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The positive effect of UV radiation on biochemical and microbiological characteristics of different altitude grown Darjeeling tea clones

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Abstract
Fresh tea leaves (two leaves and a bud) were collected from different altitudes of Darjeeling viz. Dilaram tea estate, (5843ft msl), Maldiram (5600ft msl), Giddhapahar (4354ft msl), Upper Jungpana (4347 ft msl) and Ahaldara (4006 ft msl) which were exposed under different natural ambient UV radiation. The altitudinal heights are directly proportional to the UV irradiance measured by UV meter. Total antioxidant and UV absorbing compounds are highly correlating with higher UV exposure to high altitude grown tea. The enzymatic antioxidant Superoxide Dismutase activity is almost similar in case of high altitude and low altitude grown tea clones. The higher extent of natural UV dose upregulates the non-enzymatic antioxidant activities as flavonoids, specially flavonoid glycosides. This flavonoid glycosides, specially Rutin serves as aroma precursor as well as better antimicrobial components. The increased extent of such active component in different altitude grown tea clones were qualitatively and quantitatively detected by Thin Layer Chromatography and UV spectrophotometric scan respectively.

Keywords: high altitude, tea, UV radiation, flavonoid glycoside, antioxidant potential, antimicrobial activity

Introduction
Tea is a universally accepted social and medicinal beverage, with wider acceptance than the other beverage of choice viz. coffee and a major cash crop in India. It is manufactured from leaves of tea herb, *Camellia sinensis*, (L.) O. Kuntze. According to FAO (Food and Agriculture Organization, UN) for tea production in all over the world, India is second highest country after China, competing with, Kenya, Sri-Lanka, Turkey and Japan (www.fao.org). Among all the tea-growing areas in India, Darjeeling (27.05°N and 88.26°E) produces most aromatic world famous tea. Darjeeling tea is a unique product of international fame, sometimes known as the "The Champagne of Teas". High altitudes can be correlated with higher exposure to UV radiation which in-turn can produce oxidative stresses in living systems including plants (Kasote *et al*., 2015) [4]. Thus for survival, plants (especially high altitude grown) have evolved effective cell protective mechanisms that may function as UV screen and also as antioxidants like flavonoids which belongs to polyphenols group of compounds (Vicente and Boscaiu, 2018) [16]. Darjeeling is the place where at different altitudinal variations tea plants are grown, exposed under different ambient doses of UV radiation (Bhattacharya and Sen-Mandi, 2011) [3]. It was observed that during summer solstice when (March-July) Darjeeling area (~27°N latitude) receives maximum UV radiation with optimum solar zenith angel gives rise to most aromatic 2nd flush of tea indicating positive influence of UV radiation (Shyam Choudhury and SenMandi, 2012a) [14]. Among UV absorbing compounds the flavonoids are most effective, specially the flavonoid glycosides and their synthesis are UV upregulated (Agati and Tattini, 2010) [1]. The higher antioxidant and antimicrobial potential of flavonoid glycosides were reported by Kumar and Pandey, 2013. This flavonoid glycosides are also served as aroma precursors as secondary value addition in tea (Bhattacharya and Sen-Mandi, 2011) [3].

So our objectives of study include the measurement of ultraviolet radiation of tea estates situated at different altitudes of Darjeeling area, and to study the antioxidant (enzymatic and non-enzymatic) and antimicrobial activities of those different altitude grown tea.

Material and Methods
- **Sample collection:** The samples were collected from Ahaldara (4006ft); Giddhapahar (4354ft); Dilaram (5843ft); Maldiram (5600ft); Upper Jungpana (4347ft).
- **Measurement of UV radiation-**UV irradiance was measured with Lutron UV-340A UV LIGHT METER in μWatt/cm^2/sec in different time intervals from different altitudes of Darjeeling (Fig 1).
- **Extract preparation:** The methanolic and aqueous extracts were prepared by taking 500 mg leaf samples in 10 ml solvents.
- **Assay of total UV absorbing compound**: this assay is done according to Mazza *et al*, 2000 by making the extract using methanol: HCl in 99:1 ratio for 48 hours at -20 degree celsius and O.D. was measured at 305 nm (Fig 2).
- **Assay of total antioxidant potential:** It was done according to Ribeiro *et al*, 2002 by using 0.4% DPPH solution (Fig 3).
- **Assay of Superoxide Dismutase Activity:** SOD enzyme assay was done according to Upadhyay and Panda, 2013 by taking NBT and TEMED. The absorbance was recorded at 638.2nm (Fig 4)

- **Measurement of total polyphenol**--It was determined according to Anesini *et al*, 2008 the polyphenol content is measured with 7% Na_2CO_3 and F.C reagent (Fig 5)
- **Flavonoids analysis**--Flavonoid assay was done according to Zhisen *et al*., 1999 with75 µl of 5% Na_2CO_3, 150 µl of 10% of $AlCl_3$, 750 µl of NaOH (Fig 6).
- **Spectophotomeric scan:** for UV absorbing compound analysis the spectrophotometric scan was also done with methanolic extracts of all the tea clones within 200-400 nm (UV range) according to Saha and Shyam Choudhury,2016-- (Fig 7).
- **Flavonoid glycoside analysis by TLC:** By using Chloroform: Methanol: Acetone in ratio 98:1:1 TLC was done and observed under long UV (365nm) according to Prabhu *et al*., 2011—(Fig 8).
- **Antimicrobial assay**: The zone of inhibition was measured against E.coli in Muller Hinton agar medium according to Radji *et al*., 2013 (Fig 9 and Fig 10).

Results and discussion

The altitudinal heights are directly proportional to the UV irradiance i.e. highest elevation Dilaram receives higher level of UV- 41200 µWatt/cm^2/sec whereas Ahaldara receives lower irradiance of UV- 19300 µWatt/cm^2/sec measured by Lutron UV-340A UV LIGHT METER (Fig 1). The antioxidant potential is highest for Dilaram 70% DPPH reduction/mg fresh wt and lowest for Ahaldara 55% DPPH reduction/mg fresh wt (Fig 3). Vicente and Boscaiu, 2018 have also demonstrated the higher UV radiation can be correlated with higher flavonoid content and antioxidant potential in different plant species. In our results the non-enzymatic antioxidant activities as total flavonoid content (1.07 Rutin Equivalent mg/ml for Dilaram tea; 0.146 Rutin Equivalent mg/ml for Ahaldara tea leaves) is higher in high altitude which may be UV upregulated (Fig 6). Flavonoids belong to phenolic class of antioxidants. The total polyphenol content (Fig 5) of the highest altitude grown Dilaram tea is around 0.006 mg/ml GAE/mg fresh wt; whereas the low altitude grown Ahaldara tea leaves contain 0.003 mg/ml GAE/mg fresh wt—which reflects the sharp variation in different altitudes, the same trends were described by Preedy, 2013 with respect to bush tea plants grown at 1400 masl and 600 masl. The flavonoids also acts as UV absorbing/ UV screening compounds (Agati and Tattini, 2010—which is reflected in our study of total UV absorbing compound-0.001 O.D $_{305nm}$/mg fresh wt. in high altitude grown Dilaram tea leaves and 0.0005O.D $_{305nm}$/mg fresh wt. in low altitude grown Ahaldara tea clones (Fig 2). Agati and Tattini, 2010 also demonstrated the UV upregulation of flavonoids specially flavonoid glycosides which shows better antioxidant efficiency. Rutin is a quercetin diglycoside which serves as aroma precursor in tea leaves and being upregulated by UV they are responsible for showing high antioxidant activities in high altitude grown tea clones (Bhattacharya and SenMandi, 2011). Rutin has absorption maxima at 271.4nm (Xu *et al*, 2010) [17] our spectrophotometric scan data within 200-400nm shows the peaks near 270nm (Fig 7) approximately and the peak height is higher for high altitude grown Dilaram tea (Absorbance 0.18) and lower peak is observed for low altitude grown Ahaldara tea (Absorbance 0.9). Kaya *et al*, 2012 have demonstrated different types of flavonoid glycosides with different R_f values which are more or less similar with our data where the silica plate was exposed to 365nm (Fig 8)—the more intense spots/bands are found in high altitude grown tea clones. Moreover around 0.4 Rf values the intensity of the spot/band is higher in high altitude grown tea clone viz. Dilaram and others than that of low altitude grown Ahaldara tea clones—this Rf value is similar to Rutin according to Prabhu *et al*., 2011. The enzymatic antioxidant Superoxide Dismutase activity is almost similar in case of high altitude grown and low altitude grown tea clones--2.78 U/mg of total protein- Dilaram tea; and 2.9 U/mg of total protein in Ahaldara tea clones (Fig 4).

The antimicrobial potential is higher in high altitude grown tea (measured as zone of inhibition against *E.coli* – 1.2 cm-maximum and for Dilaram than low altitude grown Ahaldara—0.6cm which is minimum)—(Fig 9 and Fig 10). Sati *et al*., 2018 have demonstrated that different altitude (3900ft-6500 ft msl) grown *Gingko biloba* shows variations in flavonoid glycoside content—the higher content of which is responsible to show higher antibacterial properties, similar to our data.

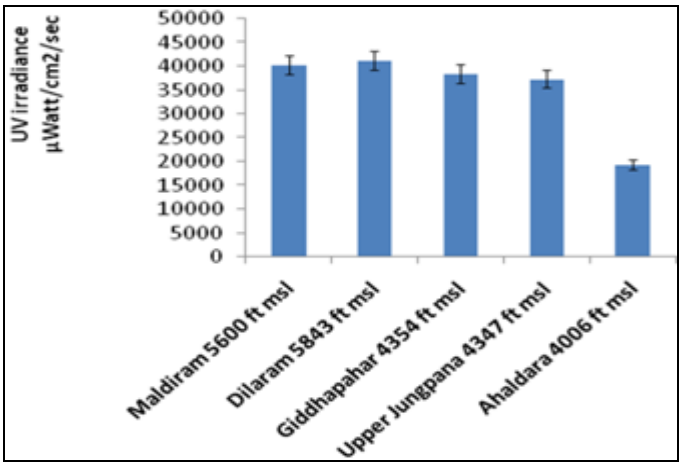

Fig 1: Measurement of UV radiation on different altitudes of Darjeeling.

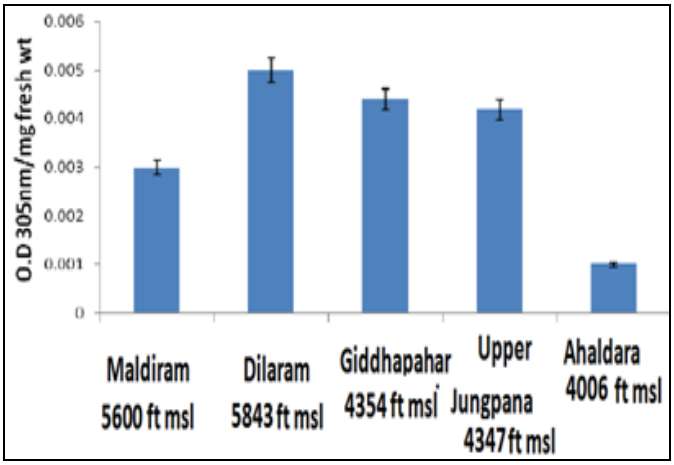

Fig 2: Assay of UV absorbing compounds.

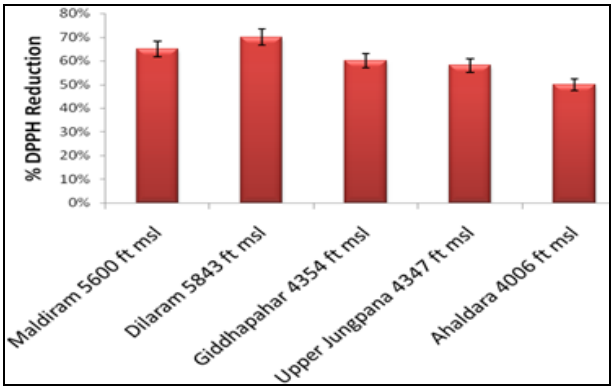

Fig 3: Antioxidant potential of different altitude tea clones

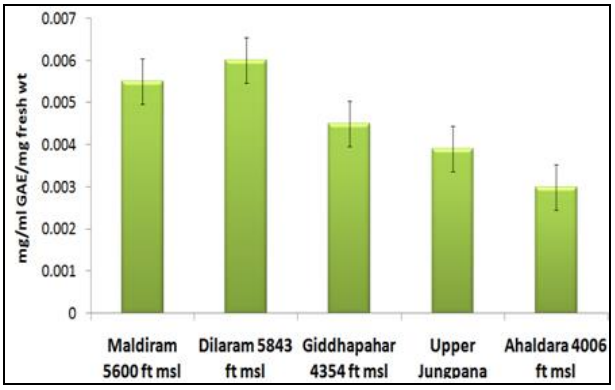

Fig 5: Total polyphenol content of different tea clones

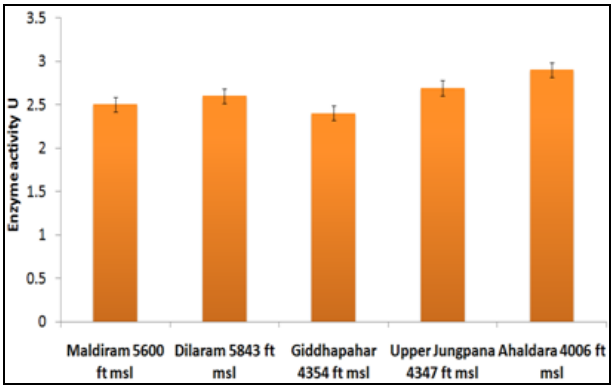

Fig 4: The SOD activity of different tea clones

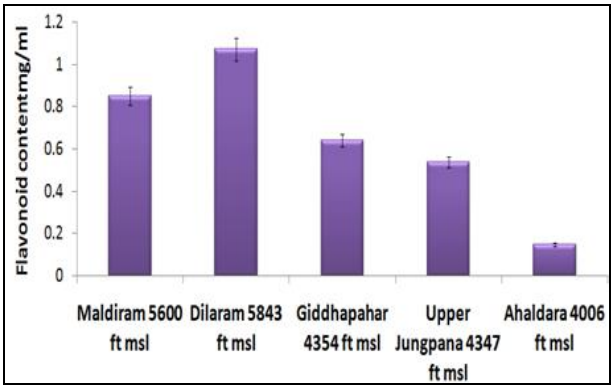

Fig 6: Total flavonoid content

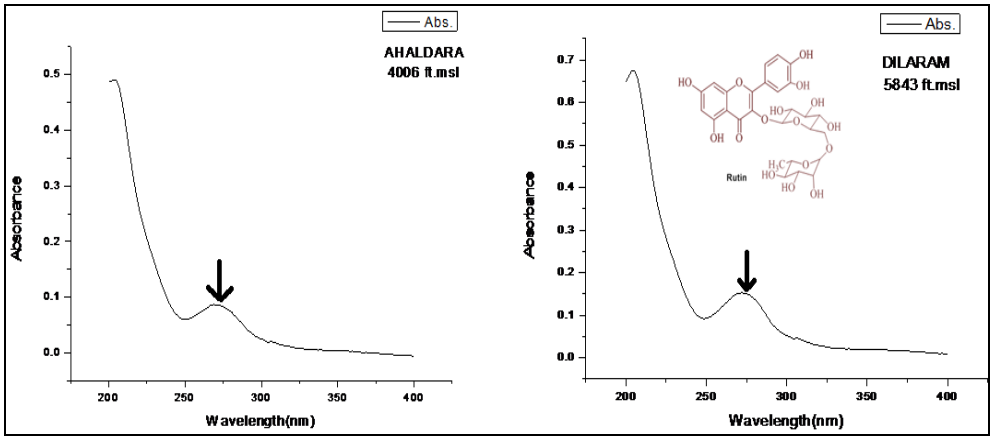

Fig 7: Spectophotomeric scan for UV absorbing compound within 200-400 nm

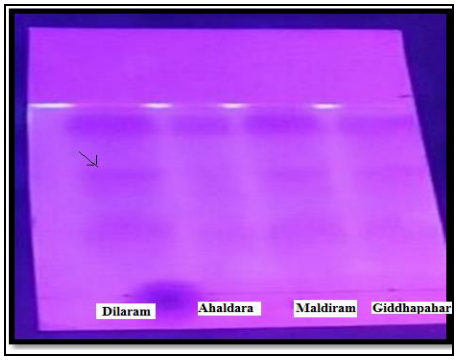

Fig 8: TLC sheet under UV long wavelength (R_f indicated with arrow indicates the position of Rutin)

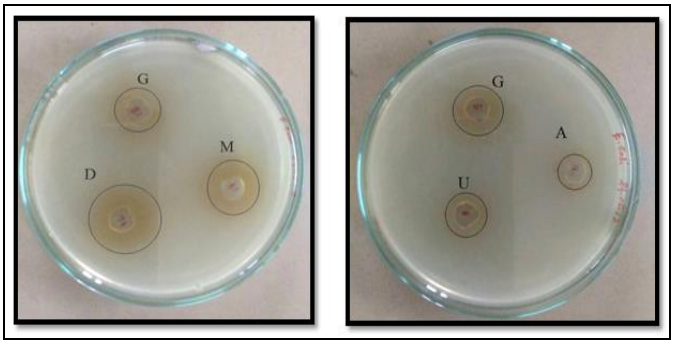

Fig 9: Zone of inhibition produced by different methanolic extracts of tea against *E.coli*

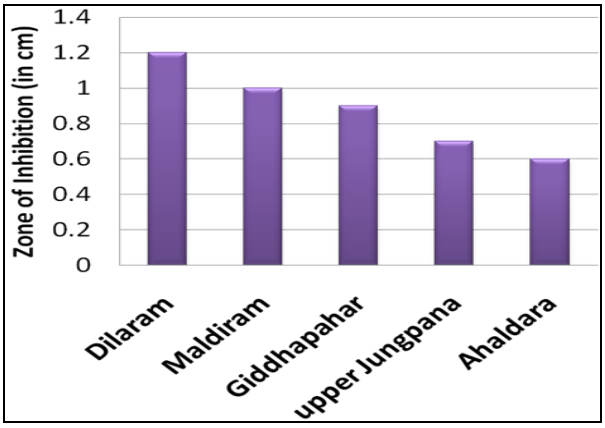

Fig 10: Histogram of antimicrobial activities (zone of inhibition in cm)

Conclusion

The trend reflects that the high altitude grown tea are exposed under high natural UV irradiance which upregulates the synthesis of flavonoids, which are UV absorbing compounds (specially flavonoid Glycosides) and which serve as aroma precursor in tea as well as responsible to show high antioxidant and antimicrobial capacities, which can be assigned as positive effects of UV radiation.

Acknowledgment

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