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# The effect of ambient UV radiation on high and low altitude north Bengal Tea

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**Abstract**
The natural UV radiation is one of the environmental factor to upregulate qualities of the tea. Tea grown in the North Bengal area starting from high altitude Darjeeling to the Himalayan foothills to Dooars and Terrai region including Darjeeling, Jalpaiguri and Siliguri, are exposed to different doses (Irradiance) of natural UV radiation. Such UV radiation can upregulate the secondary active metabolites like polyphenols *viz.* catechin, gallic acid etc. These up-regulated secondary metabolites contribute to the elevated therapeutic qualities like antioxidant and antimicrobial properties. Different types of solvents like aqueous, methanol and ethanol can extract different contents of active metabolites which can be further continued towards the drug designing process. Here the Dooars and Terrai grown (Low altitude grown) teas are having less antioxidant and antimicrobial potentials and active components than high altitude grown Darjeeling tea.

**Key words:** Tea, Dooars, Darjeeling, altitude, antioxidant, antimicrobial

## Introduction
Tea is not only a popular beverage but also very much known for its therapeutic values. North Bengal is one of the well-known tea growing place of India which contributes, around 640 million kg of tea last year.
North Bengal tea growing India is divided into two main regions-one is high altitude area in Darjeeling. Seven valleys (Kurseong South, Kurseong North, Darjeeling East, Darjeeling West, Mirik, Tessta and Upper Fagu) of Darjeeling are the land of elite tea growing areas (around 4500 ft msl) (Ray *et al*., 2020) [1]. These teas are famous for their aroma and therapeutic potentials like antioxidant and antimicrobial properties. The effects of optimum UV radiation upregulates the secondary metabolites like flavonoid glycoside which are the responsible candidate of such high therapeutic potentials. The garden factories are famous to manufacture orthodox black teas and other types (Green, Oolong, White etc.) (Saha and Shyam Choudhury, 2020) [2].
The second site is Dooars-Terrai regions where many of the well-known gardens are there to manufacture orthodox and CTC black tea. Most of the Jalpaiguri tea gardens are situated under this site as well as the Siliguri tea gardens are also situated at these low altitude region (around 292-400 ft msl) (Malik and Ghosh, 2021) [3].
UV radiation is one of the well-known factor to upregulate the quality components in high altitude grown Darjeeling tea to impart aroma and therapeutic potentials (Bhattacharya and SenMandi, 2011) [4]. Total polyphenols, flavonoids are the quality parameters to contribute towards antioxidant and antimicrobial potentials of tea. Spectrophotometric analysis and Thin layer chromatography could show the variation of different types of tea which may be fresh leaves or manufactured tea (Shyam Choudhury *et al*., 2018) [5].
The quality of these low altitudes grown tea are lesser than Darjeeling orthodox tea though they also contribute towards the repute of North Bengal tea in terms of commercial and therapeutic qualities (Halder *et al*., 2020) [6]. This paper aims to correlate the natural ambient UV radiation with the quality parameters of the tea leaves and manufactured tea of the same gardens.

## Materials and Methods
### Materials
Two leaf a bud tea leaf samples were collected acquired as S1A and S1B from Ellenbarie Tea Estate (Altitude 266 ft msl), Jalpaiguri; S1A consisted of young apical tissue of 2 leaf

and a bud, S1B are old leaves, sample S2A (Apical tissue of 2 leaf a bud)and S2B (old leaves from Sunrise Tea Garden (122 ft msl), Siliguri. Fresh tea leaves S3 A and manufactured teas S3B are collected from high altitude grown Balasun Tea garden (4511ft msl), Darjeeling. Manufactured teas were acquired from the corresponding garden (Ellenbarie black tea S1C, Sunrise black tea S2C, S3C is Balsun Black tea).

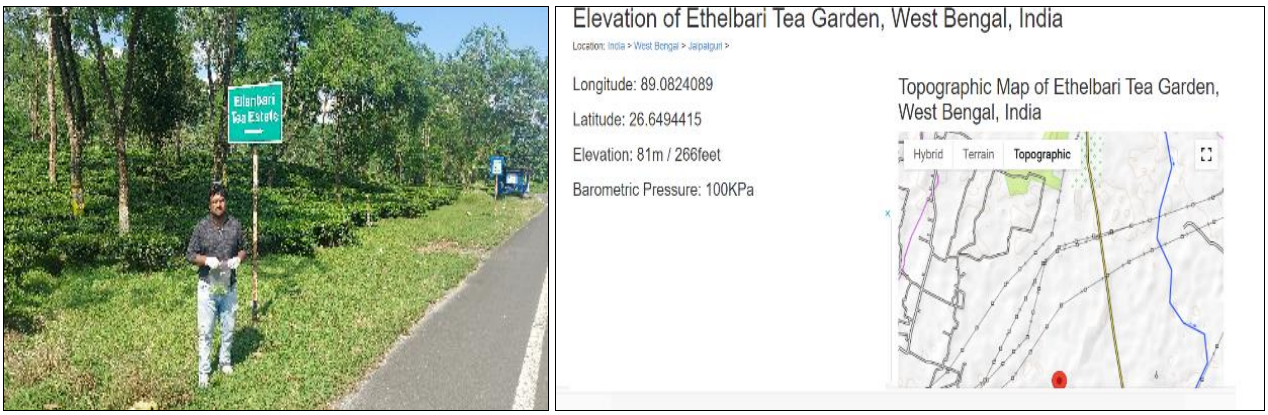


**Fig A:** Ellenbari tea garden, Jalpaiguri

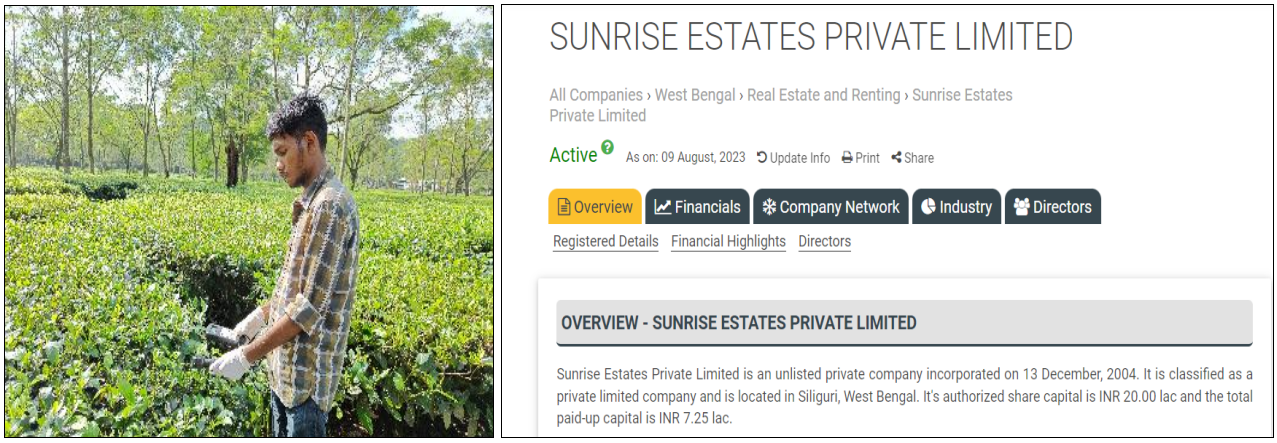


**Fig B:** Measurement of ambient UV radiation in Sunrise tea garden, Siliguri

**Fig C:** Tea leaf samples of Ellenbari and Sunrise tea garden


**Fig D:** Manufactured tea samples of Ellenbari and Sunrise tea garden


**Fig E:** Balasun Tea garden


**Fig F:** Balasun leaf and manufactured tea

**UV radiation and total ambient radiation measurement:**
UV irradiance was monitored in all the mentioned sites were measured by UV Light meter (Model: UV-340 ISO-9001, CE, IEC1010) for UVA & UVB measurement, spectrum: 290-390 nm (Fig 1).

**Preparation of extracts**
The leaf (leaf and manufactured 500 mg) extracts were made using a mortar and a pestle. At first leaves are taken and crushed with the solvent (Aqueous/Ethanol/Methanol-20ml each) slowly being added after each period of crushing.

**Measurement of Total Polyphenol**
The total polyphenol contents are determined according to the Anesini *et al*., 2008 [7]. In 1ml of each manufactured tea extracts along with 4 ml of 1 M $Na_2CO_3$ and 1 ml of Folin Ciocaltau phenol reagent are added. The absorbance is taken at 765 nm. Gallic acid was used as standard. The results were expressed in terms of mg/ml gallic acid equivalent (Fig 2).

**Spectrophotometric scan**
U-2900 Spectrophotometer was used for the spectral scan of tea samples. The spectral scan was run from 200 nm to 400 nm, Gallic acid is taken as standard (Fig 3) (Atomssa and Ghoslap, 2015) [8] (Fig 3, Table 1)

**Thin layer chromatography**
The catechin spots are detected on the silica plate and the n-butanol-acetone-acetic acid 5:5:3 (v/v) mobile phase is used. To identify the spots Vanillin-HCl reagent was used, Tannic acid (0.5 mg/ml) was taken as standard (Fig 4) (Pramiastuti and Joraho, 2020 [9].

**Antioxidant potential**
Antioxidant potential is assayed (According to Rebeiro *et al*. 2002 [10] by taking 1 ml of the tea sample extracts and the percent reduction of 0.004% DPPH solution in MeOH is assayed at 517 nm absorbance by spectrophotometer (Fig 5)

**Antimirobial potential**
The antimicrobial testes were done according to Bose and Bose, 2008 [11] by doing Agar cup plate method against *E. coli* and the zone of inhibition were measured after 24 hours. Ampicillin solution (5 mg/ml) is taken as positive control and as negative control solvents in each case (Fig 6a, 6b, 6c, 6d, 6e, 6f, 6g, 6h Table 2)

**Results and discussion**

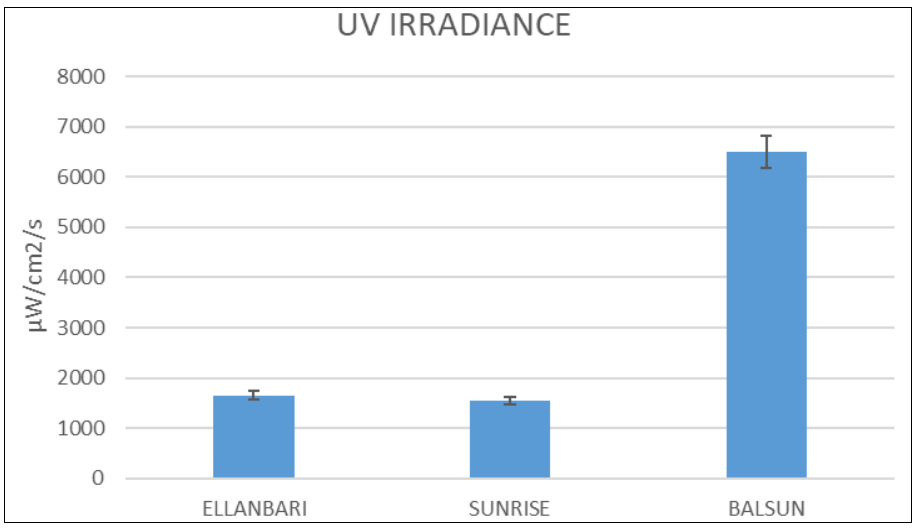


**Fig 1:** Natural UV radiation at different tea gardens

Fig 1 shows that the highest natural UV irradiance is found in the higher altitude Balasun tea garden. Bhattacharya and SenMandi, 2011 [4] had explained the different altitudinal variation in UV radiation doses in Darjeeling.

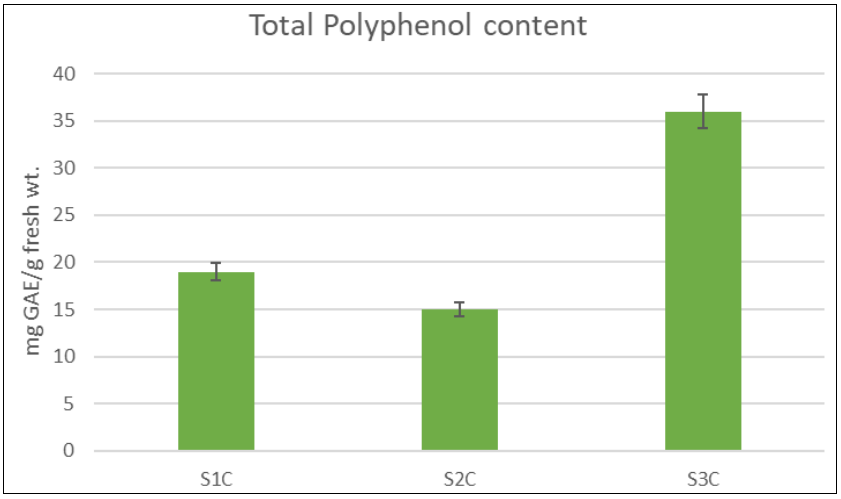


**Fig 2:** Total polyphenol content of manufactured teas

Fig 2 shows the higher total polyohenolic content in Balasun grown manufactured black tea (S3C). It is pertinent to mention here that the Sunrise manufactured tea (S2C) has lower total polyphenolic content than Ellanbari manufactured black tea (S1C). Shyam Choudhury *et al*., 2018 [5] had described such altitudinal variation in Darjeeling tea and Gomes *et al*., 2015 [12] had demonstrated such variation in Dooars tea.

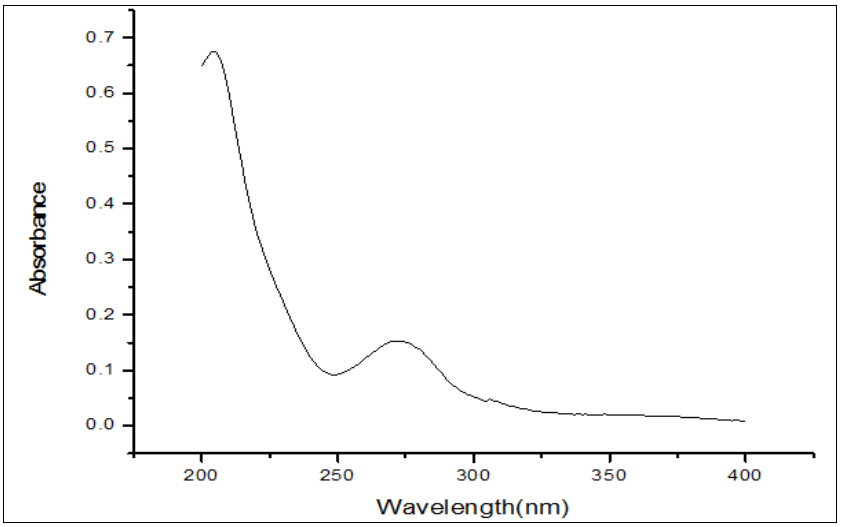


Fig 3a

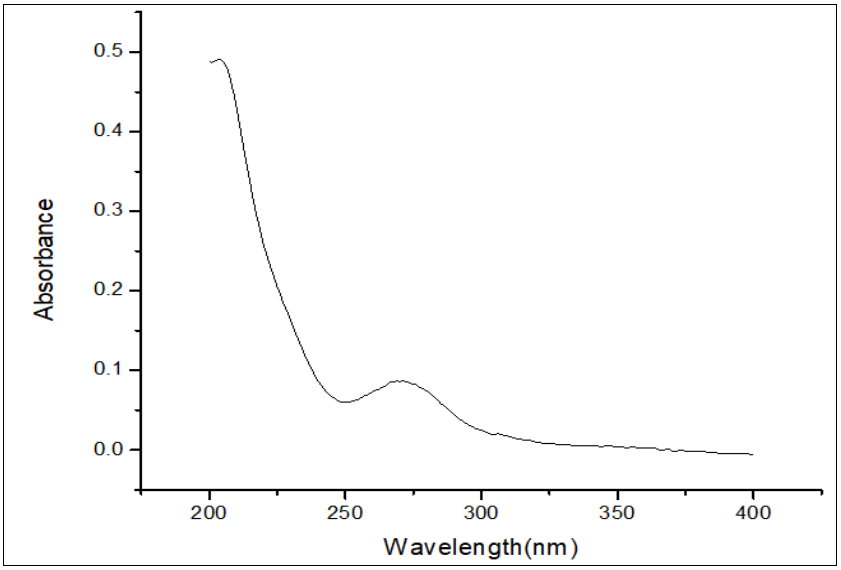


Fig 3b

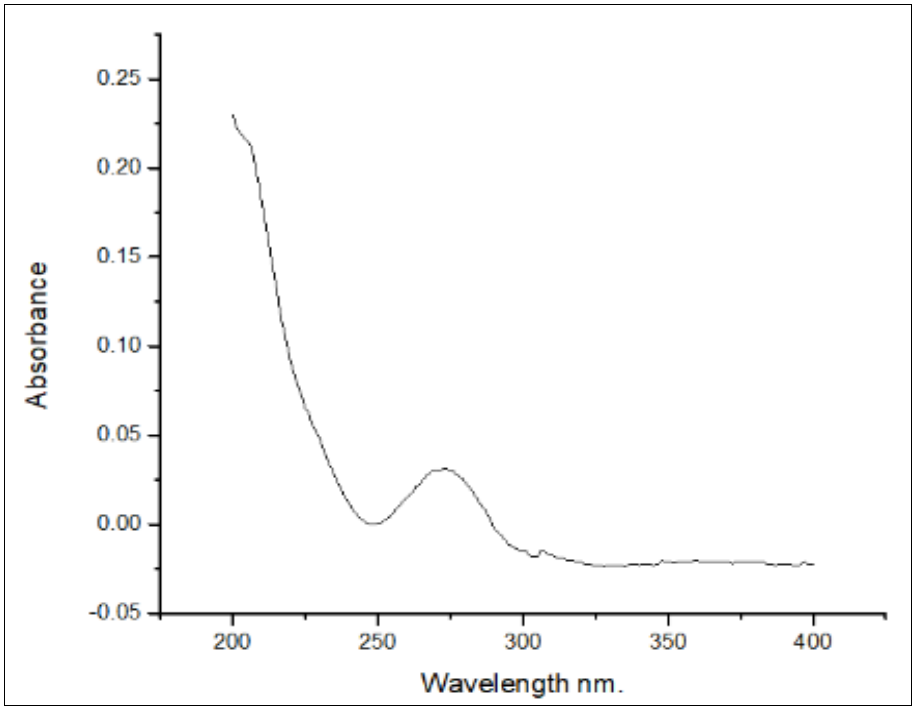


Fig 3c

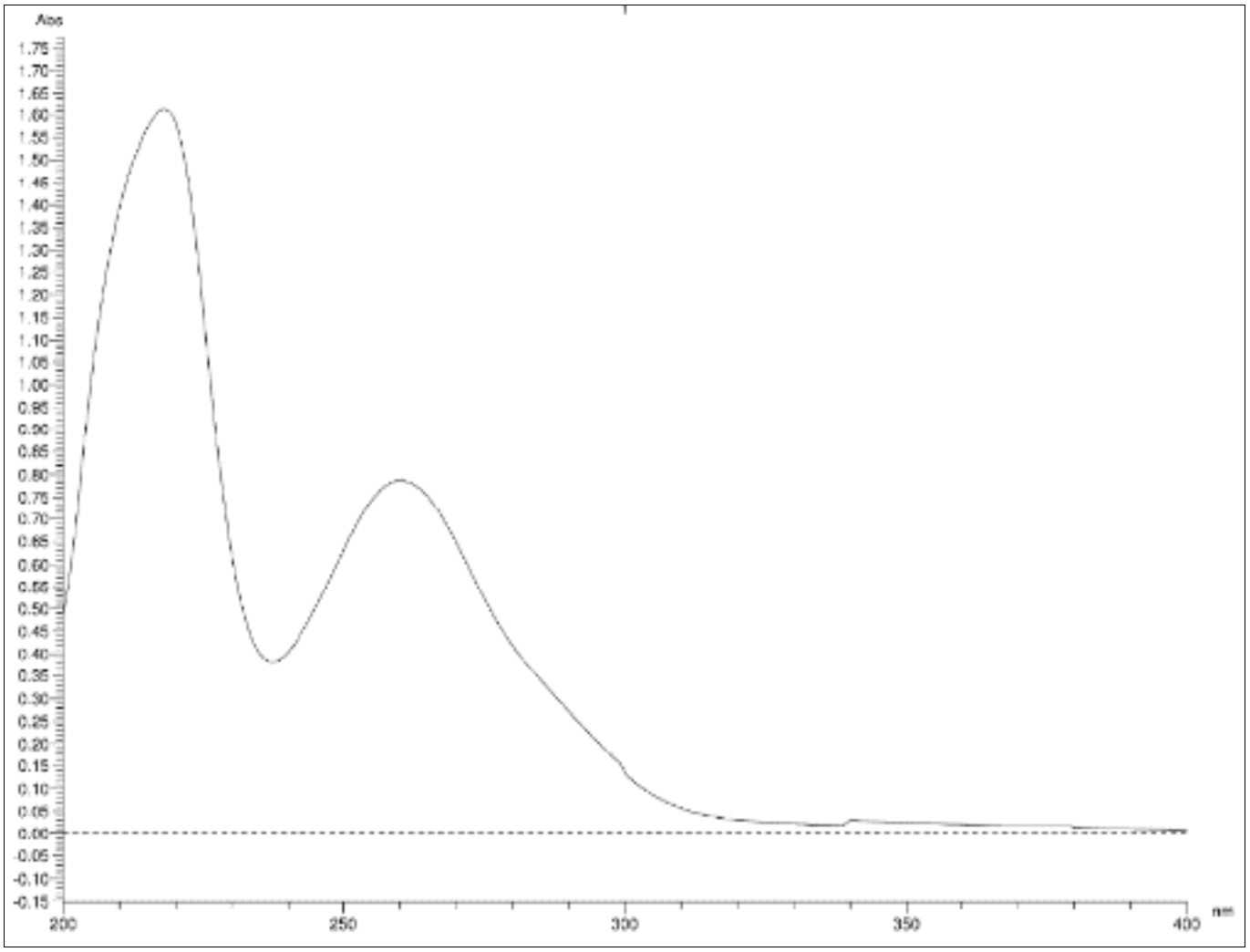


Fig 3d

**Fig 3a, 3a, 3b, 3c, 3d:** Spectrophotometric scan between 200-400 nm S3C,S1C,S2C, Gallic acid standard respectively having absorption peak at 265 nm.

**Table 1:** Polyphenol content from spectrophotometric scan at 265nm

| Sample | Absorbance | Concentration |
|---|---|---|
| Gallic acid | 0.75 | 0.0025 mg/ml |
| S1C | 0.1 | 0.00033 mg/ml of 1 ml extract |
| S2C | 0.03 | 0.0001 mg/ml of 1 ml extract |
| S3C | 0.18 | 0.0006 mg/ml of 1 ml extract |

Fig 3a, 3b, 3c, 3d and Table 1 shows that comparing with Gallic acid as standard the highest content in Balasun manufactured tea S3C. Table 1 shows the concentrations of the polyphenols from the standard peak at 265nm of Gallic acid (0.0025 mg/ml).

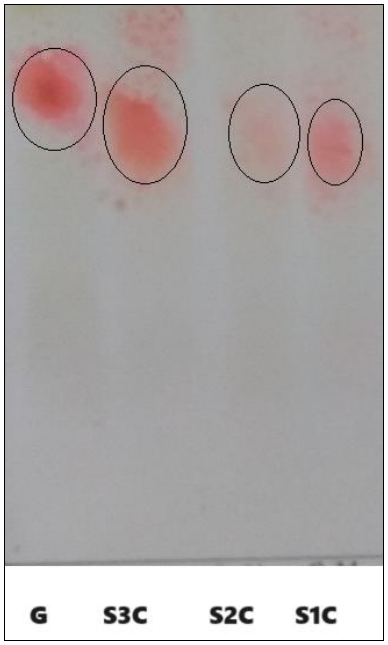


**Fig 4:** Thin layer chromatography of manufactured tea

Fig 4 shows that Rf value of standard Gallic acid at 0.55 cm is almost equivalent to S3C, S2C and S1C samples (At 0.52cm) clearly stating the presence of polyphenolic contents in those samples but most prominent spot is observed for S3C. Kumar *et al*., 2011 [13] had described different methods to measure variation of polyphenol contents in different tea.

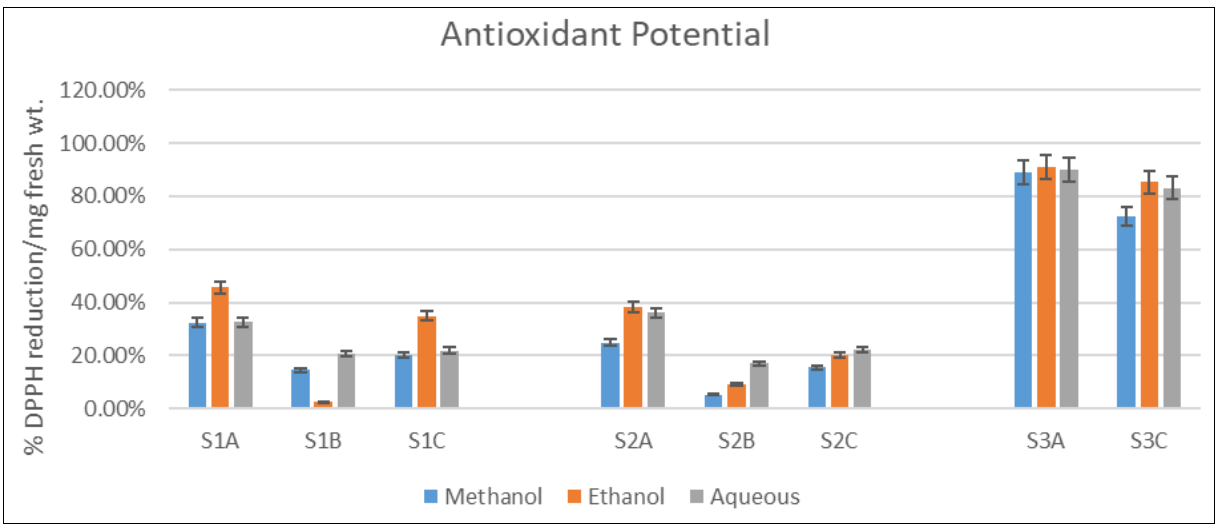


**Fig 5:** Antioxidant potential of different types of tea (fresh and old leaves and manufactured tea)

Fig 5 shows higher antioxidant potential in Balasun tea (both leaves and manufactured). It is pertinent to mention here that in older leaves have lesser antioxidant values in all the solvents, methnolic, ethanolic and aqueous extracts show variable kind of efficacies of different types of tea. Dasgupta *et al*., 2013 [15] had described the variation in Darjeeling grown teas at different altitude. With respect to antioxidant potential.

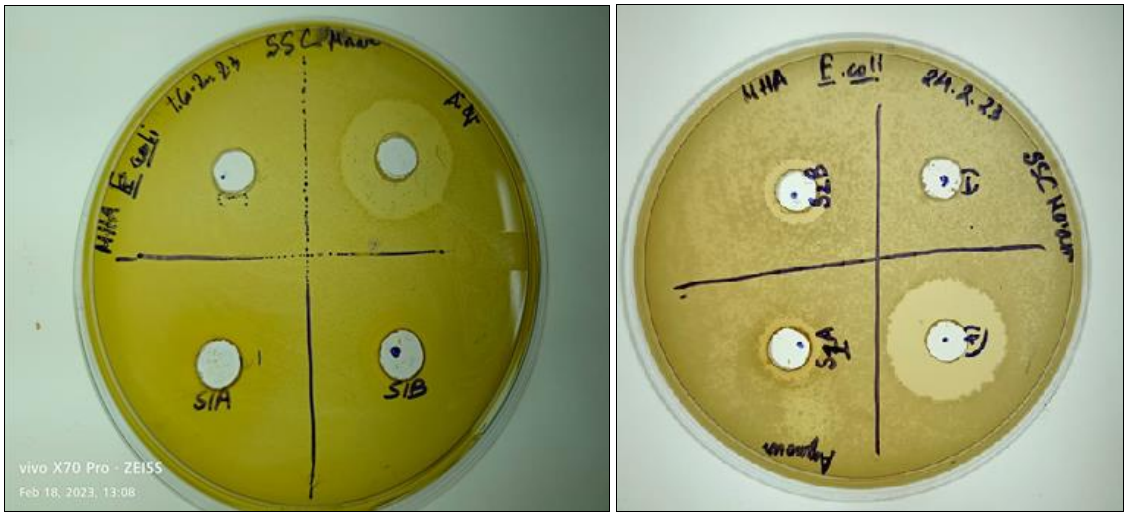


**Fig 6a:** The antimicrobial activities of S1A, S1B, S2A, S2B all aqueous solutions against *E.Coli*


**Fig 6b:** The antimicrobial activities of S1A, S1B, S2A, S2B all aqueous solutions against B.Subtilis

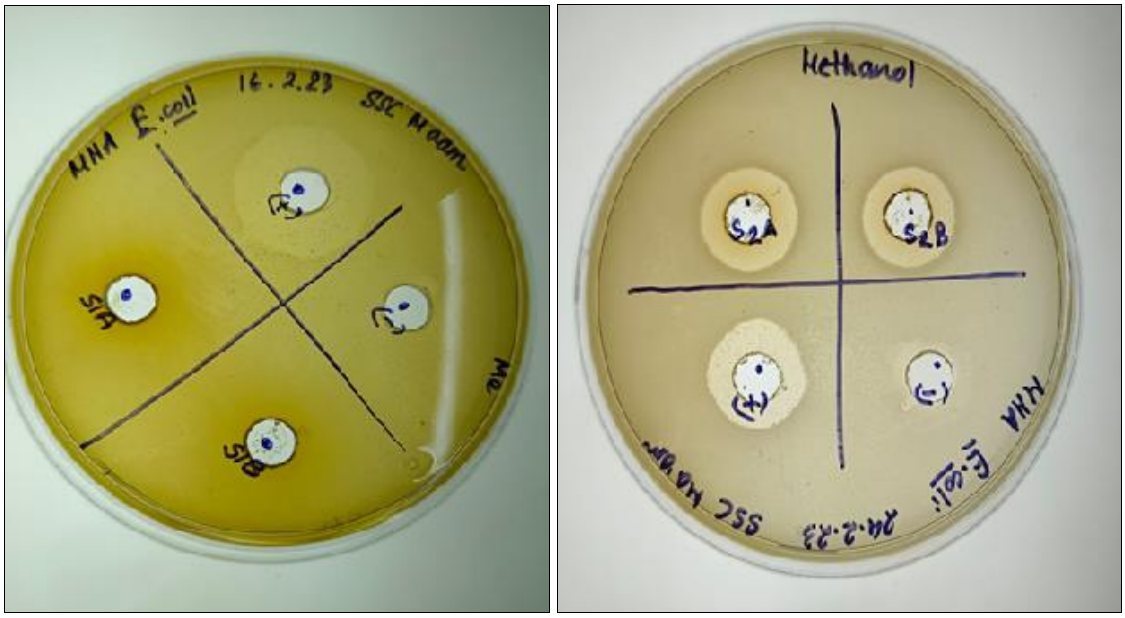


**Fig 6c:** The antimicrobial activities of S1A, S1B, S2A, S2B all methanolic solutions against E.Coli


**Fig 6d:** The antimicrobial activities of S1A, S1B, S2A, S2B all methanolic solutions against B.Subtilis


**Fig 6e:** The antimicrobial activities of S1A, S1B, S2A, S2B all ethanolic solutions against E.coli

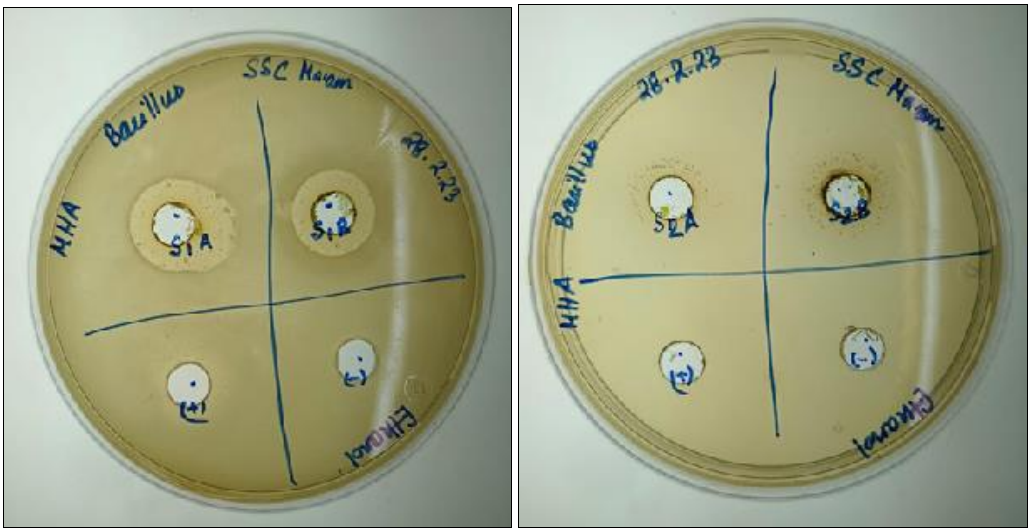


**Fig 6f:** The antimicrobial activities of S1A, S1B, S2A, S2B all ethanolic solutions against B.subtilis

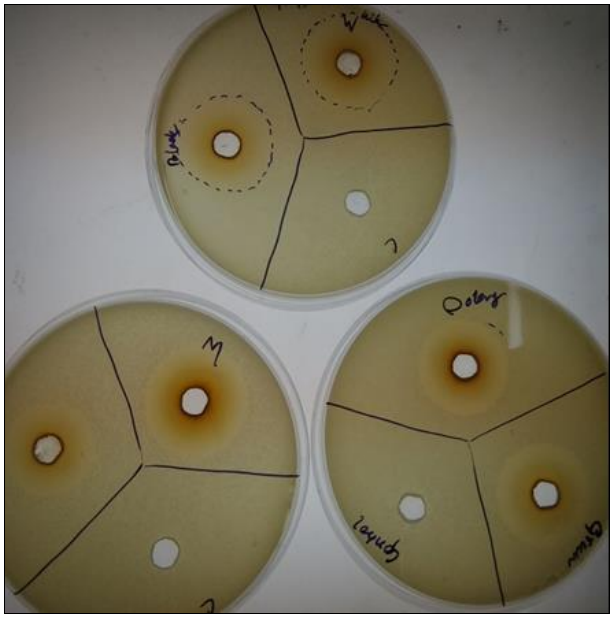

**Fig 6g:** The antimicrobial potential of S3A in ethanol, methanol and aqoues solvents against *E.Coli*

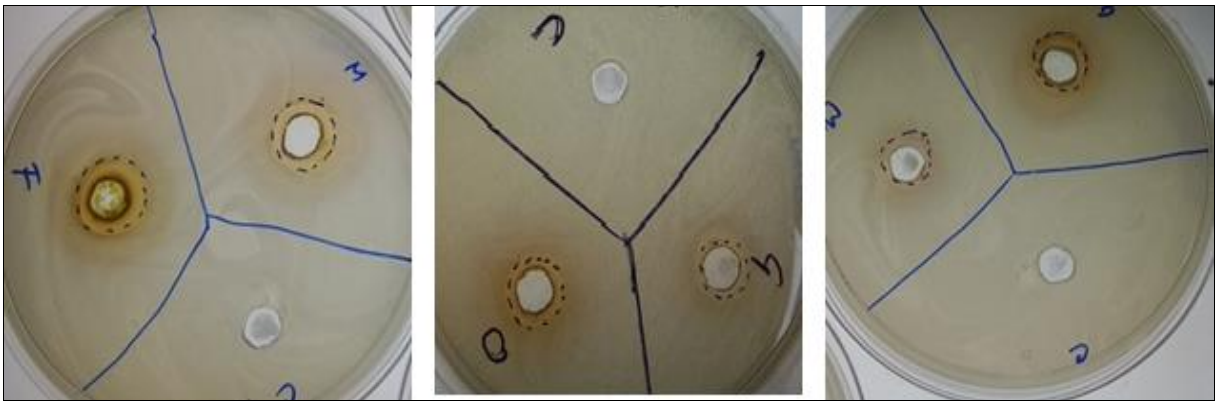

**Fig 6h:** The antimicrobial potential of S3C in ethanol, methanol and aqoues solvents against *E. Coli*

**Table 2a:** Antimicrobial potential (Zone of inhibition in cm) of S1A, S1B, S1C, S2A, S2B, S2C

| SAMPLE | Zone Of Inhibition of inhibition in cm against *E. Coli* (cm) | Zone Of Inhibition of inhibition in cm against *B. Subtilis* (cm) |
|---|---|---|
| Positive control Ampicillin | 0.53 | 0.41 |
| Negative control water | - | - |
| Negative control methanol | - | - |
| Negative control ethanol | - | - |
| S1A (Aqueous) | 0.3 | 0.2 |
| S1B (Aqueous) | 0.1 | 0.1 |
| S1C (Aqueous) | 0.08 | 0.05 |
| S2A (Aqueous) | 0.1 | 0.25 |
| S2B (Aqueous) | 0.2 | 0.25 |
| S2C (Aqueous) | 0.05 | 0.05 |
| S1A (Methanol) | 0.3 | 0.4 |
| S1B (Methanol) | 0.3 | 0.1 |
| S1C (Methanol) | 0.1 | 0.1 |
| S2A (Methanol) | 0.4 | 0.4 |
| S2B (Methanol) | 0.4 | 0.3 |
| S2C (Methanol) | 0.08 | 0.05 |
| S1A (Ethanol) | 0.3 | 0.4 |
| S1B (Ethanol) | 0.3 | 0.3 |
| S1C (Ethanol) | 0.09 | 0.09 |
| S2A (Ethanol) | 0.35 | 0.2 |
| S2B (Ethanol) | 0.4 | 0.1 |

**Table 2b:** Antimicrobial potential (Zone of inhibition in cm) of S3A, S3C

| Sample | Zone Of Inhibition of inhibition in cm against *E. Coli* (cm) |
|---|---|
| Positive control Ampicillin | 0.5 |
| Negative control all solvents | - |
| S3A (Methanol) | 0.5 |
| S3A (Aqueous) | 0.3 |
| S3A (Ethanol) | 0.4 |
| S3C (Methanol) | 0.3 |
| S3C (Aqueous) | 0.1 |
| S3C (Ethanol) | 0.2 |

Fig 6a, 6b, 6c, 6d, 6e, 6f, 6g, 6h and Table 2a and 2b shows that among all the highest zone of inhibition against *E. coli* imparted by S3A Balasun fresh leaf methanolic extract.

**Table 3:** Regression analysis of Balasun tea garden throughout the year:

| Month | Antioxidant Potential (y) | Annual UV Radiation dose(microwatt/cm2/sec) (x) | |
|---|---|---|---|
| January | 66 | 2800 | Regression equation y = 24.43061 + 0.015815x |
| February | 73 | 2900 | |
| march | 78 | 3100 | |
| April | 89 | 4000 | |
| May | 92 | 4300 | |
| June | 98 | 4500 | |
| July | 94 | 4490 | |
| August | 80 | 3700 | |
| September | 79 | 3600 | |
| October | 76 | 3200 | |
| November | 70 | 2900 | |
| December | 67 | 2800 | |
| Month | Antimicrobial potential ZOI in cm (y) | Annual UV Radiation dose(microwatt/cm2/sec) (x) | |
| January | 0.2 | 2800 | Regression equation y = - 1.92098 + 0.000793x |
| February | 0.6 | 2900 | |
| march | 0.8 | 3100 | |
| April | 1 | 4000 | |
| May | 1.3 | 4300 | |
| June | 1.9 | 4500 | |
| July | 1.8 | 4490 | |
| August | 1 | 3700 | |
| September | 0.7 | 3600 | |
| October | 0.6 | 3200 | |
| November | 0.4 | 2900 | |
| December | 0.2 | 2800 | |
| Month | Polyphenol content (mgGAE/gDW) (y) | Annual UV Radiation dose(microwatt/cm2/sec) (x) | |
| January | 155 | 2800 | Regression equation y = - 155.89 + 0.116805x |
| February | 170 | 2900 | |
| march | 210 | 3100 | |
| April | 310 | 4000 | |
| May | 345 | 4300 | |
| June | 360 | 4500 | |
| July | 350 | 4490 | |
| August | 295 | 3700 | |
| September | 285 | 3600 | |
| October | 270 | 3200 | |
| November | 160 | 2900 | |
| December | 159 | 2800 | |

It is evident from the above results that the high altitude grown Balasun tea has greater antioxidant and antimicrobial potential which is also evident in polyphenol and catechin content. So it could be inferred that the exposure to higher natural UV radiation is responsible to upregulate all those secondary metabolites which are in turn responsible to show the therapeutic values. Laber, 2022 [14] had explained such variation of medicinal values of Darjeeling and Dooars tea.

**Conclusion:** The effect of natural UV radiation is reflected in the polyphenol contents of Darjeeling and Dooars-Terrai grown tea which is ultimately manifested as differential therapeutic potentials (Antioxidant, antimicrobial values)

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