INTRODUCTION

Tea decocion is the most preferred beverage in the world next to water\(^1\). Tea decocion is generally prepared by brewing tea in hot water or milk or both. Among the several commonly available varieties, green and black teas are preferred most throughout the world. Green and black teas are preferred for aroma, taste, brew colour and various positive physiological functions\(^2\). Health benefits of tea consumption have been well known to human civilization for centuries. Since the early days hot tea infusions was very much popular for improving mood, cognition ability, blood flow, detoxification and prevention of different diseases\(^3\). Phytochemical screening of tea leaves have shown the presence of more than 700 chemicals viz. polyphenols, flavonoids, alkaloids, tannins, volatile constituents and amino acids as the main active ingredients having potent antioxidant activities, anti-ulcer, anti-cancer and hepatoprotective activities\(^4,5\). Among the several bioactive components of tea, polyphenols and flavonoids play an important role in maintaining health and wellness by boosting up the body’s antioxidant system. Antioxidant is a group of compounds that help to protect the cells in from free radical damage, and thereby controlling stress and the rate of ageing. Scot et al showed that tea decocion can reduce fatigue of the team members during their high altitude expedition. During their journey hot brewed tea formed a major part of the required fluid intake where brewing tea at hot was possible, but at unfavourable condition for tea brewing, tea was excluded from their diet\(^6\) because from the time unknown drinking hot tea decocion becomes the only popular way to consume the tea. Recent research shows that heating sometime deactivates or destroys several important heat sensitive bioactive molecules mainly flavonoids class of compounds present in the tea or other green vegetables or fruits\(^7\). It is also observed that people of several high altitudes or extreme environments are devoid of all such facilities to prepare hot tea brew. In this backdrop finding an easiest low cost method become essential to serve tea and its bioactive components to all without any bar. Hence, the aim of this study was to screen total polyphenolics content (TPC), flavonoids content and free radical scavenging property of cold water decocion of tea and to compare with the traditional hot water decocion.

MATERIAL AND METHODS

**Instruments and Apparatus**

Analytical digital weighing balance (Wensor), UV-Visible Spectrophotometer (Systronics117), Centrifuge (Remi PR 23) was used for the experiments.

**Chemicals and Reagents**

For quantitative estimation of polyphenols content, flavonoids content and their anti-oxidants activity in tea samples the following reagents and chemicals were used: Gallic Acid standard (Sigma), Ascorbic acid (Sigma), Sodium carbonate (Merck), Sodium Hydroxide(Merck), Anhydrous Sodium Nitrite (Merck), Anhydrous

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Different tea samples were collected from the locally situated shop in Kolkata, West Bengal. Name of the tea brands are summarized in the table 1:

**Preparation of tea infusion**

Prior to extraction, tea samples were grinded by using mortar pestle and pass through a sieve in order to obtain a homogeneous fine powder. For preparing hot infusion 100 ml boiling water was added into 2 gm of powdered tea in 250 ml conical flask and kept in 90 degree C temperature for 05 min, then clarified through Whatman filter paper (Grade 1). Similarly, cold tea brew was prepared by adding 100 ml water (at room temperature) into 2 gm of powdered tea in 250 ml conical flask and kept in 4 degree C for 06 hr with intermittent shaking, then clarified through Whatman filter paper (Grade 1). The clear flow through was collected in a 100 ml volumetric flask, volume was made up with water and used for subsequent assay.

**Determination of Total Polyphenolics Content (Folin-Ciocalteu Assay)**

Tea infusions prepared from different samples were used to determine the total polyphenolics content using standard method with slight modification. Folin-Ciocalteu (1:10) reagent was added to each samples of both experiments followed by addition of 7.5% Sodium Carbonate. This reaction was incubated for 2 h. The absorbance was recorded at 765 nm. The calibration curve was prepared using Gallic acid as standard.

**Determination of Flavonoids Content (Aluminium chloride Assay)**

Aluminium chloride colorimetric method was used for determination of flavonoids with slight modification. Tea extract was mixed with 5% sodium nitrate followed by 10% aluminium chloride. After 6 min 1M sodium hydroxide was added to it. Absorbance was recorded at 510 nm. The calibration curve was prepared by using Catechin as standard.

**DPPH Radical Scavenging Assay**

DPPH Scavenging Assay was performed according to standard method with some modification to measure antioxidants properties present in natural products with slight modifications. DPPH (1, 1 – Diphenyl-2-picrylhydrazyl) is a stable free radial. 0.1mM DPPH solution was prepared in methanol solution and its OD was adjusted in between 0.9 to 1.000. 100 µL of each Tea samples (of suitable dilution) were added to DPPH solution and incubated for 30 min in dark. Absorbance was noted at 517 nm. The calibration curve was prepared by using Ascorbic acid as standard.

**Statistical Analysis**

Graph Pad Prism7 has been used for statistical analysis of the data. Paired t test has been performed to study the significant changes among the data <0.005.

**RESULTS**

**Total polyphenols content (TPC)**

From the present study it was observed that the TPC in black tea has no such significant difference when brewed in cold and hot water. However a significant difference in hot and cold decoction of green tea was observed (Fig.1.).
It was observed that like hot decoction a cup of cold green and black tea contains a good amount of flavonoids (Table 2).

**Total Flavonoids content (FC)**

From the present study it was observed that the FC in black tea has no such significant difference when brewed in cold and hot water. However a significant difference in hot and cold decoction of green tea was observed (Fig.2). With respect to hot green tea decoction cold green tea decoction contain significantly high amount of flavonoids as seen in the Fig.2.

Interestingly, like TPC hot decoction a cup of cold green and black tea contains a good amount of flavonoids (Table 3).

**Total Ascorbic Acid Equivalent Content (TAAEC)**

In this experiment TAAEC in different tea decoction has been calculated based on % DPPH radical scavenging assay where Ascorbic Acid was used as standard. Both in the case of green and black tea extracts infused in hot and cold water on average have shown no such significant difference statistically.

Another interesting point was observed that like hot decoction a cup of cold green and black tea contains a good amount of antioxidant (Total ascorbic Acid Equivalent Content).

**DISCUSSION**

Work stress is one of the most common factors nowadays. Antioxidants, flavonoids and polyphenols are very much needed for proper maintenance of health. Generally, vegetables, fruits and beverages have been suggested for better health as they full of important antioxidants, flavonoids and polyphenols. There are many medicinal plants whose bark, stem are being soaked in water for whole night and the water is drunk in the whole day. Our present work aimed to compare antioxidant status of cold brewed tea with the traditional hot tea infusion. Tea is chosen for that study because tea is full of good antioxidants and easy to prepare. People of every economical background can procure tea as its price range from very low to high which based on the cultivation process, region, production process, tea cultivar etc. But one thing is true that all kind of tea contains good polyphenols that have huge health promoting activities. In common practice tea is commonly prepared by pouring hot or boiling water over cured leaves of the *Camellia sinensis* But problem occurs when there is scarcity of fuel or electricity. Basically high altitude areas are devoid of all the facilities for preparing tea. Moreover many secondary metabolites that are heat sensitive might get destroyed during hot decoction preparation time. In this situation drinking cold brewed tea is only the option. But this method is not popular like hot brewing procedure. So detailed scientific study only can popularize the cold brewed tea to common people. Very few works has been done to date. Hence in the present have been designed to see the antioxidant status of cold brewed tea in compared to hot. We found that the almost similar amount of polyphenolics, flavonoids and anti-oxidants are released in both the brewing condition. However, it was observed that polyphenols and flavonoids content are significantly higher in cold brewed green tea with respect to hot decoction. However, it was observed that cold brewed tea...
Table 4: TAAEC in Hot and Cold decoctions of black and green varieties of tea. Here 5 black teas and 5 green teas were used for this experiment.

<table>
<thead>
<tr>
<th>Tea Type</th>
<th>TAAEC in Hot Decoction/cup of tea (mg AAE/ cup)</th>
<th>TAAEC in Cold Decoction/cup of tea (mg AAE/ cup)</th>
</tr>
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<tbody>
<tr>
<td>Black Tea</td>
<td>260.06 ± 76.26</td>
<td>231.35 ± 89.18</td>
</tr>
<tr>
<td>Green Tea</td>
<td>338.77 ± 47.93</td>
<td>341.86 ± 32.46</td>
</tr>
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is more or less comparable with hot decoction with respect to antioxidant activity. Ascorbic acid equivalent for cold brewed green and black tea is 341.86 ± 32.46 mg/cup and 231.35 ± 89.18 mg/ cup respectively which is pretty high with respect to average daily intake of vitamin C (107 mg)\(^9\). Therefore, we can say that after brewing the tea overnight in cold condition can also fulfill the antioxidant requirement of our body.

ACKNOWLEDGEMENT
Authors are very much thankful to Prof. Sandip K. Bandyopadhyay, Ex. Professor, KPC Medical College and Dr. B. Bera, Director (Research), Tea Board for unconditional help throughout the work. Authors also grateful to Chairman, Techno India University, West Bengal for all necessary lab facilities for research work.

CONFLICT OF INTEREST
No conflict of interest

REFERENCES