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*Research Article*

# BIOCHEMICAL CHARACTERIZATION OF NOVEL MOONLIGHT TEA OF MAJHERDABRI TEA GARDEN, ALIPURDUAR, NORTH BENGAL, INDIA: A SIGNATURE OF BIODYNAMIC FARMING

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**Abstract:**
Lunar radiation significantly acts as a signalling cue to plants like tea (*Camellia sinensis*). The full moon plucking in three different flushes at Majherdabri tea garden, Alipurduar, North Bengal gives rise to moonlight tea. There is significant variation in secondary metabolite content like polyphenols and flavonoids between freshly harvested and manufactured tea in different seasonal and diurnal phases. The biochemical characterization, metabolite profiling was done with spectrophotometric and spectrofluorimetric scan, HPLC, FTIR analysis, Gas chromatography. The manufactured tea was graded according to commercial parameters with E-nose (N.I Nor aroma Index) and E-tongue (T.S Taster's Score) devices. Among all the flushes and phase, lunar 2nd flush fresh leaves showed highest flavonoid and polyphenol content along with higher commercial parameters like higher E-nose and E-tongue parameters (N.I and T.S) which corroborated with the higher volatile aroma components measured with Gas chromatography. The higher antioxidant potential of the 2nd flush lunar harvests indicated the higher therapeutic qualities contributed by the presence of enhanced secondary metabolites (polyphenols and

flavonoids). Thus the age-old practice of ethnic Biodynamic farming (harvest on a cool full moon night when cell saps accumulate at leaves) establishes the enhancement of tea quality in moonlight tea of Majherdabri, Alipurduar tea garden.

## 1. Introduction

Tea (*Camellia sinensis*) contains a diverse range of bioactive compounds (as a part of secondary metabolism), including flavonoids, catechins, and polyphenols, which contribute to its superior commercial and therapeutic qualities like antioxidant, antimicrobial, anti-inflammatory, and anticancer properties (Luo et al., 2024). Such tea qualities and its growth, development (morphological and agricultural parameters) are determined by the gene along with environment interactions specifically with both abiotic (natural UV radiation) and biotic factors (rhizospheric and phyllospheric microbiota) of environment combined contributing in natural biotic and abiotic conditioning of tea (Samynathan et al., 2024). The enhancement of the tea quality occurs due to the superior microenvironment contributed by the topographic and geographic space. The example is the terroir cultivation of tea in Darjeeling where the contribution of agro-climatic parameters like soil, mist, slope, cloud cover, mist, temperature, sunshine hours, and rainfall result in production of world famous GI tagged aromatic tea. *Bhattacharya et al.,2011* and *Biswas et al., 2025* had described the effects of natural UV radiation (part of ambient solar radiation) prevailing at different natural doses at different altitudes of Himalayan region on the quality enhancement of Darjeeling and Dooars-grown tea respectively (natural abiotic conditioning) (Bhattacharya and Sen-Mandi, 2011 , Biswas et al., 2025).

The concentration of active metabolites during harvest of tea leaves is influenced by environmental factors like diurnal variations, circadian rhythms, and harvest timing which could be characterized and determined by HPLC, TLC, LC-MS, FTIR and GC MS analysis to give a complete picture of stress-upregulated biochemical characterization and metabolomics profile (Wang et al., 2021, Wen et al., 2023). Diurnal variation (solar and lunar harvesting), seasonal variation of different flushes, specifically the differences between day-harvested (solar) and night-harvested (lunar) leaves may show a sharp differential metabolome profile. During daylight hours in plant, high photosynthetic activity enhances carbon assimilation, supporting microbes involved in nitrogen fixation, phosphate solubilization, and phyto-hormone production (Zhao et al., 2024). At night, reduced transpiration and stress recovery pathways favour microbes, responsible for organic matter mineralization and defence regulation (Vega et al., 2023). Current research had shown that the plants perceive the moonlight as a stress signal which triggers the developmental transition characterized which brings in changes in genome, cellular structure and function contributing in enhanced growth and qualities (Sannidhi et al., 2026). *Díaz-Chuquizuta et al., 2023* had shown the effects of sowing corn towards growth performances at various lunar phases, showing highest yield, better pest management and growth during full moon (Díaz-Chuquizuta et al.,2023).

This study aims to find the quality enhancement by lunar radiation of Dooars garden grown moonlight tea variety which are usually plucked during full moon night. Majherdabri tea garden in Alipurduar (low altitude Dooars garden) is the place where the moonlight tea is plucked during midnight of full moon in the synchronization of moon's and star's celestial rhythm. It is believed that under the moonlit sky, tea leaves are at their most pristine state. The lunar radiation (consisting of limited natural dose of UV and other cosmic rays) along with the cool night air together can upregulate the production of volatile aroma components in tea enhancing is mystique aroma. The nocturnal reduced transpiration, cooler night, slower oxidation, lunar rhythm and sap flow together contribute in better volatile aroma component production in tea leaves. The magic of cosmic energy is transformed in the brewed cuppa. The gravitational and electromagnetic

effects of full moon in its synodic cycle affects plant metabolism as stressor to photoreceptors leading to altered cellular activities as described with the effects of full moonlight on the transcriptome level of *Coffea arabica*) (Breitler et al., 2020). As it was evidenced that some of the flowers open up and release their fragrance at midnight, moonlit harvested teas are also furnished with aroma components upregulated during full moon night (Fenske and Imaizumi ,2016). The preliminary studies had shown that at night during decelerated photosynthetic conditions of tea plants, the lunar radiation (as optimum cosmic influence of full moon ray) could upregulate the polyphenols and amino acids which in turn serve as the precursors for aroma (commercial quality) and antioxidants (therapeutic quality) of tea (Priyanka et al., 2024). In previous research reported, full moonlight acts as a signalling cue to regulate photo-periodism and ROS scavenging in tobacco plants where full moonlight also contributes to greater energy availability to promote plant growth and development(Singiri et al., 2023 ,Wingler and Henriques ,2022).The idea of research that enhanced metabolite concentration in lunar tea under potential applicative biodynamic cultivation practice where strategic timing of harvest might optimize health-benefiting compounds thus enhancing the commercial potential of Indian tea still does not have specific scientific literature report and still remains unexplored(Singiri et al., 2023). Our aim of this paper is to biochemically characterize (metabolome profile) the lunar harvest (full moon) tea at three different seasons from a similar site and differentiate them from similar lunar cultivations which could further help to develop better quality tea.

## 2. Materials and Methods

### 2.1. Collection of Fresh and Manufactured Tea samples grown at specific environmental conditions.

The solar and lunar harvest of fresh leaves have been done thrice a year during three full moon phases on 1st (2.2.2026), 2nd (11.5.2025) and autumnal (7.10.2025) (the timespan of study is from May 2025 to February 2026) flushes along with their corresponding daylight (solar) harvest from Majherdabri Tea Estate, located in the humid subtropical zone of the Dooars region (Alipurduar, West Bengal, India) Latitude:26.5396565 ºN. Longitude: 89.5568382 ºE, 180 ft msl elevation. Along with the fresh samples, the solar and lunar harvested manufactured tea (1st, 2nd and autumnal flushes) samples were collected from Majherdabri Tea lounge (Dabri tea lounge, Majherdabri, Alipurduar-Pin-736123). (Figure 1 a and 1b)

**Table 1.** Environmental parameters

| Environmental Parameters | DAY 1st Flush | NIGHT 1st Flush | DAY 2nd Flush | NIGHT 2nd Flush | DAY Autumn Flush | NIGHT Autumn Flush |
|---|---|---|---|---|---|---|
| Temperature | 18-22ºC Dew Point 10-14 | 10-15ºC Dew Point 8-12 | 25-30ºC Dew Point 20-24 | 18-22ºC Dew Point 18-22 | 21-33ºC Dew Point 15-18 | 21-23 C Dew Point 12-15 |
| Relative Humidity | 70-80% | 85-95% | 75-90% | 85-95% | 70-85% | 80-90% |
| Precipitation | 20-40 cm | | 80-120 cm | | 30-60cm | |
| Illuminance | 20000-50000 lux | 0.01-0.1 lux | 40000-80000 lux | 0.05-0.3 lux | 25000-60000 lux | 0.01-0.1 lux |

[1] The specific environmental parameters under which Moonlight Tea is cultivated

During the moonlight tea plucking, the lunar phase was a full moon (100% illumination) providing soft, uniform natural lighting across the tea fields. Dew-point temperatures for this period fall while radiative cooling lowers leaf-surface temperatures by 0.5º-2 º C. The specific environmental parameters are shown in a tabular form below (Table1)

### 2.2. Biochemical Analysis and Profiling by Spectrophotometric and Spectrofluorimetric scan:

In U-2900 Spectrophotometer, the spectrophotometric scan was done of tea samples with respect to specific standard polyphenol and flavonoids like Epigallocatechin Gallate (EGCG), Rutin, Quercetin and Gallic acid. The spectral scan ran from 200-400 nm with the scan speed of 400nm/min, sampling interval of 0.5 nm and slit width of 1.50 nm (Atomssa and Gholap, 2015) (Fig 2a-p, Table 2a and 2b). In F-7000 FL Spectrofluorimeter, the scan was done for the for different tea samples with a specific standards as spectrophotometric scans having excitation wavelength with respect to specific standard polyphenol and flavonoids like Epigallocatechin Gallate (EGCG), Rutin, Quercetin and Gallic acid at 275nm and emission wavelength at 350-400 nm having emission peak -351nm; 450/530nm (Khanchi et al., 2007) (Fig 3a-p and Table 3a and 3b)




1a




1b

**Figure 1.** Collection of Fresh samples of Moonlight tea samples (Figure 1a). Different solar and lunar manufactured tea samples collected from Majherdabri Tea Lounge, Alipurduar, Pin-736123 (Figure 1b).

### 2.3. Characterization of Secondary metabolites by HPLC and FTIR analysis:

Characterization with HPLC-high performance liquid chromatography (Waters® Empower™) was done with methanolic extracts in Hypersil ODS column, UV detector at 285nm to detect secondary non-volatile flavonoid and catechin group of compounds. Ultimate 3000/Amazon SL ion trap mass spectrometry was undertaken using Acclaim 2.2 µm 120 A 2.1–150 mm columns (Thermo Fisher Scientific, Waltham, MA). (Shyam Choudhury and Sen-Mandi , 2012) (Fig 4a-4p and Table 4a and 4b). The upregulated secondary polyphenol, flavonoid, groups of secondary metabolites were characterized with FTIR (Fourier Transform Infrared Spectroscopy) and detected on a C-18 column equilibrated at 30°C using an isocratic mobile phase of acetonitrile: 0.1% formic acid (15: 85), prior to introduction to the deposition interface which was linked to the FTIR detector. (Kokali et al., 2014) (Fig 5a-p, Table 5)

### 2.4. Characterization of Volatile Components

This experiment was performed with Gas chromatography was outsourced from IISER (), Kolkata with Agilent 8890 GC coupled to 5977C HES MSD, a single quadrupole analyzer, MS and FID detector. The injection volume of the chloroform extracts of samples will be5 µl, Counts vs. acquisition time was 35 minutes (Hassan et al., 2024). (Fig 6a-c and Table 6)

### 2.5. Determination of total polyphenol and flavonoid Content

Total flavonoid content was analyzed spectrophotometrically at 510nm with 0.5 ml of test samples' solution in methanol mixed with 2ml of distilled water and 150 µl of 5% sodium nitrate (Fig 8). Total polyphenol was measured spectrophotometrically at 715 nm with 200 µl Folin- Ciocalteu reagent and 1 mL of aqueous $Na_2CO_3$ (Anesini et al., 2008 ,Zhishen et al., 1999) (Fig 7)

### 2.6. Assay of antioxidant potential

Spectrophotometric analysis of antioxidants with 0.4% DPPH (α, α-diphenyl-β-picrylhydrazyl) solution and observing the reduction at 517nm. (Shyam Choudhury and Sen-Mandi , 2012) (Fig 9)

### 2.7. E-nose and E-tongue assay

The volatile and non-volatile components and aroma/therapeutic index scores could be assessed from CDAC, Kolkata to detect therapeutic indices of all tea samples. (Zhi et al., 2017) (Fig 10)

### 2.8. Statistical Analysis

In every experiment, standard deviation (S.D) was calculated (n=3). The PCA analysis, Expression analysis of the metabolites were done with Heatmapper2.ca (Fig 11c and Fig 11d), 2D score plot analysis and Dendrogram analysis were done with lab.learnbin.net (Fig 11a, Fig 11b).

## 3. Results and Discussions

### 3.1. Spectrophotometric scan analysis

The lunar leaf sample showed higher absorbance value (1st flush lunar fresh leaves – 1.53±0.012 at 230 nm, 2nd flush lunar leaf – 1.4±0.013 at 230 nm & autumn flush lunar fresh leaves – 1.45±0.002 at 230 nm) compared to solar plucking fresh leaves (1st flush solar leaves – 1.48±0.023 at 230nm & 0.43±0.01 at 280 nm; 2nd flush solar leaves – 0.63±0.003 at 230 and 0.18±0.002 at 280 & autumn flush solar leaves – 0.65±0.002 at 280 nm) in three different flushes. Fresh leaf (lunar 1st flush fresh leaf –1.53±0.012 at 230; lunar 2ndflush fresh leaf- 1.4±0.013 at 230 nm; autumn flush lunar fresh leaf – 1.45±0.002 at 230 nm and autumn flush solar fresh leaf- 1.43±0.013 at 230 nm) had more polyphenol and flavonoids as it showed higher absorbance value in most cases than manufactured (solar 1st flush manufactured – 0.19±0.01 at 280 nm; solar and lunar autumn flush manufactured – 0.55±0.015 and 0.55±0.023 at 300 nm) whereas 2nd flush lunar manufactured gives higher peak ( 1.73±0.023 at 300 nm, 1.17±0.012 at 435 nm & 1.04±0.01 at 380 nm) significantly. As the leaves are oxidized during drying and fermentation and also have some processing factors. For first flush the absorbance peaks are mainly observed at 230nm

(1.43±0.012,1.45±0.002,1.05±0.02,0.63±0.001) and280nm (0.64±0.009,0.43±0.01,0.41±0.05,0.19±0.01) for both fresh leaves and manufactured which confirmed the presence of phenolic content. For the second flush which is mainly in summer season indicates a rich phytochemical content due to higher exposure of sunlight as it gives significant peak at 230nm (0.63±0.003,1.4±0.013),280nm (0.88±0.013,0.56±0.023,0.18±0.002),300nm (1.73±0.023,0.55±0.023),380nm (1.04±0.01) & 435nm (1.17±0.012) nm. Here lunar manufactured contains peaks at 230nm, 380nm and 435nm which is not present in other. For autumnal flush, both the solar and lunar fresh leaves, there was a little variation for the peaks at 230nm (1.43±0.012 and 1.45±0.0021) and for manufactured they give the same result at 300 nm (0.55±0.015) showing no such variation. All the leaf and manufactured samples of three different flushes showed the presence of gallic acid as it gave the absorbance peak within 420nm. Lunar 2nd flush manufactured and solar and lunar autumnal flush manufactured gave the absorbance peak at 300(1.73±0.023,0.55±0.023) nm which indicates the presence of quercetin (1.03±0.02 at 300 nm). All the leaf and manufactured samples of three different flushes showed the presence of EGCG as it contains its absorbance peak at (1.05±0.02 at 276 nm,0.53±0.023 at 330nm, 0.52±0.01 at 430 nm) where lunar 2nd flush manufactured leaves (1.73±0.023 at 300 nm,1.04±0.01 at 380nm, 1.17±0.012 at 435 nm) showed higher amount of EGCG present. (Fig 2(a-p) and Table 2a and 2b).

**Table 2a.** Spectrophotometric scan analysis of tea samples

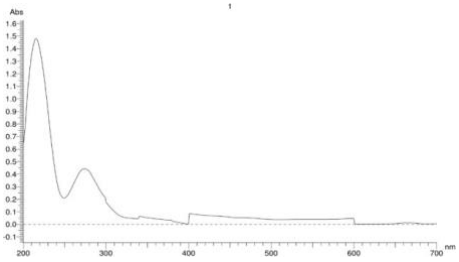
| Sample | Flush | types | Peaks at 230 | Peak at 280nm | Peak at 300 nm | Peak at 380 | Peak at 435 nm |
|---|---|---|---|---|---|---|---|
| Solar | 1st | leaf | 1.48±0.023 | 0.43±0.01 | | | |
| Lunar | 1st | leaf | 1.53±0.012 | 0.41±0.05 | | | |
| Solar | 1st | Manufactured | 0.63±0.003 | 0.19±0.01 | | | |
| Lunar | 1st | Manufactured | 1.05±0.02 | 0.64±0.009 | | | |
| Solar | 2nd | leaf | 0.63±0.001 | 0.18±0.002 | | | |
| Lunar | 2nd | leaf | 1.4±0.013 | 0.88±0.013 | | | |
| Solar | 2nd | Manufactured | | 0.56±0.023 | | | |
| Lunar | 2nd | Manufactured | | | 1.73±0.023 | 1.04±0.01 | 1.17±0.012 |
| Solar | Autumn | leaf | | | 0.55±0.015 | | |
| Lunar | Autumn | leaf | | | 0.55±0.023 | | |

| | | | | |
|---|---|---|---|---|
| Solar | Autumn | Manufactured | 1.43±0.012 | 0.65±0.002 |
| Lunar | Autumn | Manufactured | 1.45±0.002 | 0.65±0.002 |

**Table 2b.** Spectrophotometric scan analysis of standards

| Sample | Abs of Peak at 270-276nm | Abs of Peak at 300 nm | Abs of Peak at 320 nm | Abs of Peak at 330nm | Abs of Peak at 375 nm | Abs of Peak at 420 nm | Abs of Peak at 430 nm |
|---|---|---|---|---|---|---|---|
| Gallic acid | | | | 1.1±0.05 | 0.97±0.01 | 1±0.02 | |
| Quercetin | | 1.03±0.02 | | | | | |
| Rutin | | | 1.03±0.02 | | | | |
| EGCG | 1.05±0.02 | | | 0.53±0.023 | | | 0.52±0.01 |

**Spectrophotometric scan analysis**



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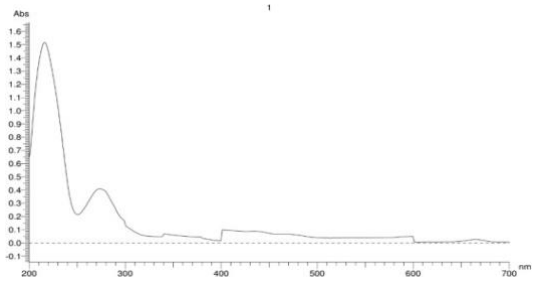

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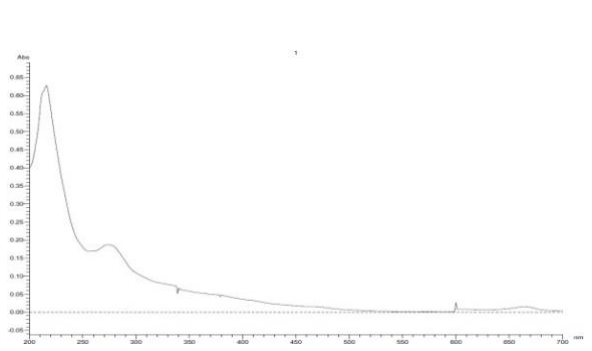

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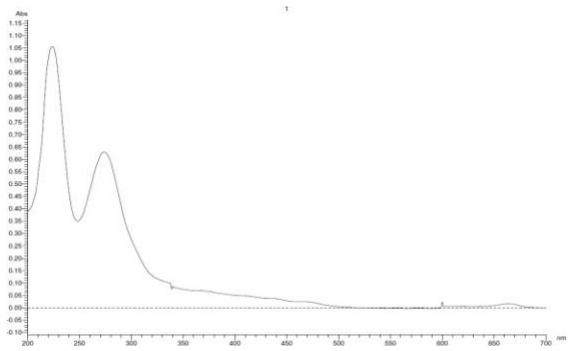

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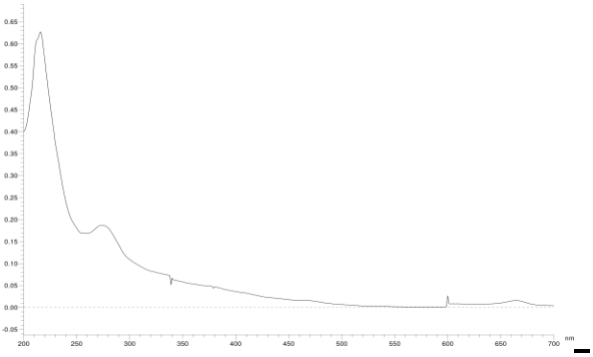

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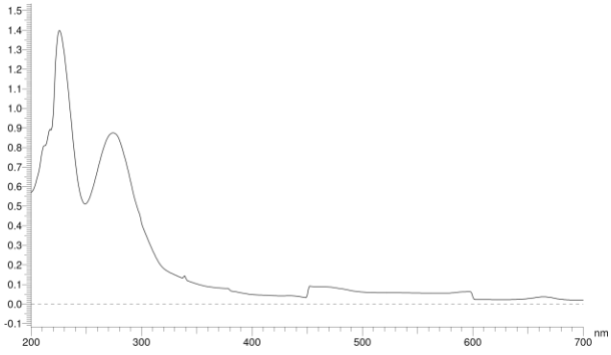

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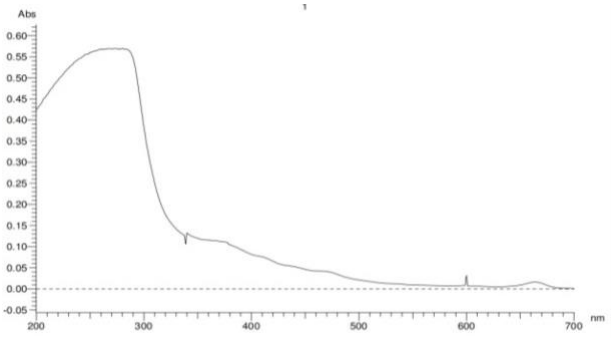

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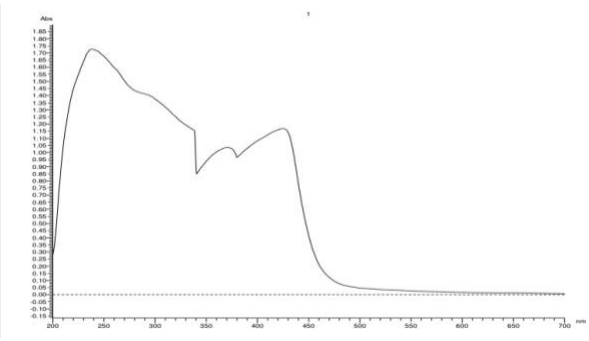

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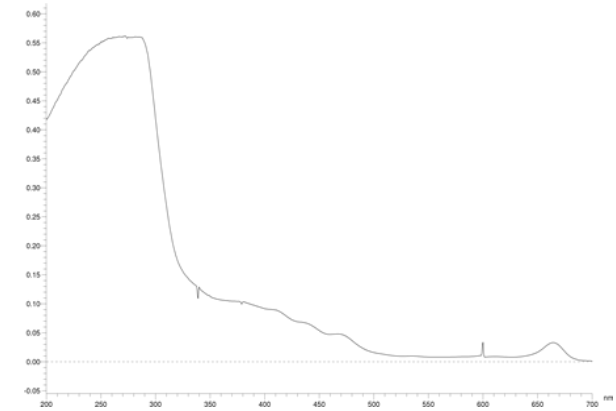

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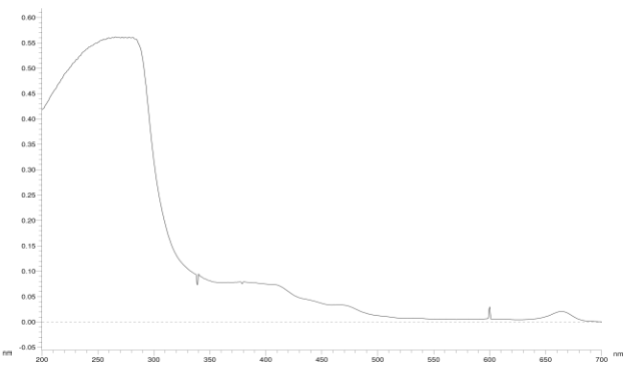

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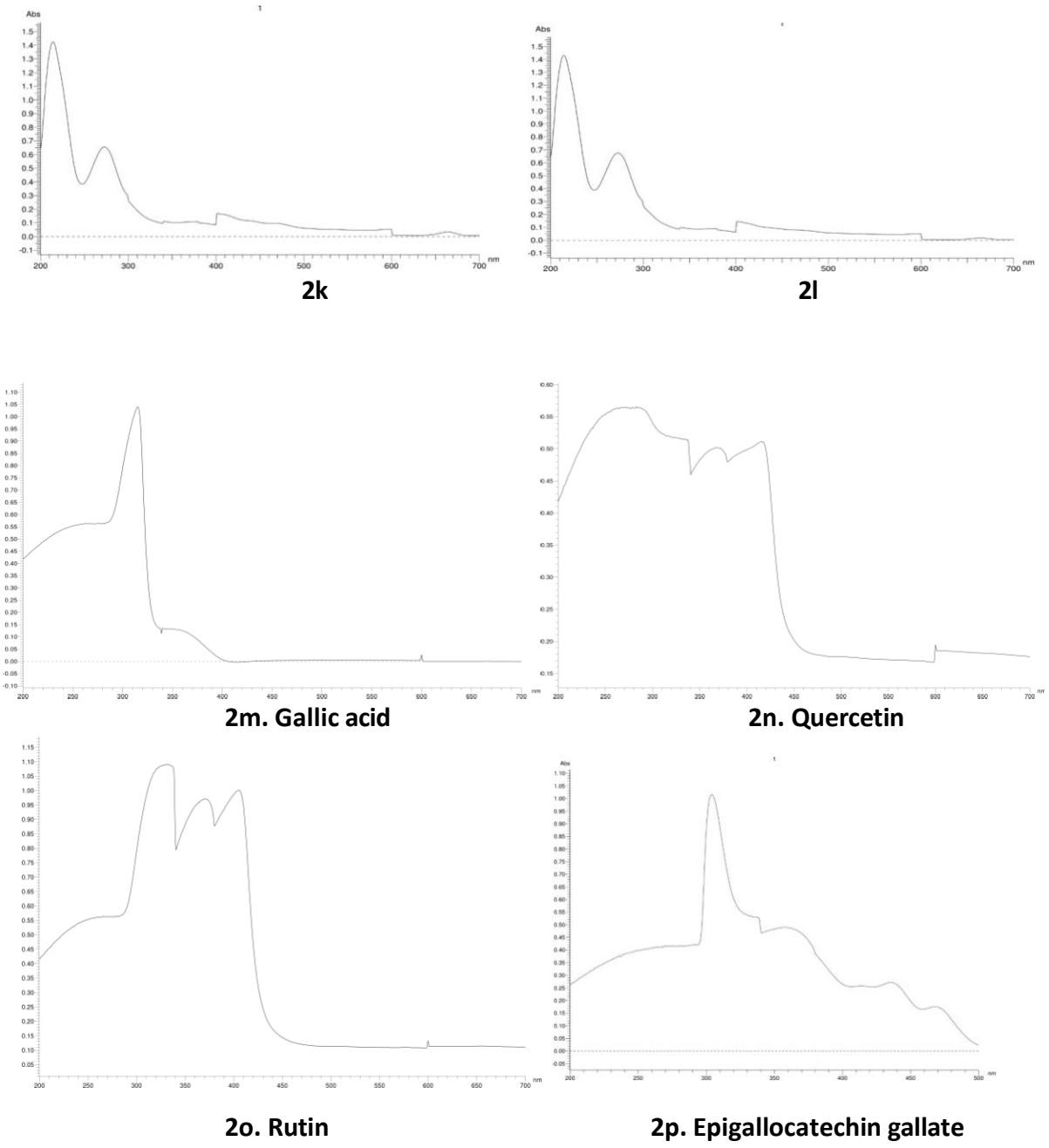



**Figure 2.** Spectrophotometric scan of Solar 1st flush fresh leaf(2a), Lunar 1st flush fresh leaf(2b), Solar 1st flush manufactured(2c), Lunar 1st flush manufactured(2d),Solar 2nd flush fresh leaf(2e), Lunar 2nd flush fresh leaf(2f),Solar 2nd flush manufactured(2g),Lunar 2nd flush manufactured(2h),Solar autumn flush fresh leaf(2i),Lunar autumn flush fresh leaf(2j),Solar autumn flush manufactured(2k),Lunar autumn flush manufactured(2l),Gallic acid(2m), Quercetin(2n), Rutin(2o) and EGCG Epigallocatechin gallate(2p).

### 3.2. Spectrofluorimetric scan analysis

The lunar leaf sample showed higher fluorescence intensity in most cases (1st flush lunar fresh leaves – 77±0.03 at 325 nm, 2nd flush lunar leaf – 61±0.02 at 325 nm and autumn flush lunar fresh leaves – 48±0.03 at 570 nm) compared to solar plucking fresh leaves. But exceptionally the 1st flush solar leaf (255±0.02 at 325 nm) & autumn flush solar leaf (30.5±0.02 at 330 nm& 23±0.06 at 570nm) shows higher

emission peak. In fresh leaf( lunar 1st flush fresh leaf – 77±0.03 at 325nm; lunar 2nd flush fresh leaf- 61±0.02 at 325 nm; autumn flush lunar fresh leaf – 48 at 570 nm; lunar 2nd flush fresh leaf – 61 at 325nm and solar 1st flush fresh leaf- 255±0.02 at 325 nm) it showed higher fluorescence value in most cases than manufactured ( solar 1st flush manufactured – 35±0.02 at 350 nm; solar 2nd flush manufactured – 17.5±0.05 at 325 nm and solar autumn flush manufactured 39±0.01 at 342 nm) where 2nd flush lunar manufactured gave higher peak ( 149±0.05 at 315 nm) significantly. For first flush, the absorbance peaks were mainly observed at 325(255±0.02 & 77±0.03) nm for fresh leaves, 335(83±0.01) nm & 350 (35±0.02) nm for manufactured leaves. For the second flush, both the solar plucking leaf and manufactured gave the same value at 325nm (17.5±0.02). Here lunar manufactured contains higher fluorescence intensity at 315 nm (149±0.05). For autumn flush, both the solar and lunar fresh leaves showed 2 significant peaks at 330nm (30.5±0.02),340nm (18±0.01) and 570nm (23±0.03,48±0.03) and for manufactured leaves they showed the peak intensity at 342nm (39±0.01,41±0.03). Autumn flush solar and lunar fresh leaves showed its peak at 570nm (23±0.06 & 48±0.03) respectively which assured the presence of gallic acid (2.2±0.03 at 570nm), rutin (2.98±0.01 at 570nm) & quercetin (2.2±0.04 at 580nm) (Fig 3 (a-o) and Tables 3a and 3b).

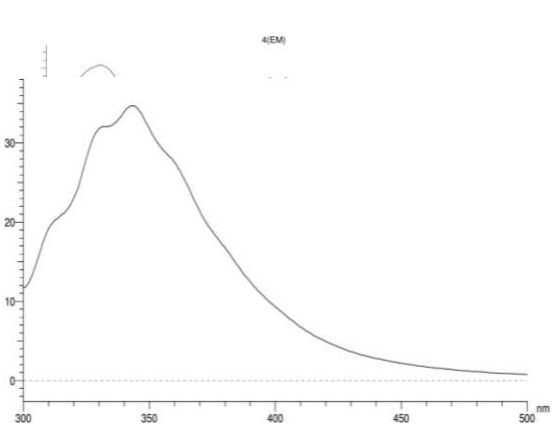
**Table 3a.** Spectrofluorimetric scan analysis of tea samples

| Sample | Plucking season | Plucking types | Wavelength(nm) | Absorbance |
|---|---|---|---|---|
| Solar | 1st | Leaf | 325 | 255±0.02 |
| Lunar | 1st | Leaf | 325 | 77±0.03 |
| Solar | 1st | Manufactured | 350 | 35±0.02 |
| Lunar | 1st | Manufactured | 335 | 83±0.01 |
| Solar | 2nd | Leaf | 325 | 17.5±0.02 |
| Lunar | 2nd | Leaf | 325 | 61±0.02 |
| Solar | 2nd | Manufactured | 325 | 17.5±0.05 |
| Lunar | 2nd | Manufactured | 315 | 149±0.05 |
| Solar | Autumn | Leaf | 330 | 30.5±0.02 |
| | | | 570 | 23±0.06 |
| Lunar | Autumn | Leaf | 340 | 18±0.01 |
| | | | 570 | 48±0.03 |
| Solar | Autumn | Manufactured | 342 | 39±0.01 |
| Lunar | Autumn | Manufactured | 342 | 41±0.03 |

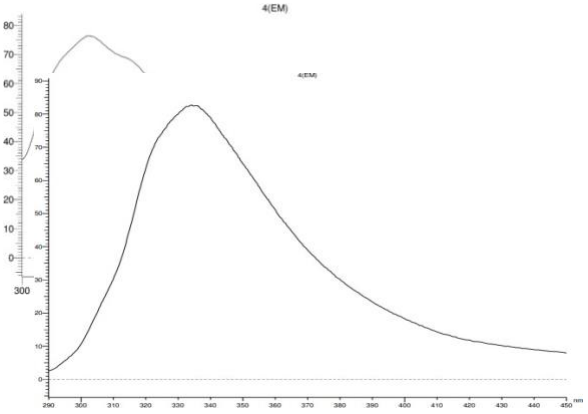
**Table 3b.** Spectrofluorimetric scan analysis of standards

| Sample | Wavelength(nm) | Absorbance |
|---|---|---|
| Gallic acid | 380 | 4.2±0.02 |
| | 570 | 2.2±0.03 |
| Quercetin | 580 | 2.2±0.04 |
| Rutin | 570 | 2.98±0.01 |
| Epigallocatechin gallate | 370 | 54±0.02 |

**Spectroflourometric analysis**



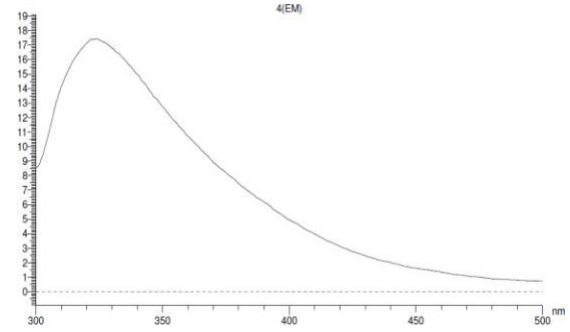

**3a**




**3b**

**3c**

**3d**

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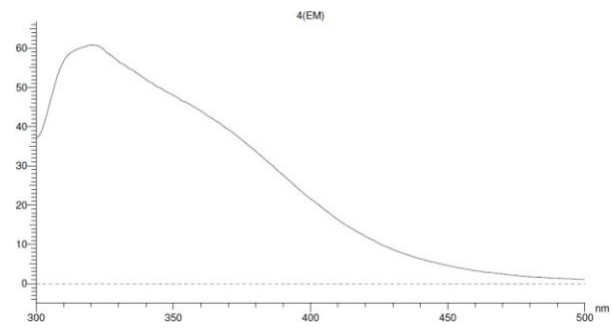
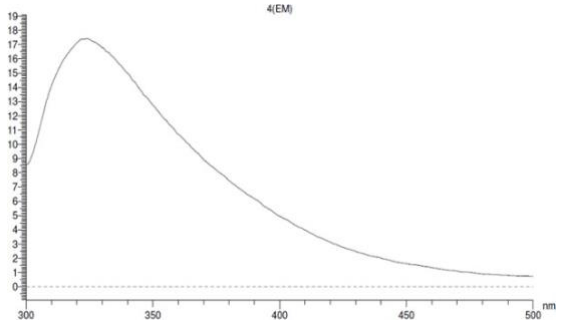



**3f**

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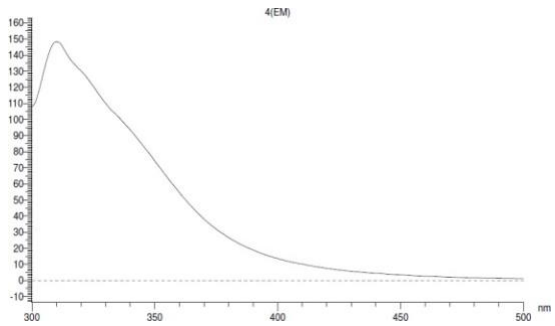


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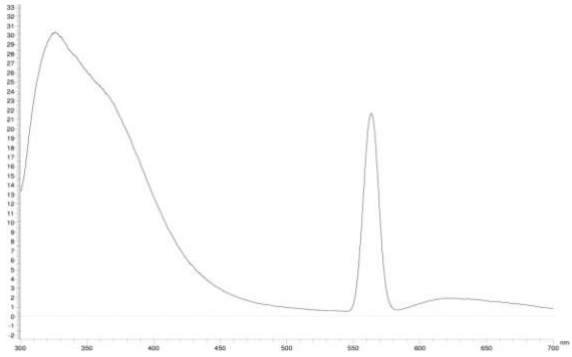

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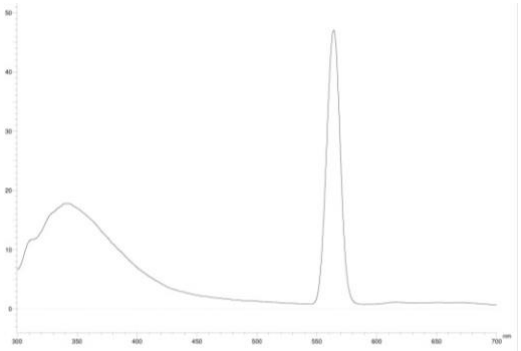

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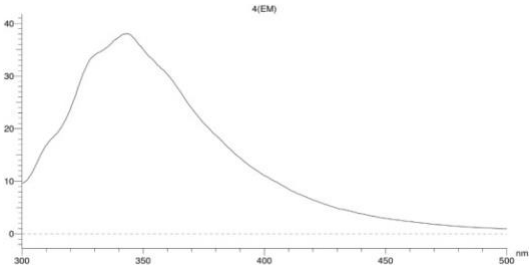


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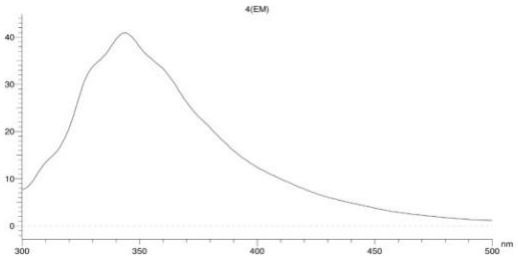


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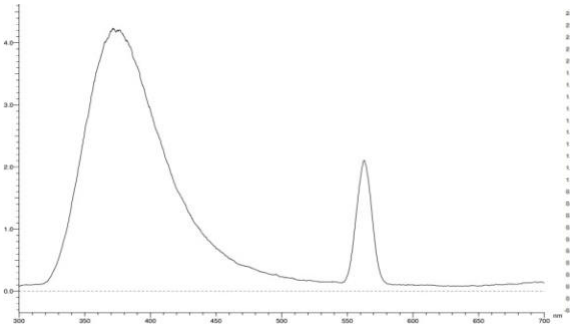

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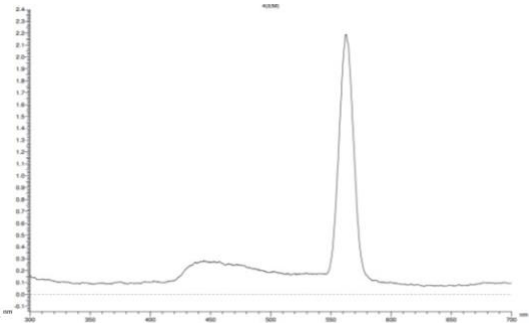
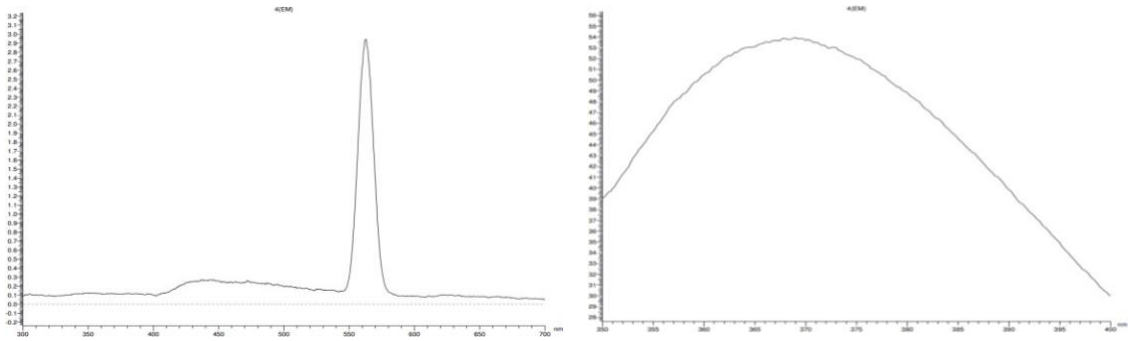


**3n**



**3o** **3p**

**Figure 3.** Spectrofluorimetric scan of Solar 1st flush fresh leaf(3a), Lunar 1st flush fresh leaf(3b), Solar 1st flush manufactured(3c),Lunar 1st flush manufactured (3d),Solar 2nd flush fresh leaf(3e), Lunar 2nd flush fresh leaf(3f),Solar 2nd flush manufactured(3g),Lunar 2nd flush manufactured(3h),Solar autumn flush fresh leaf(3i),Lunar autumn flush fresh leaf (3j), Solar autumn flush manufactured(3k),Lunar autumn flush manufactured (3l), Gallic acid (3m), Quercetin(3n),Rutin(3o) and Epigallocatechin gallate(3p).

### 3.3. HPLC Analysis

The lunar fresh leaves showed more and sharp intense peak. The peaks at ~8.2, ~8.9 (lunar 1st flush fresh leaves) and ~8.6 (lunar autumn flush fresh leaves) minutes remain sharp and high in intensity (reaching~0.38±0.003,0.56±0.001,1.3±0.004AU) respectively. Whereas the solar plucking fresh leaves showed its peak at 8.9,8.2&9.5 minutes reaching 0.76±0.001,0.30±0.001 and 0.14±0.001 AU respectively (solar 1st flush fresh leaves); at 2.062,2.553 minutes to 0.014±0.001,0.0052±0.001 AU (solar 2nd flush fresh leaves). But autumn flush (2.2 min - 0.67±0.003 AU,7.9 min – 0.5±0.002 AU, 8.5 min – 1.37±0.002 AU) and 1st flush solar plucking leaves showed more and higher peak than solar 2nd flush fresh leaves. Autumn flush solar (8.5 min – 1.37±0.002 AU) and lunar (8.5 min – 1.3±0.004 AU) fresh leaves showed highest peak compared to other different leaves. Fresh leaf (lunar 1st flush 8.9,8.2,7.8 minutes with 0.56±0.001,0.38±0.003,0.28±0.003 AU; solar 1st flush 8.9,8.0,8.24 minutes with 0.76±0.001,0.51±0.003,0.30±0.001 AU; autumn flush lunar 8.5 minutes with 1.3±0.004 AU and autumn flush solar 8.5,2.2,7.9,7.5 mintutes with 1.37±0.002,0.67±0.003,0.5±0.002,0.28±0.001 AU)showed its higher intensity value in most cases than manufactured (solar 1st flush 5.8,7.8 mins with 0.002±0.001, 0.0003±0.001 AU; solar 2nd flush 9.6,2.3,3.5 minutes with 0.067±0.001,0.024±0.001,0.02±0.001 AU, solar autumn flush 7.5,1.13 minutes with 0.23±0.002,0.15±0.002 AU; lunar 2nd flush 2.6,1.8 minutes with 0.0067±0.001,0.0045±0.001 AU). But there were some exceptions like lunar 1st flush manufactured showed some distinct peaks at 8.7,7.6,8.1 & 7.3 minutes at 1.8±0.003,1.38±0.003,0.78±0.005 and 0.43±0.002respectively. The 2nd flush solar manufactured gave more number of peaks but with less absorbing unit (0.003,0.001,0.020,0.024 AU). The autumn flush solar manufactured leaves showed the highest absorbing unit (2.1±0.003) at 8.8 minutes followed by lunar 1st flush (1.8±0.003 AU at 8.7 minutes) and autumn flush (1.15±0.002 AU at 8.7 minutes) manufactured. The 1st flush both solar (1.4,2.0,7.6,8.0,8.2,8.9,9.5,10.2 minutes with 0.11±0.001,0.05±0.002,0.14±0.002,0.51±0.003, 0.30±0.001,0.76±0.001,0.14±0.001,0.08±0.002 AU) and lunar (2.2,7.8,8.2,8.9,9.5,10.2,10.5 minutes with 0.15±0.001,0.28±0.003,0.38±0.003,0.56±0.001,

0.12±0.003,0.09±0.001,0.05±0.002 AU) fresh leaves gave the maximum number of peaks with high intensity compared to both the manufactured leaves. Significantly lunar 1st flush manufactured gave 4 distinct peaks (7.3,7.6,8.1,8.7 minutes with 0.43±0.002,1.38±0.003,0.78±0.005,1.8±0.003 AU) compared tosolar manufactured. The 2nd flush solar manufactured leaves showed the maximum number of peaks (2.3,3.5,5.5,7.2,9.6,13.7 minutes with 0.024±0.001,0.020±0.001,0.001±0.001,0.003±0.001,0.067±0.001,0.003±0.001 AU) compared to other 2nd flush. For autumn flush, both solar (2.2,3.0,7.1,7.5,7.7,7.9,8.5 minutes with 0.67±0.003,0.10±0.004,0.20±0.003,0.28±0.001, 0.25±0.001,0.50±0.002,1.37±0.004 AU) and lunar (1.2,2.1,2.9,7.1,7.5,8.0,8.5 minutes with 0.1±0.001,0.09±0.001,0.09±0.001,0.18±0.001,0.17±0.002,0.16±0.003,1.3±0.004 AU) fresh leaves gave the prominent and maximum number of peaks than manufactured. But the solar manufactured showed the highest peak at 8.8 minutes with 2.1±0.003 AU. 2nd flush solar and lunar fresh leaves and lunar manufactured showed the intensity peak near 2.5 minutes with 0.0052±0.001, 0.0185±0.001, 0.0067±0.001 AU respectively which proved the presence of gallic acid at 2.5 minutes with 1.18±0.002 AU. The 1st flush solar fresh leaves (0.14±0.002,0.76±0.001 AU at 7.7, 8.9 minutes) and manufactured (0.0003±0.001 AU at 7.7 minutes) & lunar fresh leaves (0.28±0.003,0.56±0.001 AU at 7.7,8.9 minutes ) and manufactured (1.38±0.003AU at 7.7 minutes) and autumn flush solar fresh leaves ( 0.25±0.001 AU at 7.7 minutes), solar (2.1±0.003 AU at 8.8 minutes) and lunar manufactured (1.15±0.002 AU at 8.9 minutes) showed the nearest peak at 7.7 and 8.9 minutes which confirmed the presence of rutin and quercetin (0.038±0.001 AU at 7.7 minutes, 0.17±0.003 AU at 8.9 minutes & 1.5±0.002 AU at 8.9 minutes).( Fig 4 (a-o) and Tables 4a and 4b )

**Table 4a.** HPLC scan analysis of tea sample:

| Sample | Plucking Season | Plucking type | Retention time(min) | AU |
|---|---|---|---|---|
| Solar | 1st | Leaf | 1.402 | 0.11±0.001 |
| | | | 2.001 | 0.05±0.002 |
| | | | 7.617 | 0.14±0.002 |
| | | | 8.018 | 0.51±0.003 |
| | | | 8.244 | 0.30±0.001 |
| | | | 8.950 | 0.76±0.001 |
| | | | 9.582 | 0.14±0.001 |
| | | | 10.240 | 0.08±0.002 |
| Lunar | 1st | Leaf | 2.22 | 0.15±0.001 |
| | | | 7.87 | 0.28±0.003 |
| | | | 8.229 | 0.38±0.003 |

| | | | | |
|---|---|---|---|---|
| | | | 8.946 | 0.56±0.001 |
| | | | 9.577 | 0.12±0.003 |
| | | | 10.227 | 0.09±0.001 |
| | | | 10.550 | 0.051±0.002 |
| Solar | 1st | Manufactured | 5.857 | 0.002±0.001 |
| | | | 7.855 | 0.0003±0.001 |
| Lunar | 1st | Manufactured | 7.387 | 0.43±0.002 |
| | | | 7.699 | 1.38±0.003 |
| | | | 8.103 | 0.78±0.005 |
| | | | 8.709 | 1.8±0.003 |
| Solar | 2nd | Leaf | 2.062 | 0.014±0.001 |
| | | | 2.553 | 0.0052±0.001 |
| Lunar | 2nd | Leaf | 1.833 | 0.0155±0.001 |
| | | | 2.009 | 0.015±0.002 |
| | | | 2.418 | 0.0185±0.001 |
| Solar | 2nd | Manufactured | 2.333 | 0.024±0.001 |
| | | | 3.547 | 0.020±0.001 |
| | | | 5.526 | 0.001±0.001 |
| | | | 7.256 | 0.003±0.001 |
| | | | 9.682 | 0.067±0.001 |
| | | | 13.770 | 0.003±0.001 |
| Lunar | 2nd | Manufactured | 1.868 | 0.0045±0.001 |
| | | | 2.698 | 0.0067±0.001 |
| Solar | Autumn | Leaf | 2.258 | 0.67±0.003 |

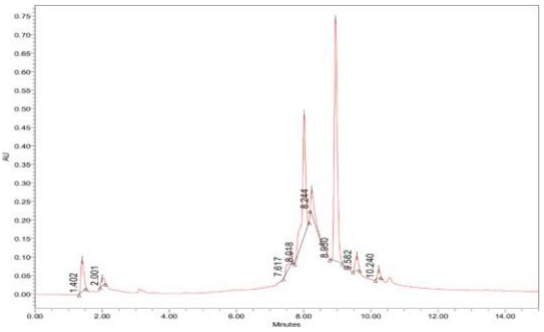
| | | | | |
|---|---|---|---|---|
| | | | 3.020 | 010±0.004 |
| | | | 7.186 | 0.20±0.003 |
| | | | 7.583 | 0.28±0.001 |
| | | | 7.750 | 0.25±0.001 |
| | | | 7.992 | 0.50±0.002 |
| | | | 8.543 | 1.37±0.002 |
| Lunar | Autumn | Leaf | 1.244 | 0.10±0.001 |
| | | | 2.128 | 0.09±0.001 |
| | | | 2.981 | 0.09±0.001 |
| | | | 7.192 | 0.18±0.001 |
| | | | 7.598 | 0.17±0.002 |
| | | | 8.012 | 0.16±0.003 |
| | | | 8.559 | 1.3±0.004 |
| Solar | Autumn | Manufactured | 1.134 | 0.15±0.002 |
| | | | 7.575 | 0.23±0.002 |
| | | | 8.870 | 2.10±0.003 |
| Lunar | Autumn | Manufactured | 1.419 | 0.09±0.001 |
| | | | 1.989 | 0.085±0.001 |
| | | | 7.596 | 0.15±0.001 |
| | | | 8.918 | 1.15±0.002 |

**Table 4b.** HPLC analysis for standards:

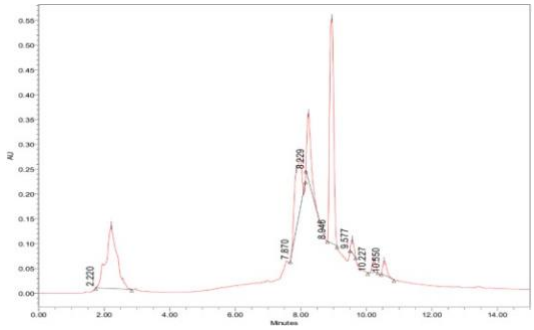
| **Standards** | **Retention time(min)** | **AU** |
|---|---|---|
| Gallic acid | 2.594 | 1.18±0.002 |
| Epigallocatechin gallate | 2.82 | 1.25±0.001 |

| | | |
|---|---|---|
| Rutin | 0.810 | 0.025±0.001 |
| | 7.700 | 0.038±0.001 |
| | 8.185 | 0.075±0.001 |
| | 8.972 | 0.17±0.003 |
| Quercetin | 8.932 | 1.5±0.002 |

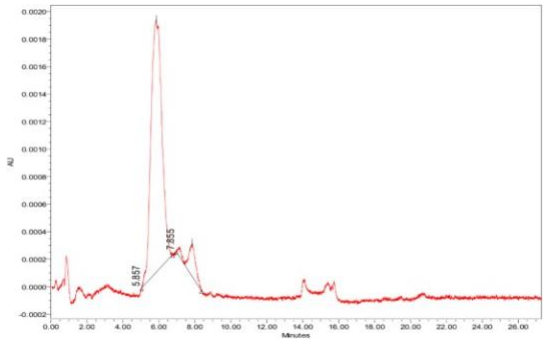
**HPLC Analysis**



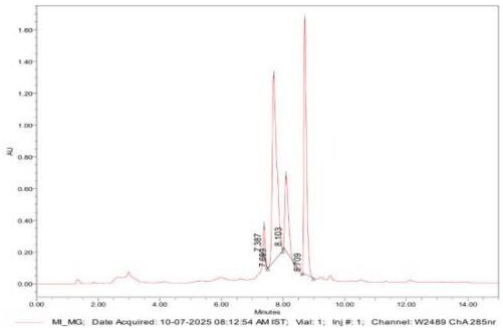
**4a**



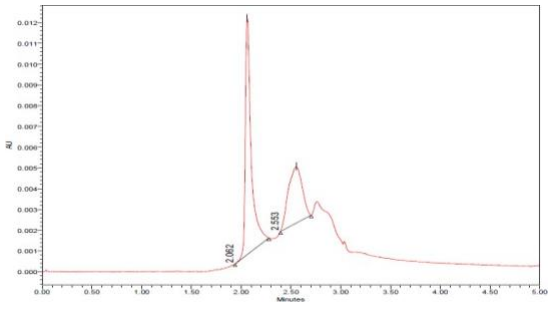
**4b**



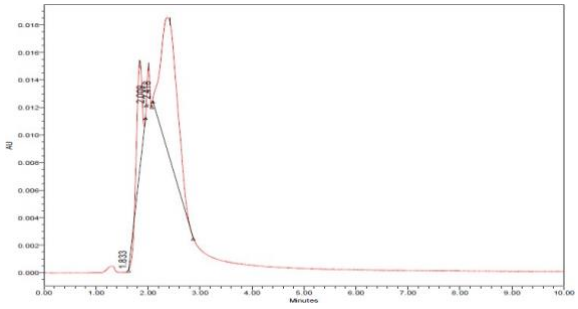
**4c**



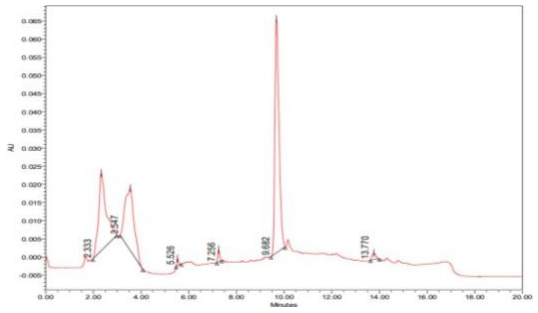
**4d**



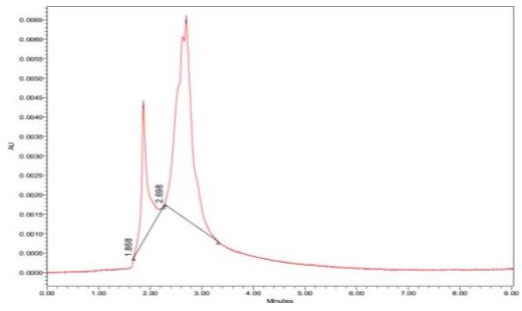
**4e**



**4f**




**4g**

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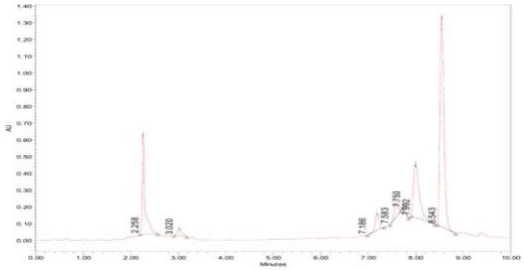
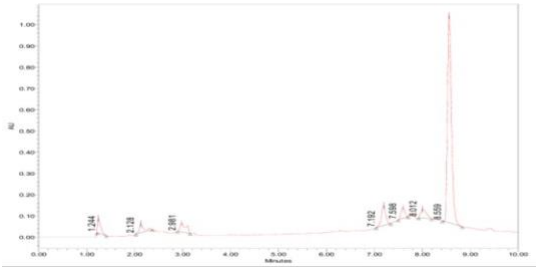
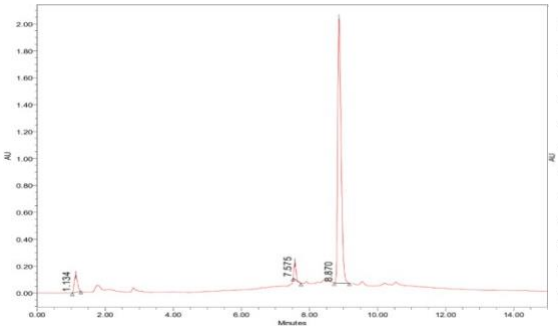


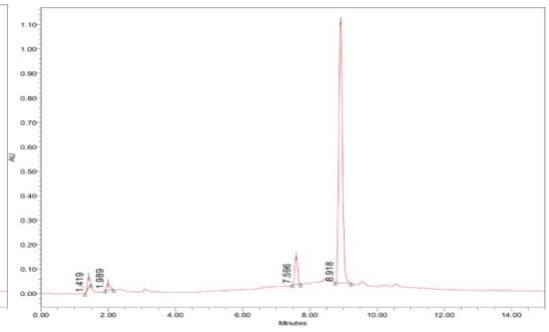

**4i**



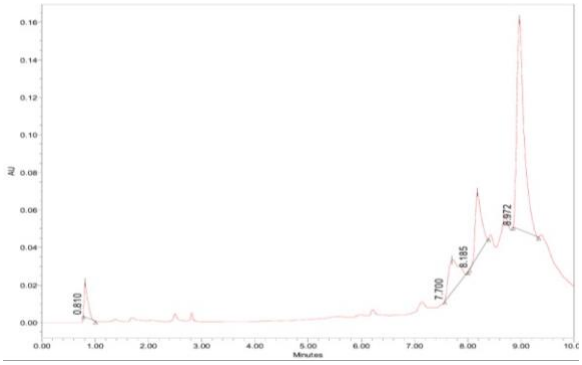

**4j**



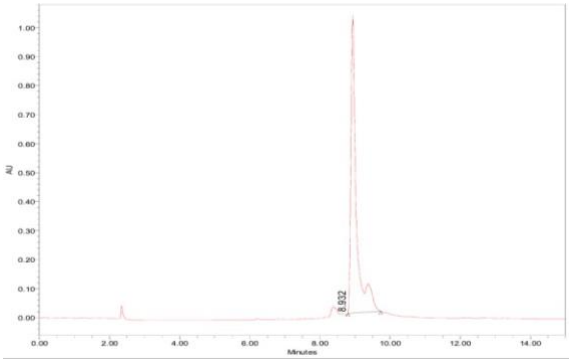

**4k**




**4l**




**4m**




**4n**

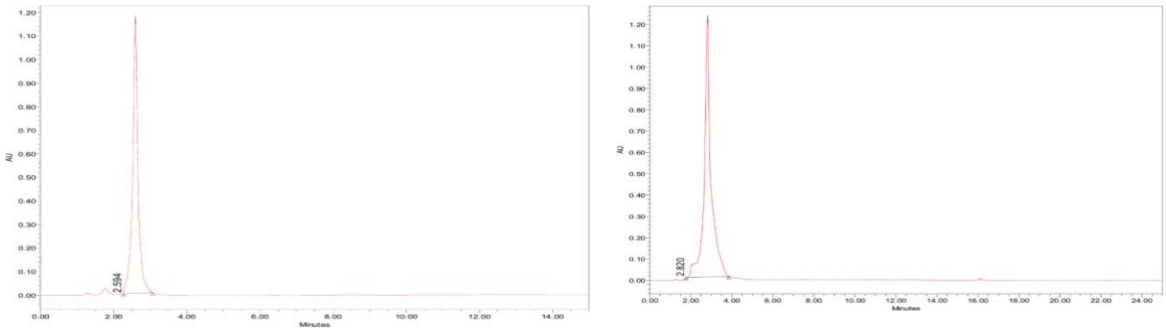



**Figure 4.** HPLC scan of Solar 1st flush fresh leaf(4a), Lunar 1st flush fresh leaf(4b), Solar 1st flush manufactured(4c), Lunar 1st flush manufactured(4d), Solar 2nd flush fresh leaf(4e), Lunar 2nd flush fresh leaf(4f), Solar 2nd flush manufactured(4g), Lunar 2nd flush manufactured(4h), Solar autumn flush fresh leaf(4i), Lunar autumn flush fresh leaf(4j), Solar autumn flush manufactured(4k), Lunar autumn flush manufactured(4l), Gallic acid(4m), Quercetin(4n), Rutin(4o) and Epigallocatechin gallate (4p).

### 3.4. FTIR scan analysis

The lunar fresh leaf samples showed higher percentage of transmittance intensity than solar samples. As seen in the first flush, lunar leaves showed strong bands around 3327, 2942.53, 2831.05 & 1448.82 $cm^{-1}$ at 80±0.04%,88.85±0.02%,86±0.03%& 90±0.06% respectively. The solar samples on the other hand showed peaks at 3787.44, 3661.53,3190.45,1529.63,1109.82,1658.77$cm^{-1}$at100.5±0.02,100±0.023,99±0.05,100.5±0.03,99.7±0.03,99.6±0.01 % respectively. In the second flush, both solar and lunar samples had similar fluorescence transmittance values at 3331.83 (80±0.036%) and 1020.38 (40±0.05%) $cm^{-1}$. In the autumn flush, both solar (around3428.1, 3184.9,1110.36,565.60 $cm^{-1}$ at. 97±0.014, 96±0.02,99±0.036,85±0.023%respectively) and lunar fresh leaves (3420.61, 3184.69 at 99±0.03,98±0.05% respectively) showed multiple peaks. Fresh leaves have shown stronger and broader absorption bands than manufactured leaves (lunar 1st flush 3327.50,506.80,1021.45 $cm^{-1}$at 80±0.04,56±0.02,30±0.02%) mainly in the first flush solar (3787.44, 3661.53, 3190.45,1529.63,1109.82,1658.77$cm^{-1}$at100.5±0.02,100±0.023,99±0.05,100.5±0.03,99.7±0.03, 99.6 ±0.01% respectively) and lunar leaves (3327,2942.53, 2831.05 & 1448.82 $cm^{-1}$ at 80±0.012%,87±0.02%,88±0.05% & 90±0.041%respectively). Lunar 2nd flush manufactured samples show distinct peaks at 1091.51,519.47 $cm^{-1}$ at 40±0.03 and 50±0.015%. Autumn flush solar and lunar manufactured leaves showed higher absorbance at 1021.52,1021.58 $cm^{-1}$ with 20±0.02 % for both. In comparison with the manufactured leaves, the fresh leaves sample showed lower transmittance values for lower wavenumbers. The first flush shows prominent absorption peaks in the wavenumber range 3787.44 to 620.55 $cm^{-1}$ for fresh leaves and 2360.24 to 510.04 $cm^{-1}$ for manufactured ones. The second flush predominantly showed fewer peaks but consistent absorption around 3331.83 (80±0.036%), 2942.94 (85±0.03%) and 1021.14 (30±0.012%)$cm^{-1}$. Autumn flush showed multiple peaks at 3330.72 (78±0.02%), 2944.01 (85±0.023%), 1653.16 (98±0.04%) and 1019.51(40±0.03%) $cm^{-1}$ in fresh leaves, and around 3420.61 (99±0.03%), 3184.69(98±0.05%) and 1021.52(20±0.021%)

$cm^{-1}$ in manufactured leaves. (Figure 5 (a-o) and Table 5)

**Table 5.** FTIR analysis of tea samples and standards:

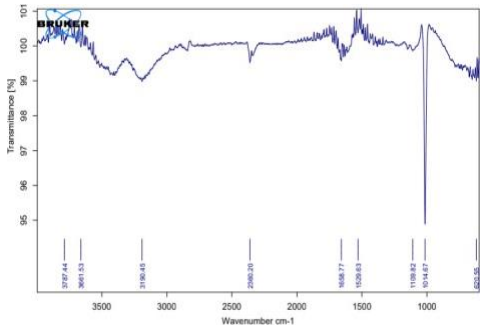
| Flush | Method | State | Wavelength | Transmittance% |
|---|---|---|---|---|
| 1st Flush | Solar | Fresh | 3787.44 | 100.5±0.02 |
| 1st Flush | Solar | Fresh | 3661.53 | 100±0.023 |
| 1st Flush | Solar | Fresh | 3190.45 | 99±0.05 |
| 1st Flush | Solar | Fresh | 2360.2 | 99.5±0.06 |
| 1st Flush | Solar | Fresh | 1658.77 | 99.6±0.01 |
| 1st Flush | Solar | Fresh | 1529.63 | 100.5±0.03 |
| 1st Flush | Solar | Fresh | 1109.82 | 99.7±0.03 |
| 1st Flush | Solar | Fresh | 1014.67 | 95±0.023 |
| 1st Flush | Solar | Fresh | 620.55 | 99±0.05 |
| 1st Flush | Lunar | Fresh | 3327 | 80±0.04 |
| 1st Flush | Lunar | Fresh | 2942.53 | 88.85±0.02 |
| 1st Flush | Lunar | Fresh | 2831.05 | 86±0.03 |
| 1st Flush | Lunar | Fresh | 1448.82 | 90±0.06 |
| 1st Flush | Lunar | Fresh | 1021.45 | 30±0.02 |
| 1st Flush | Lunar | Fresh | 620.07 | 79±0.05 |
| 1st Flush | Lunar | Fresh | 506.8 | 63±0.05 |
| 1st Flush | Solar | Manufactured | 2360.24 | 101±0.032 |
| 1st Flush | Solar | Manufactured | 1237.35 | 102±0.045 |
| 1st Flush | Solar | Manufactured | 907.46 | 102±0.036 |
| 1st Flush | Solar | Manufactured | 535.62 | 103±0.022 |
| 1st Flush | Solar | Manufactured | 523.5 | 104±0.063 |
| 1st Flush | Solar | Manufactured | 510.04 | 105±0.045 |
| 1st Flush | Lunar | Manufactured | 3327.5 | 80±0.012 |

| | | | | |
|---|---|---|---|---|
| 1st Flush | Lunar | Manufactured | 2942.53 | 87±0.02 |
| 1st Flush | Lunar | Manufactured | 2831.05 | 88±0.05 |
| 1st Flush | Lunar | Manufactured | 1448.82 | 90±0.041 |
| 1st Flush | Lunar | Manufactured | 1021.45 | 30±0.06 |
| 1st Flush | Lunar | Manufactured | 620.07 | 78±0.052 |
| 1st Flush | Lunar | Manufactured | 506.8 | 56±0.02 |
| | | | | |
| 2nd Flush | Solar | Fresh | 3331.83 | 80±0.036 |
| 2nd Flush | Solar | Fresh | 1020.38 | 40±0.05 |
| 2nd Flush | Solar | Fresh | 592.43 | 70±0.03 |
| 2nd Flush | Lunar | Fresh | 3331.83 | 80±0.06 |
| 2nd Flush | Lunar | Fresh | 1020.38 | 40±0.036 |
| 2nd Flush | Lunar | Fresh | 592.43 | 70±0.07 |
| 2nd Flush | Solar | Manufactured | 3319.03 | 80±0.065 |
| 2nd Flush | Solar | Manufactured | 2942.94 | 85±0.03 |
| 2nd Flush | Solar | Manufactured | 2831.36 | 87±0.02 |
| 2nd Flush | Solar | Manufactured | 1448.82 | 90±0.023 |
| 2nd Flush | Solar | Manufactured | 1021.14 | 30±0.012 |
| 2nd Flush | Solar | Manufactured | 575.93 | 25±0.04 |
| | | | | |
| Autumn | Solar | Fresh | 3330.72 | 78±0.02 |
| Autumn | Solar | Fresh | 2944.01 | 85±0.023 |
| Autumn | Solar | Fresh | 1449 | 90±0.05 |
| Autumn | Solar | Fresh | 1019.51 | 40±0.03 |
| Autumn | Solar | Fresh | 519.47 | 50±0.015 |

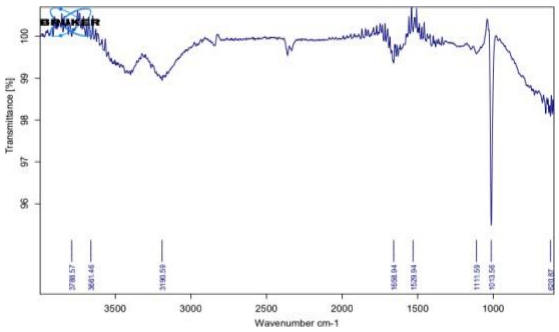
| | | | | |
|---|---|---|---|---|
| Autumn | Lunar | Fresh | 3428.1 | 97±0.014 |
| Autumn | Lunar | Fresh | 3184.9 | 96±0.02 |
| Autumn | Lunar | Fresh | 1654.37 | 96±0.03 |
| Autumn | Lunar | Fresh | 1110.36 | 99±0.036 |
| Autumn | Lunar | Fresh | 1006 | 86±0.038 |
| Autumn | Lunar | Fresh | 574.28 | 94±0.042 |
| Autumn | Lunar | Fresh | 565.6 | 85±0.023 |
| Autumn | Solar | Manufactured | 3420.61 | 99±0.03 |
| Autumn | Solar | Manufactured | 3184.69 | 98±0.05 |
| Autumn | Solar | Manufactured | 1653.16 | 98±0.04 |
| Autumn | Solar | Manufactured | 1109.96 | 98±0.03 |
| Autumn | Solar | Manufactured | 1005.11 | 91±0.023 |
| Autumn | Lunar | Manufactured | 3320.09 | 70±0.01 |
| Autumn | Lunar | Manufactured | 2943.14 | 78±0.02 |
| Autumn | Lunar | Manufactured | 2831.44 | 79±0.036 |
| Autumn | Lunar | Manufactured | 1448.87 | 80±0.042 |
| Autumn | Lunar | Manufactured | 1021.52 | 20±0.02 |
| Rutin | | | 1654.72<br>626.86<br>609.56<br>574.86<br>545.19<br>529.02<br>513.97 | 100±0.045<br>100±0.023<br>99±0.031<br>99±0.014<br>99±0.025<br>98±0.032<br>100±0.023 |
| Quercetin | | | 1000.61<br>626.30<br>574.40<br>560.06<br>526.16<br>511.49 | 100±0.02<br>95±0.05<br>95±0.02<br>97±0.045<br>97±0.02<br>87±0.01 |

| | | |
|---|---|---|
| Gallic acid | 3422.23 | 99±0.02 |
| | 3183.32 | 99±0.023 |
| | 1652.80 | 99±0.01 |
| | 1001.67 | 96.5±0.01 |
| | 627.51 | 94±0.04 |
| | 597.57 | 94±0.03 |
| | 573.83 | 92±0.09 |
| | 560.14 | 92±0.08 |
| | 543.52 | 91±0.078 |
| | 526.42 | 92±0.012 |
| | 513.24 | 93±0.01 |
| EGCG | 3910.77 | 102.2±0.01 |
| | 3894.86 | 102.1±0.03 |
| | 3876.77 | 102.0±0.05 |
| | 3846.36 | 101.9±0.056 |
| | 3828.14 | 101.8±0.03 |
| | 3810.93 | 101.7±0.05 |
| | 3790.31 | 101.6±0.01 |
| | 3740.36 | 101.5±0.02 |
| | 3716.38 | 101.4±0.08 |
| | 3696.34 | 101.3±0.01 |
| | 3682.71 | 101.2±0.03 |
| | 3661.89 | 101.1±0.05 |
| | 3637.08 | 101.0±0.06 |
| | 3623.12 | 100.9±0.056 |
| | 3602.56 | 100.8±0.01 |
| | 3574.24 | 100.7±0.085 |
| | 3425.75 | 100.5±0.012 |
| | 3190.06 | 100.3±0.01 |
| | 2847.27 | 100.2±0.032 |
| | 2735.36 | 101.0±0.023 |

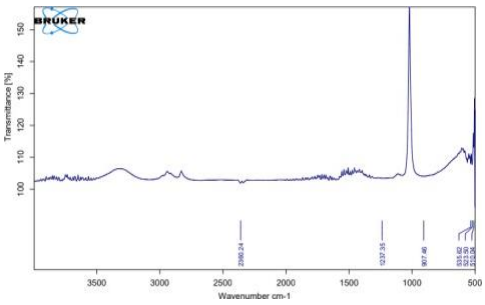
**FTIR scan analysis**




**5a**




**5b**

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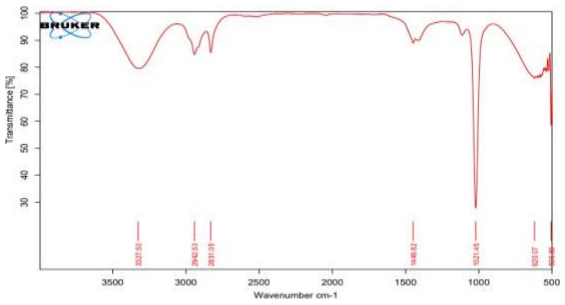


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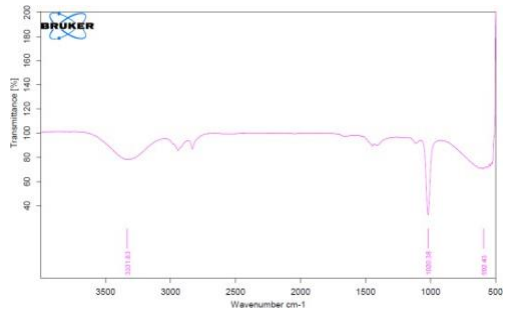


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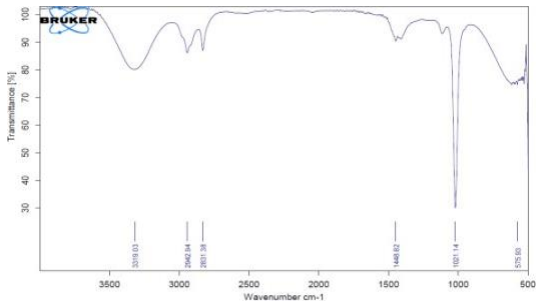


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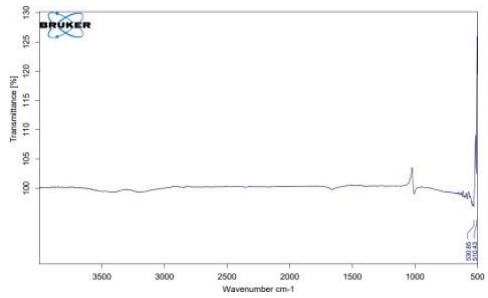


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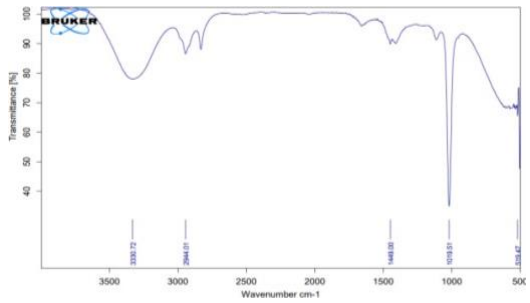
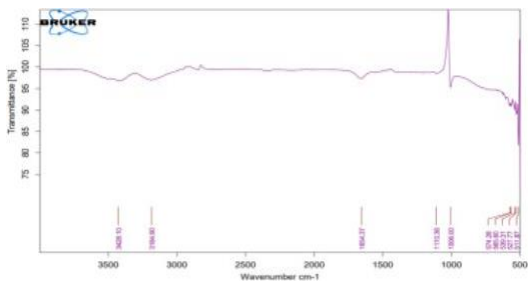
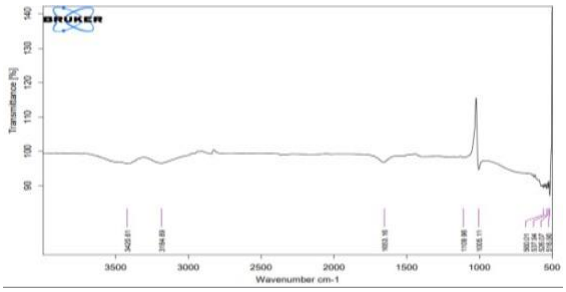



**5h**



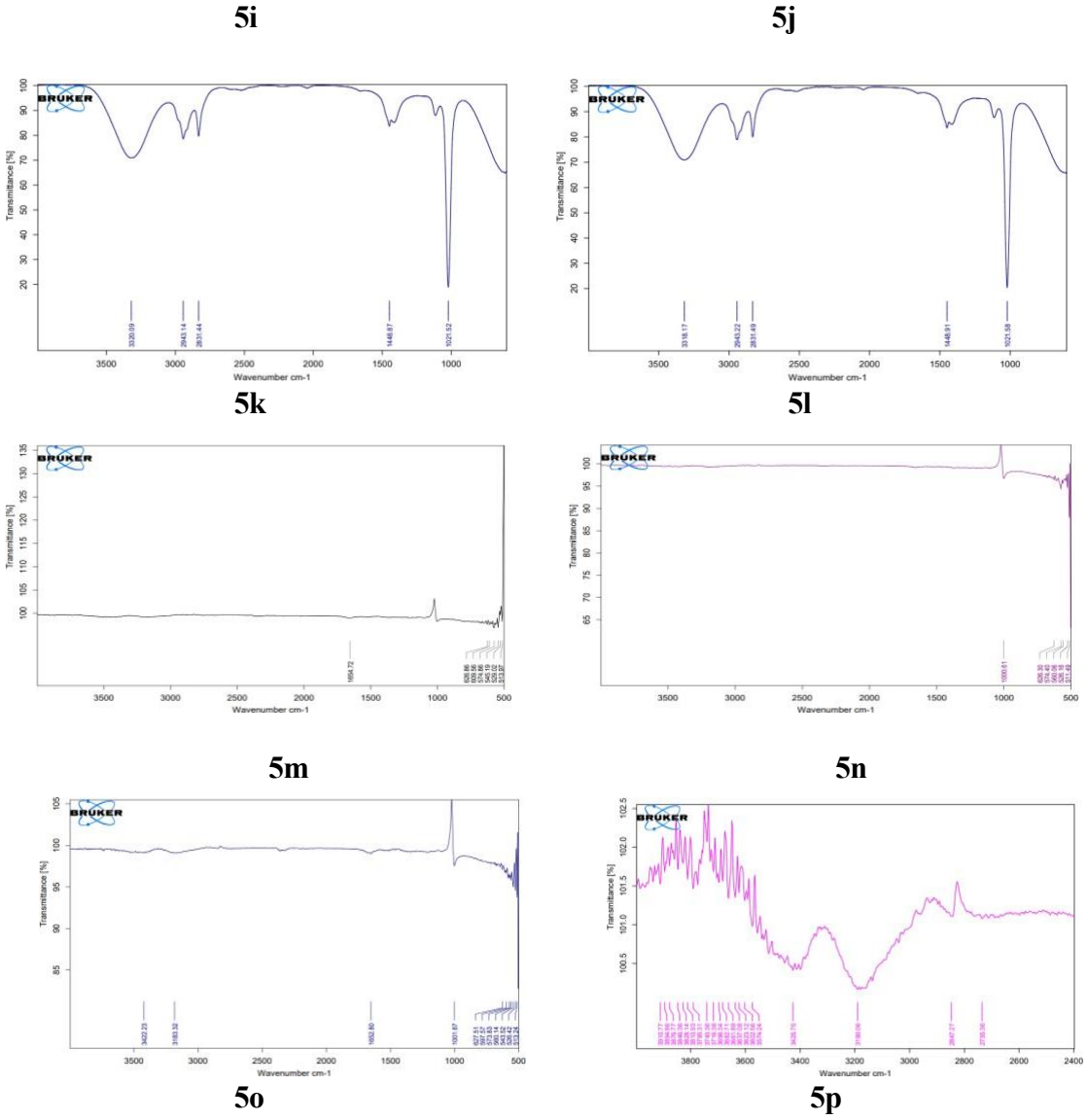



**Figure 5.** FTIR scan of Solar 1st flush fresh leaf (5a), Lunar 1st flush fresh leaf(5b), Solar 1st flush manufactured(5c), Lunar 1st flush manufactured(5d), Solar 2nd flush fresh leaf (5e), Lunar 2nd flush fresh leaf (5f), Solar 2nd flush manufactured (5g), Lunar 2nd flush manufactured (5h), Solar autumn flush fresh leaf (5i), Lunar autumn flush fresh leaf(5j), Solar autumn flush manufactured(5k), Lunar autumn flush manufactured(5l), Rutin (5m), Quercetin (5n), Gallic acid (5o) and Epigallocatechin gallate (5p).

### 3.5. Gas Chromatography

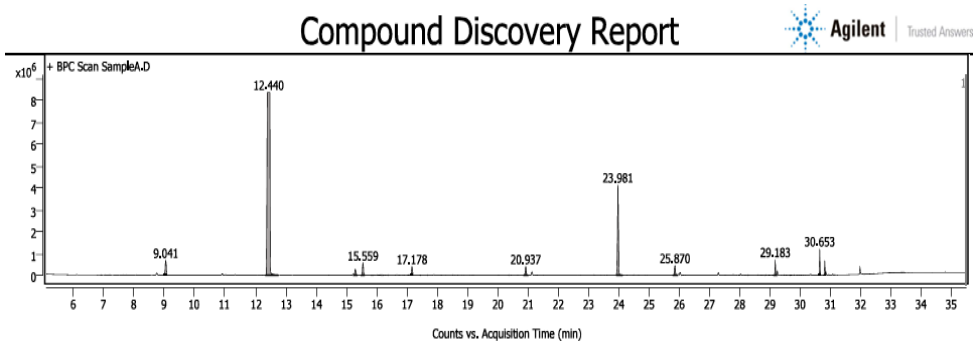
The higher number of peaks (12) than 2nd flush solar fresh leaves (10) of volatile components are observed by GC MS analysis. Compared to standard linalool at acquisition time X$10^6$ around 9.041 (standard) 9.018 (lunar 2nd flush fresh leaf), 9.013 (solar 2nd flush fresh leaf) is around 0.8±0.001, 0.8±0.001, 0.7±0.003 counts respectively showing lesser content in 2nd flush solar fresh leaf harvests. The similar trend is followed for 25.870 for linalool), 2nd flush lunar fresh leaf for 25.853 and for 2nd flush solar fresh leaf 25.853 Acquisition x $10^6$ are around 0.8±0.002, 0.8±0.001 and 0.5±0.009 respectively (Figures 6 a, 6b and 6c and Table 6).

**Table 6.** Analysis of GC MS of different tea sample and standard

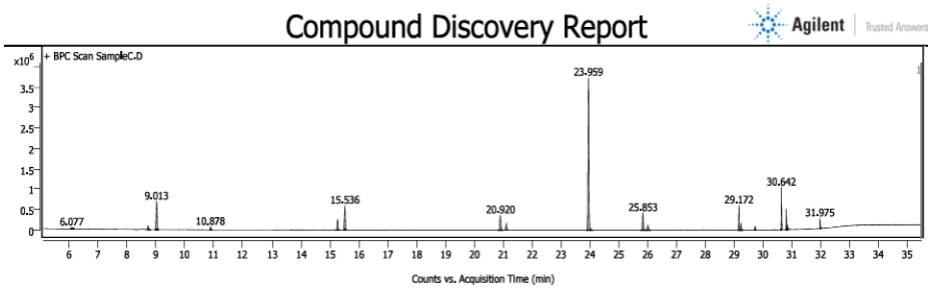
| SAMPLE | TOTAL | ACQUISITION TIME $X10^6$ | COUNTS |
|---|---|---|---|
| Linalool (standard) | 9 | 9.041 | 0.8±0.001 |
| | | 12.440 | 8±0.002 |
| | | 15.359 | 0.8±0.002 |
| | | 17.178 | 0.5±0.005 |
| | | 20.937 | 0.5±0.001 |
| | | 23.981 | 4.5±0.004 |
| | | 25.870 | 0.8±0.002 |
| | | 29.183 | 1.5±0.001 |
| | | 30.653 | 2±0.003 |
| | | | |
| $2^{nd}$ flush lunar fresh leaf | 12 | 9.018 | 0.8±0.001 |
| | | 10.884 | 0.1±0.002 |
| | | 15.536 | 0.7±0.003 |
| | | 17.155 | 0.2±0.004 |
| | | 20.926 | 0.4±0.001 |
| | | 23.964 | 3.8±0.002 |
| | | 25.853 | 0.8±0.001 |
| | | 27.289 | 0.05±0.001 |
| | | 28.177 | 1±0.003 |
| | | 29.738 | 2.8±0.001 |
| | | 30.648 | 1.5±0.003 |
| | | 31.981 | 0.3±0.001 |

| SAMPLE | TOTAL | ACQUISITION TIME X10$^6$ | COUNTS |
|---|---|---|---|
| 2nd flush solar fresh leaf | 10 | 6.077 | 0.09±0.001 |
| | | 9.013 | 0.7±0.003 |
| | | 10.878 | 0.09±0.005 |
| | | 15.536 | 0.8±0.001 |
| | | 20.920 | 0.4±0.001 |
| | | 23.959 | 3.8±0.006 |
| | | 25.853 | 0.5±0.009 |
| | | 29.172 | 1±0.001 |
| | | 30.642 | 1.2±0.002 |
| | | 31.975 | 0.1±0.001 |

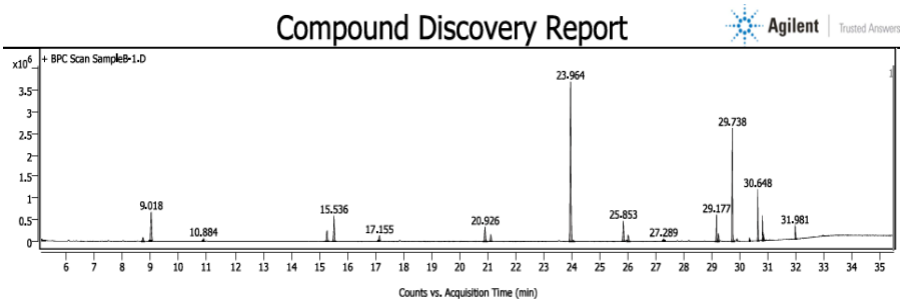
**Gas Chromatography:**




**6a**




**6b**




**6c**

**Figure 6.** GC MS analysis of Linalool(6a),2nd flush lunar fresh leaf(6b) and 2nd flush solar fresh leaf(6c).

### 3.6. Total Polyphenol content

The highest polyphenol content is observed in lunar 2nd flush fresh leaves around 185±0.2 GAE/g fresh wt., the lower content of polyphenols are observed in 1st flush solar or lunar fresh leaves (120±0.1 GAE/g fresh wt. and 135±0.1 GAE/g fresh wt. respectively). Among the manufactured leaves, lunar 2nd flush manufactured showed highest content around 135±0.1 GAE/g fresh wt. and the lowest content is shown by 1st flush solar harvest (98±0.1 GAE/g fresh wt.). Solar and lunar autumn harvest showed equal amount of total polyphenol content 120±0.2 GAE/g fresh wt. whereas their corresponding fresh leaves showed variation (solar autumn flush 140±0.1 GAE/g fresh wt., lunar autumn flush 150±0.1 GAE/g fresh wt.) (Figure7).

### 3.7. Total Flavonoid Content

As shown in Figure 8 the total flavonoid content was highest in lunar 2nd flush fresh leaves around 49±0.01 QE mg/g, and the lowest content was observed in 1st flush solar harvest 31±0.02 QE mg/g. Among the manufactured tea, the highest content was observed in lunar 2nd flush tea 35±0.01 QE mg/g and the lowest in solar harvest 1st flush fresh leaves (20±0.01 QE mg/g).

### 3.8. Total Antioxidant potential

The total antioxidant potential was highest in lunar 2nd flush fresh leaves and manufactured tea (89±0.01% and 75±0.02% DPPH reduction/mg fresh wt.). The lower content was observed in first flush solar and lunar fresh and manufactured tea (71±0.01% for solar 1st flush fresh leaves, 75±0.02% for lunar 1st flush fresh leaves, 59±0.01% solar 1st flush manufactured and 62±0.01% lunar 1st flush manufactured tea) (Figure9).

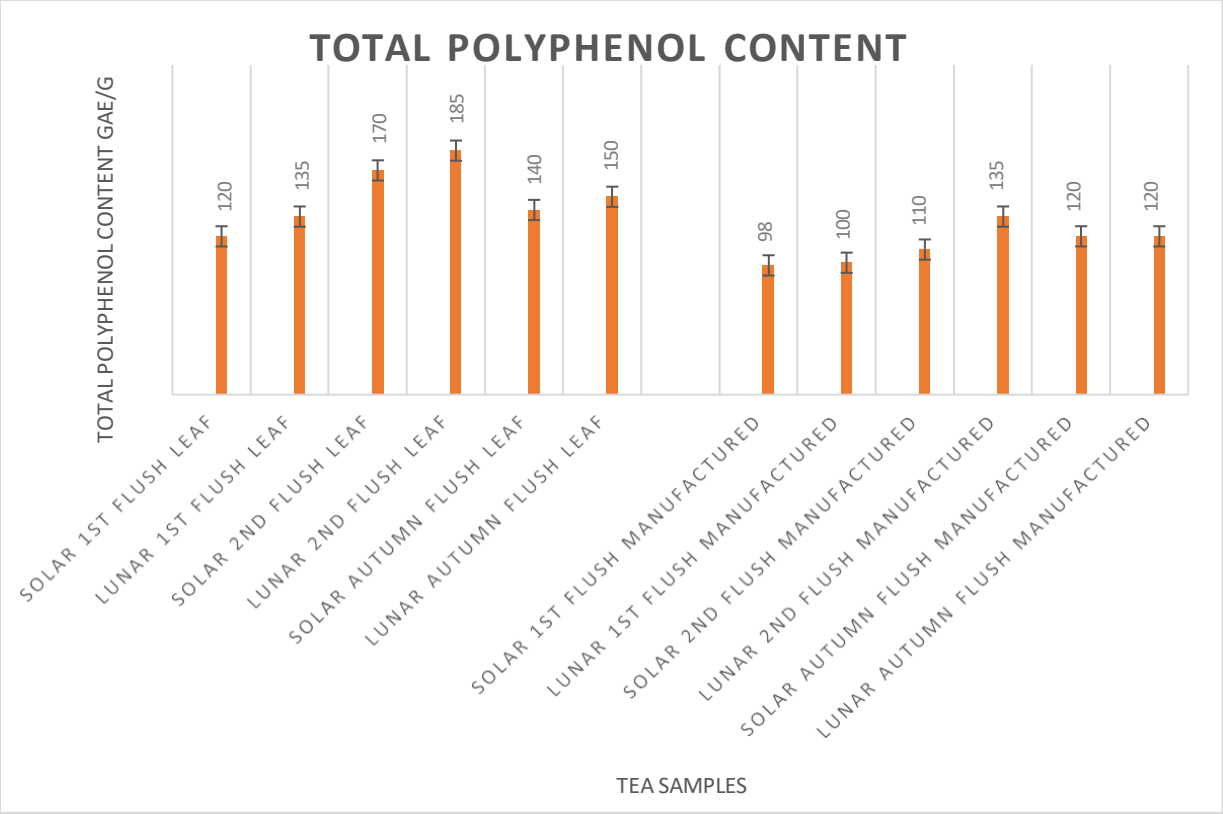



**Figure 7.** Total polyphenol content of different tea samples

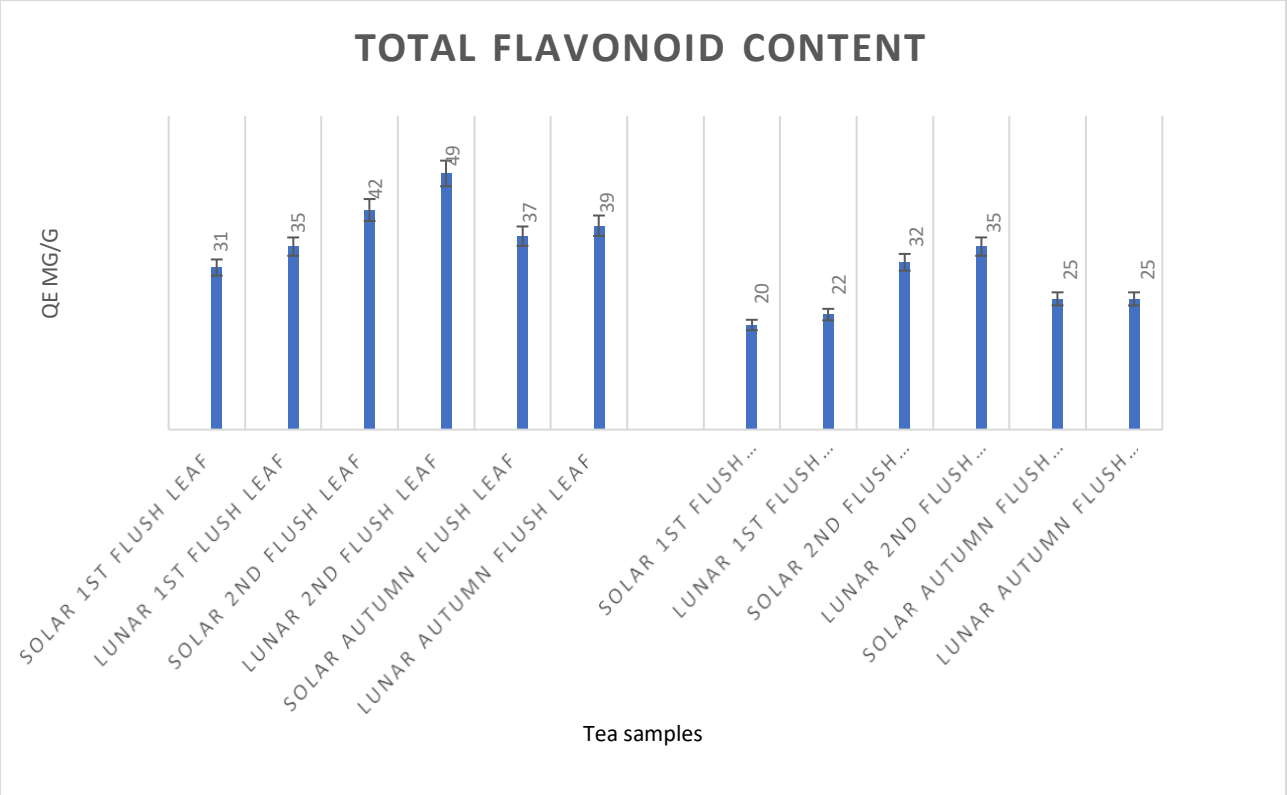


**Figure 8.** Total flavonoid content of different tea samples

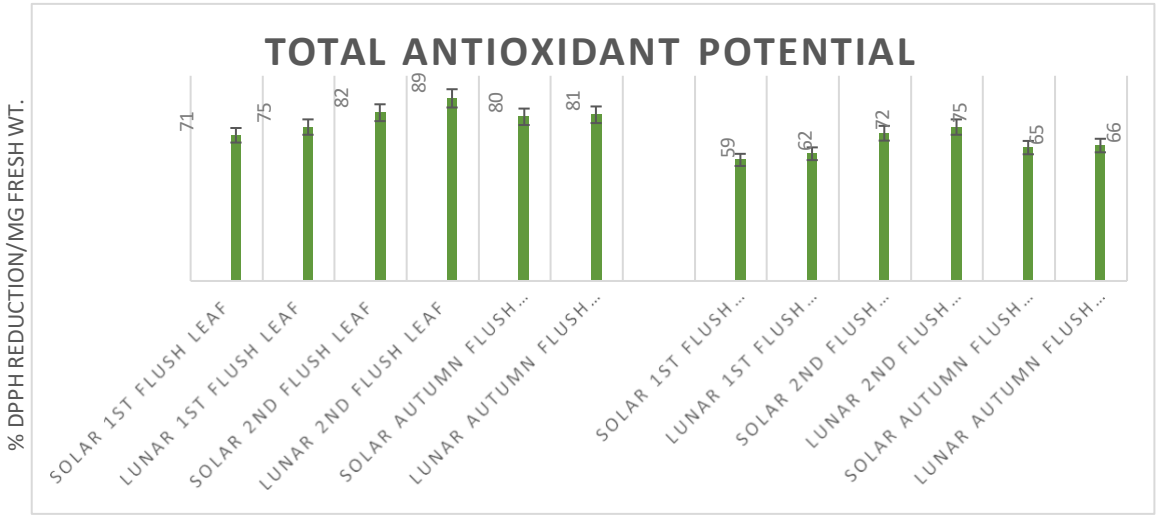



**Figure 9.** Total antioxidant potential of different tea samples

**3.9**. **The E-tongue and E-nose analysis** The E-nose and E-tongue value is highest for lunar 2nd flush manufactured tea (noraroma index N.I is 19±0.01 and taster's score around 2±0.01)(Figure10). Figure 10. shows that the E-nose and E-tongue value is highest for lunar 2nd flush manufactured tea (noraroma index N.I is 19±0.01 and taster's score around 2±0.01)

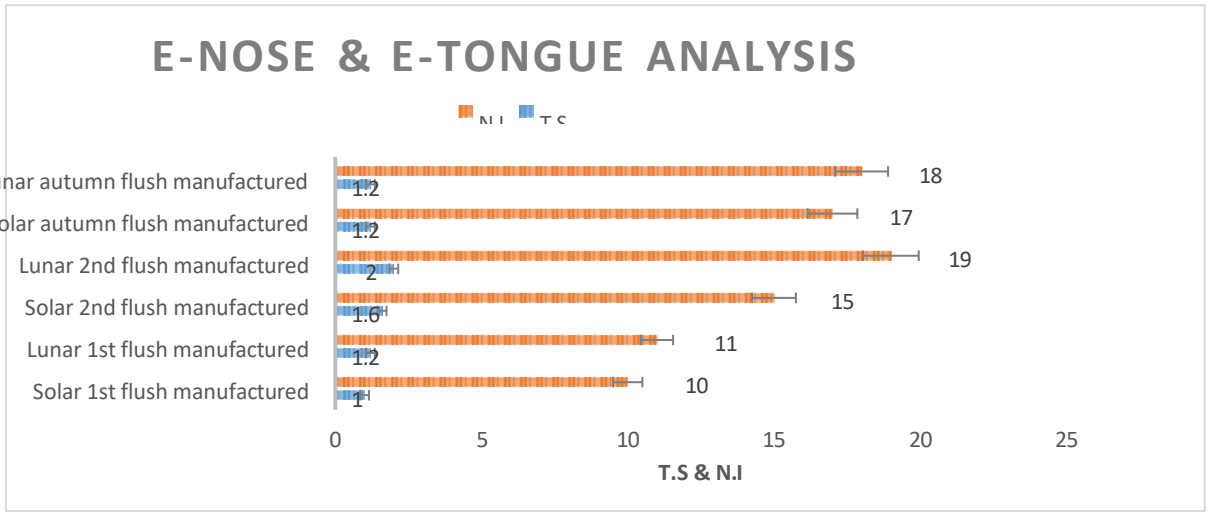



**Figure 10.** E-nose and E-tongue analysis of different tea samples

**3.10. Discussions**

***3.10.1.Variation of secondary metabolites specially polyphenols and flavonoids of fresh harvested tea samples: Seasonal variations***

Figure 2(a,b,e,f,i,j,m,n,o,p), Figure 3 (a,b,e,f,i,j,m,n,o,p), Figure 4 (a,b,e,f,i,j,m,n,o,p), Figure 5 (a,b,e,f,i,j,m,n,o,p), Figure 7 , Figure 8 and Tables 2a,2b, 3a, 3b, 4a, 4b and 5 showed significant variation in the flavonoid and polyphenol content (compared with standards like Gallic acid, Rutin, Quercetin, Epigallocatechin gallate) among different seasonal flushes (1st, 2nd and autumn flush). It

showed the 2nd flush harvest to have higher polyphenolic content. Previous report with *Biswas et al., 2025* and *Bhattacharyaet al.,2011* had shown that during summer solistice (June and July during 2nd flush harvest), the northern Himalayan Darjeeling and Dooars are receiving maximum amount of UV radiation which could upregulate the secondary metabolites like flavonoids which help to cope up high-altitude based oxidative stress. (Bhattacharya and Sen-Mandi, 2011, Biswas et al., 2025)

### *3.10.2. Variation in polyphenol, flavonoid content of fresh leaves and manufactured tea samples*

Figures 2 (a-p), 3 (a-p), 4 (a-p), 5 (a-p), 7 and 8 shows that the manufactured tea samples are showing lower polyphenol and flavonoid group of compounds than fresh leaves. Previous research had reported and shown that tea manufacturing process or oxidation causes the reduction of the polyphenol and flavonoid content during processing. Research describing the differential polyphenol and flavonoid content of differentially manufactured tea have also been reported. (Chen et al., 2023, Maitra et al., 2025)

### *3.10.3. Variation of polyphenols and flavonoids of fresh and manufactured tea samples: Diurnal variation*

Figures 2 (a-p), 3 (a-p), 4 (a-p), 5(a-p) and Figures 7-8 shows that the lunar harvest at different flushes (specially 2nd flush) and both fresh and manufactured leaves shows higher polyphenol and flavonoid content. *Sannidhi et al., 2026* had described that the synodic cycle causes the variation of the metabolite content in plants specially at full moon harvest. Moonlight is sensed as stressor towards the photoreceptor of the plants to upregulate the quality metabolites to promote growth, development and overall performance (Wingler and Henriques, 2022). Previous reports had shown that the effect of full moon as local gravimetric oscillations (circalunar oscillations) on plant growth and development. The moon can act in two ways on plants, via its gravitational effect or via the sunlight it reflects. The wavelength of full moonlight is generally cantered around 400 nm (580nm for the sun) with a very low energy level (0.2lx or 0.0024µmolm$^{-2}$ s$^{-1}$). The red: far red (R:FR) ratio of sunlight during the day is more than 1.2, while that of moonlight is between 0.18 and 0.22· (Wingler and Henriques, 2022, Sannidhi et al., 2026, Priyanka et al., 2024, Breitler et al., 2020).

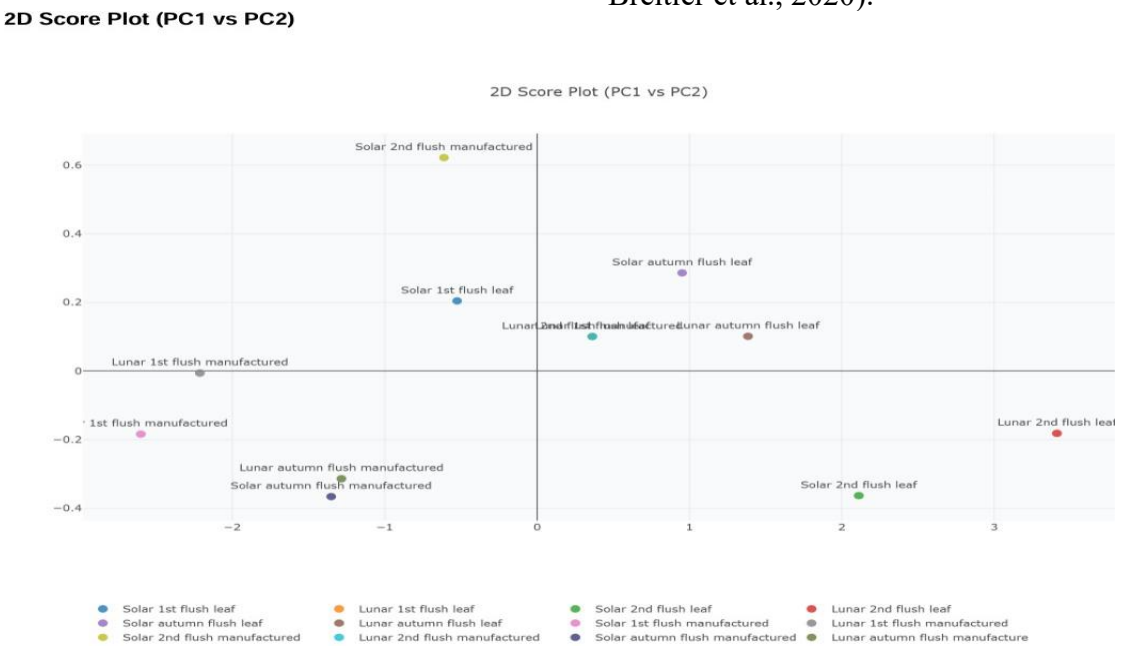



**Figure 11a.** 2D score plot of different tea samples

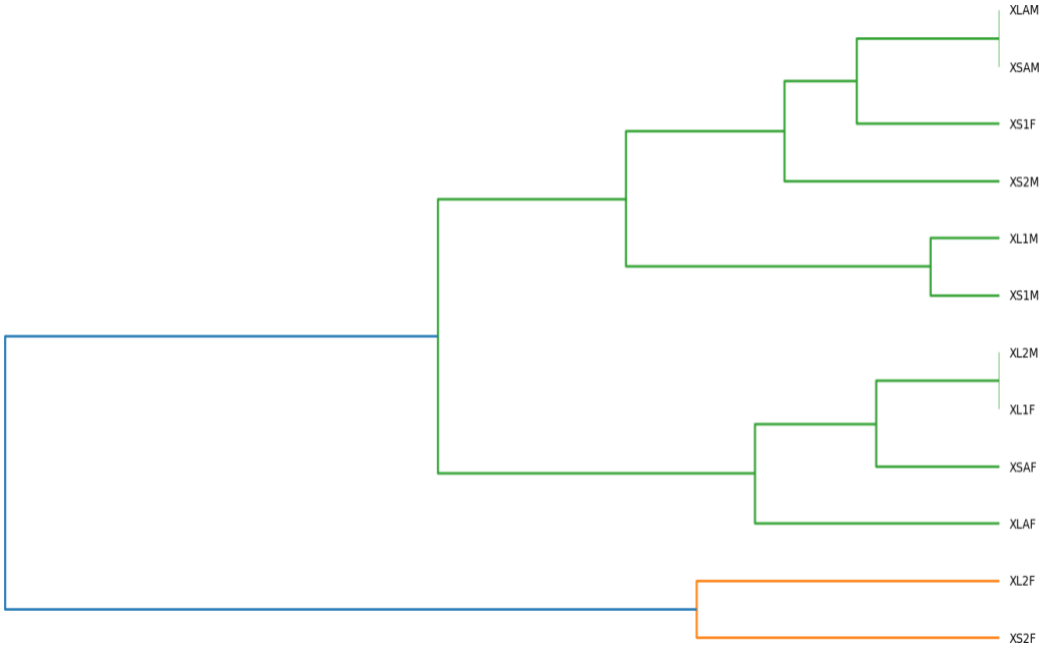



**Figure 11b.** Dendrogram analysis of different tea samples.

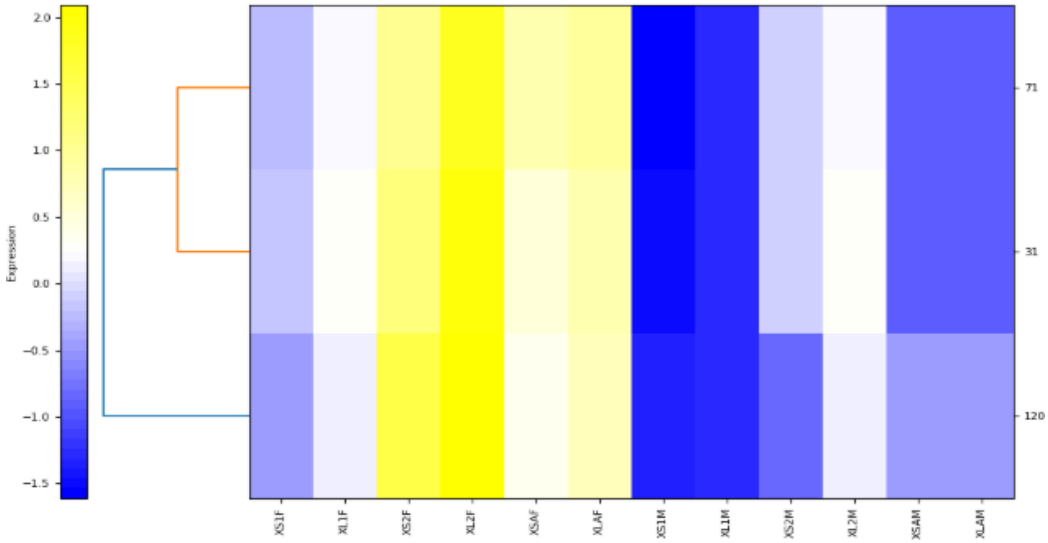



**Figure 11c.** PCA analysis of different tea samples

**Fig 11a** (2D score plot) shows that the lunar 2nd flush manufactured and lunar autumn fresh leaves are clustered together according to secondary metabolite profile and antioxidant potential. **Fig 11b** shows that Lunar autumn manufactured and solar autumn manufactured are clustered together and closely related to solar 1st flush fresh leaf and solar 2nd flush manufactured samples and the cluster is closely associated with lunar 1st flush manufactured and solar 1st flush manufactured but this cluster is away from lunar 2nd flush manufactured, lunar 1st flush fresh leaves, solar autumn fresh leaf and lunar autumn fresh leaves. The clusters are out-grouped from the clusters of lunar 2nd flush fresh leaves and solar 2nd flush fresh eaves. **Fig 11c** shows the highest expression (1.5-2) of secondary metabolites (polyphenol, flavonoids) and antioxidant values in Lunar 2nd flush leaves.

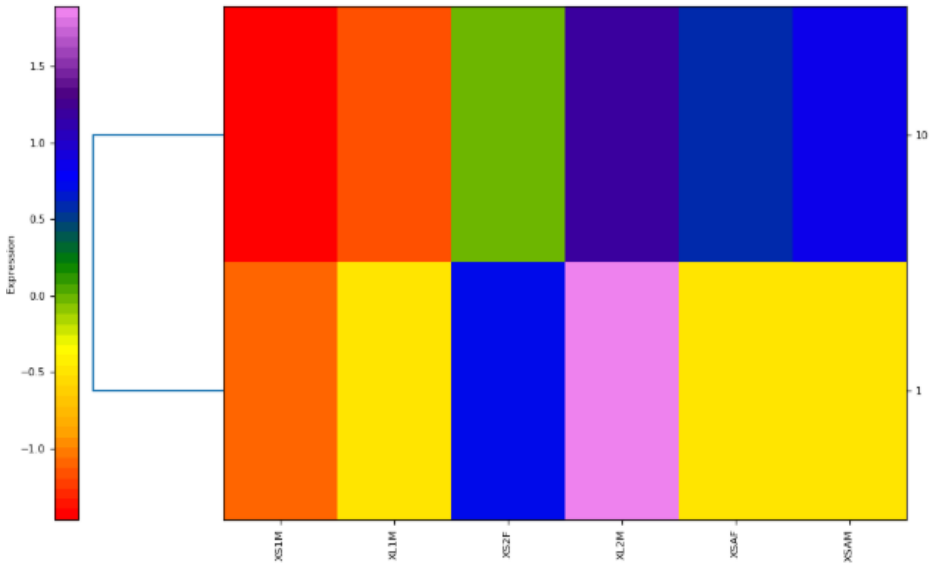



**Figure 11d.** Expression analysis of manufactured tea (E-nose N.I and E-tongue T.S values) of different seasonal flushes and different harvest time (diurnal phases)

Figure 11d shows that the expression analysis of all the manufactured tea (of different flushes and different diurnal variations like solar and lunar). It shows that the higher expression of E-nose (N.I) and E-tongue (T.S) values of Lunar 2nd flush manufactured tea.

***3.10.4. Higher content of quality components in Lunar harvest tea***

The quality components of tea depend on its commercial and therapeutic values. Higher the volatile components, higher the commercial qualities. Figures 6a, 6b, 6c and Table 6 shows that the lunar 2nd harvest manufactured tea samples contain higher volatile components contributing towards elevated commercial qualities which is corroborated with the E-nose and E-tongue analysis. Higher N.I and T.S is observed for 2nd flush lunar harvest tea (Fig 10). The data is supported by Fig 11a PCA expression analysis. The antioxidant potential (Fig 9) shows that the elevated values for 2nd flush lunar harvest tea (both fresh and manufactured tea samples) which could contribute to better therapeutic qualities. Figures 11a, 11b, 11c and 11d showed higher expression of metabolites (flavonoids and polyphenols) which are the causative agents of higher commercial and therapeutic qualities. Lunar harvest tea or "moonlight tea" or "full moon tea," is widely regarded as a superior quality tea due to its unique, biodynamic cultivation methods, night time plucking, and limited, artisanal production. Optimal harvesting time, unique flavour profile makes it a better Indian tea. Previous research has analysed the better quality enhancement in other plants during Lunar harvest (Sannidhi et al., 2026). Here in our paper we have found the best quality tea during 2nd flush lunar harvest.

## 4.Conclusion

Thus the consolidated metabolome profile of all the tea samples including fresh and manufactured tea of different diurnal variations (solar and lunar) at different seasonal flushes shows that the higher metabolite content in 2nd flush lunar harvest at Majherdabri, Alipurduar and North Bengal tea garden. The manufactured lunar 2nd flush harvested tea is established of having higher therapeutic and commercial qualities. The practice of age-old ethnic Biodynamic farming (harvest at full moon) establishes the enhancement of tea quality in Majherdabri, Alipurduar tea garden.

## 5. References

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Statistical analysis-2D score plot analysis and Dendrogram analysis - https://lab.learnbin.net/tools/principal-component-analysis-pca-tool/.

Statistical analysis-PCA analysis and expression analysis- https://heatmapper2.ca/

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