooars and Shillong
	O-tea	Shillong > Dooars > Nilgiri, Assam > Terai.
Protein	CTC-tea	Shillong > Nilgiri, Assam, Dooars and Terai
	O-tea	Nilgiri, Dooars, Shilong > Assam and Terai.
Coumarine	CTC-tea	Nilgiri, Assam, Terai > Dooars and Shillong
	O-tea	Terai > Assam, Dooars and Shillong > Nilgiri.
Terpenoids	CTC-tea	Dooars > Nilgiri, Terai > Assam and Shillong
	O-tea	Terai > Dooars > Assam > Nilgiri > Shillong.
Steroids	CTC-tea	Shillong > Nilgiri, Assam, Dooars and Terai
	O-tea	Shillong > Nilgiri > Assam > Dooars > Terai.
Phenol	CTC-tea	Nilgiri, Dooars, Shillong > Assam > Terai

	O-tea	Nilgiri, Assam> dooars, Terai, Shillong.
Reducing sugar	CTC-tea	Nilgiri, Assam, Shillong > Dooars > Terai
	O-tea	Nilgiri, Dooars, Shillong> Assam, Terai.

Conclusion

Bioactive compounds present in tea and tea infusions are variable. The differences in chemical nature of manufactured tea from tea growing regions were reflected in our results. Tea lovers have a multitude of reasons for preferring tea from specific region and undergoing specific manufacturing processes. Love for the distinctive taste of tea is so immense that test results concerning its other assets are often overlooked. 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.

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