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Chemical Profiling of Crush, Tear, Curl (CTC) Tea Waste of Eastern Sub-Himalayan Regions: An Elemental and Spectroscopic Analysis

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Tea is not only the most popular beverage in the world but also producing a large quantity untreated wastes product every year. In particular, the tea gardens of eastern Sub-Himalayan region cumulatively produce 15 million kg of crush, tear, curl (CTC)-factory tea waste (FTW) every year, which primarily includes discarded tea leaves, leaf fibers, buds and tender stems of tea plants. Beside that ~ 80% population of Indian subcontinent consume CTC tea regularly at their homes, tea stalls, market, cafe *etc.* and the waste produced from it, is called CTC domestic tea waste (DTW). Thus, not only factory tea waste but also a large quantity of domestic CTC tea waste (DTW) is exposed into the environment regularly. In present study, an attempt has been made for primary screening of the compounds in both the CTC-tea wastes. It has been shown that FTW sample contains greater amount of non-metal elements such as sulfur, calcium, phosphorus and metal elements like potassium and iron compared to DTW sample. Abundance of aromatic compounds has been seen to be higher in FTWs whereas, DTW primarily contains aliphatic compounds. Using Orbitrap-HRLCMS analysis allowed to make accurate predictions about the molecular structures of the likely organic chemicals found in tea trash. Thus, various bioactive organic compounds, micronutrients and trace elements from tea waste were found.

Keywords: Tea waste, CTC-FTW, CTC-DTW, Chemical profiling.

INTRODUCTION

Tea is the most popular beverage in the world. India is the major producer of tea. India mainly produce three types of tea *i.e.* CTC tea, green tea and an orthodox tea [1]. Among those about 70-80% tea belongs to CTC type. Green tea and orthodox tea do not produce any waste during its manufacture except only domestic tea waste (DTW). But in case of CTC tea, it produces large number of factory tea waste (FTW) during its manufacturing. There are about 400 big industrial tea gardens (BTGs) and many small tea growers (STGs) in the Sub-Himalayan Terai and Dooars regions of India, especially in Darjeeling, Kalimpong, Jalpaiguri, Alipuduar and Cooch Behar. BTGs and STGs produce about 52% and 48%, respectively, of total production of tea in North Bengal area of India. Furthermore, they not only produce CTC teas but also produce around 15 million kg of factory tea waste (CTC-FTW) per year. Beside that a large number of tea wastes are produced in the tea shops and domestic consumption of tea which can be termed as domestic tea waste. In India, CTC tea is consumed about 70% of total consumption of tea and the waste produced from it is called CTC domestic tea waste (CTC-DTW), which also exposed into the environment (Fig. 1). These pollutants, which harm the environment and are improperly disposed of, primarily consist of tea leaves, buds, and fragile stems that have been dumped [2].

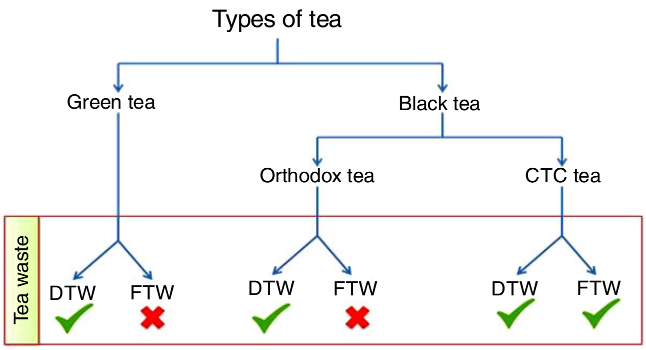


Fig. 1. Types of tea waste (CTC = crush tear curl, DTW = domestic tea waste, FTW = factory tea saste)

Earlier, several researchers have attempted to profile the volatile components of green tea, orthodox tea, CTC tea, tea blossoms, tea leaves and other varieties of tea using GC-MS technology [3-9]. Some researchers used ICP-MS, ICP-OES and ICP-AES techniques in order to identify the presence of trace elements and heavy metals in a variety of teas, tea leaves and tea infusions [10-15]. However, the metabolic profiling of tea leaves, seeds, green tea, orthodox tea, Oolong tea and other types of tea was attempted by using NMR analysis [16-24], whereas FTIR was used to analyze antioxident activity and determination of total phenol, flavonoids, chlorophyll, caffeine and pheophytin in Indian made tea [25-29].

In present study, a carefully designed experimental protocol and spectroscopic techniques for the chemical profiling of CTC tea wastes of Eastern Sub-Himalayan regions were made. Initially, for primary screening of both the CTC-tea waste *i.e.* factory tea waste (FTW) and domestic tea waste (DTW) were done by using elemental, GC and HRMS analysis. The ICP-AES technique was used for the identification and quantification of micronutrient, trace elements and heavy metals in tea wastes. The chemical composition of waste product was analyzed by UV-Vis, FTIR and NMR spectroscopy. The morphology of FTW and DTW were also performed using FESEM technique.

EXPERIMENTAL

Extraction of CTC factory tea waste (CTC-FTW): The factory tea waste was collected from different tea factories. Then, 250 g of CTC-FTW was added to 250 mL of ethanol and refluxed for 5 h. The ethanolic solution was collected by filtration, which was evaporated by rotary evaporator and the organic crude layer was dried over the inner surface of round bottom flask. The organic crude was then collected from round bottom flask by scratching and put it into Eppendorf tube for further study. About 211 μg of organic crude was obtained from 250 g of CTC-FTW.

Extraction of CTC domestic tea waste (CTC-DTW): The CTC made tea was purchased from the local market and then boiled in hot water until or unless the water becomes transparent. The residue CTC made tea was then collected and dried at room temperature. Then same steps as CTC-DTW were followed to get the organic crude of CTC-DTW and put into Eppendorf tube for further study. About 165 μg of organic crude was obtained from 250 g of CTC-DTW.

Characterization: The CHNSO analysis was done by CHNS(O) Analyzer (Thermo finnigan, Italy), GC-HRMS analysis by gas chromatography with high resolution mass spectrometer (GC-HRMS) (Jeol; Model: AccuTOF GCV), Orbit-rap-HRLCMS analysis by Q-Exactive plus biopharma-high resolution Orbitrap liquid chromatography mass spectrometer (Thermo Fischer Scientific Pte. Ltd.), ICP-AES analysis by ICP-atomic emission spectroscopy (SPECTRO Analytical Instrument GmbH, Germany), UV-Vis analysis by double beam scanning UV-Vis spectrophotometer (Perkin Elmer; Model: Lambda 35) and ESEM analysis by environmental scanning electron microscope (FEI; Model: Quanta 200) from SAIF IITB. Beside this NMR analysis was done by nuclear magnetic resonance spectrometer (Bruker) from Department of Chemistry, University of North Bengal, Siliguri, India for instrumental analysis.

CHNSO analysis

Organic carbon: The organic carbon was estimated by taking 10 g of tea waste and placed in an oven at 105 ºC for 24 h to estimate the moisture content. The tea waste was then placed in the muffle furnace for 16 h at 400 ºC to estimate the percentage of organic matter in waste through the following equation:

$$\text{Loss ignition (\%)} = \frac{A-B}{A}\times 100$$

where, A = Oven dry tea waste weight, B = ignited tea waste weight.

RESULTS AND DISCUSSION

The chemical profiling of CTC-FTW and CTC-DTW have been documented by using differential elemental and spectroscopic methods. This study provides encouraging data on the chemical profiling of CTC tea waste (CTC-FTW and CTC-DTW).

Elemental components: From the CHN analysis, it is found that domestic tea waste (DTW) contains more percentage of carbon, hydrogen and oxygen than those of factory tea waste (FTW), while the percentage of nitrogen is very less in the domestic tea waste than factory tea waste (Table-1).

TABLE-1
CARBON, HYDROGEN, NITROGEN, OXYGEN PERCENTAGES

Name of sample	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Oxygen (%)
Factory tea waste (CTC-FTW)	50.58	6.45	3.95	23.43
Domestic tea waste (CTC-DTW)	62.54	9.64	0.623	24.70

ICP-AES analysis: Upon analysis, it appears that FTW sample contains greater amount of non-metal element such as sulfur, phosphorus and metal element potassium, calcium, iron along with some other elements whereas the DTW sample contains non-metal *e.g.* sulfur and metal calcium, manganese in greater amount. The organic carbon (OC) sample contains potassium, phosphorus in greater amount and other metal element content is low.

Moreover, it appears that FTW contains more potassium, chromium, iron, cobalt, nickel and zinc than those in the DTW, while other metals like sodium, calcium, strontium *etc*. are in more amounts in DTW than in FTW. It is observed that as compared to DTW, FTW has roughly 20 times as much potassium and 25 times as much nickel, while DTW has twice as much calcium and three times as much manganese. On the other hand, non-metal phosphorous and silicon are in greater amount in DTW but sulfur is higher in FTW (Table-2).

Spectral characterization: The UV spectrum of both wastes shows that they contain highly conjugated compounds (Fig. 2). Moreover, IR spectrum of DTW (Fig. 3b) clearly indicates the presence of bonded -OH function and/or NH_2 function (3377 cm^{-1}), α,β-unsaturated carbonyl attached with aromatic nucleus (1699, 1648, 1555, 1364 cm^{-1}), methoxyl group (s) (2933, 1238, 1035 cm^{-1}) in some molecules in the tea waste. The presence of peak around 1650 cm^{-1} may be attributed due to the presence of amide group. Similar conclusions can be drawn from the IR spectrum (1037, 1234, 1484, 1625, 1715, 2853, 2924, 3011, 3388 cm^{-1}) of FTW (Fig. 3a). Hence, both wastes contain the molecules with same functionalities.

TABLE-2
ELEMENTAL ANALYSIS DATA (ppm)
IN THE DIFFERENT TEA WASTE SAMPLES

Element	FTW sample	DTW sample
Metal elements		
Sodium	215.21	532.75
Potassium	12140.70	645.90
Calcium	5514.32	10297.89
Strontium	17.20	44.57
Magnesium	1464.86	1789.21
Aluminum	857.12	1231.30
Titanium	18.82	21.70
Chromium	41.14	24.94
Manganese	265.64	718.18
Iron	500.84	451.00
Cobalt	0.930	0.693
Nickel	11.16	0.46
Cupper	12.55	19.63
Zinc	32.07	29.33
Barium	22.31	64.43
Non-metal element		
Phosphorus	1291.72	1606.09
Sulfur	3258.11	2626.55
Silicon	685.60	809.16

^1H NMR spectrum of FTW displays signal at 7.2 ppm due to presence of aromatic protons, signals at 3.59 (m, 1H), 3.49 (m, 2H), 3.41 (m, 1H) attribute to methoxyl functions

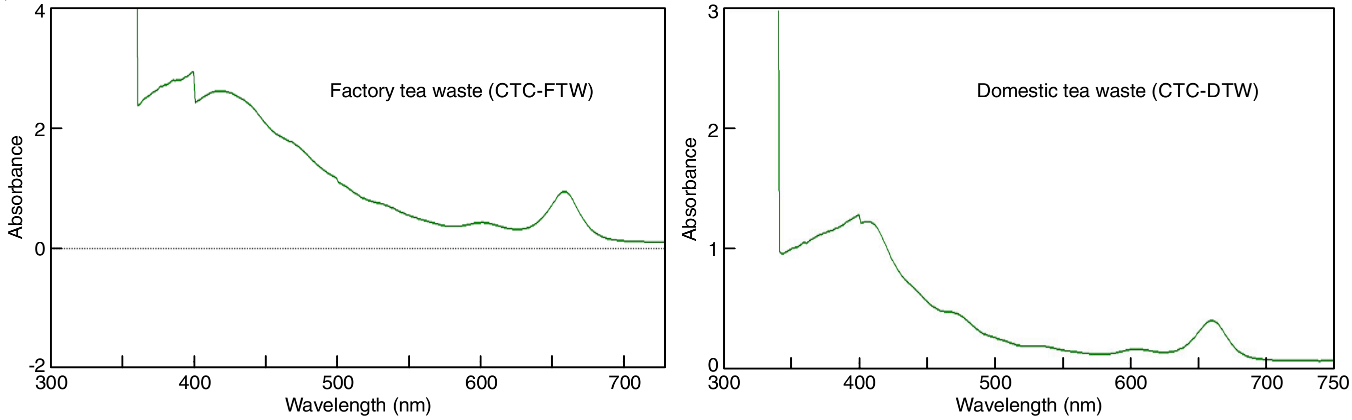


Fig. 2. UV-vis spectra of tea waste samples

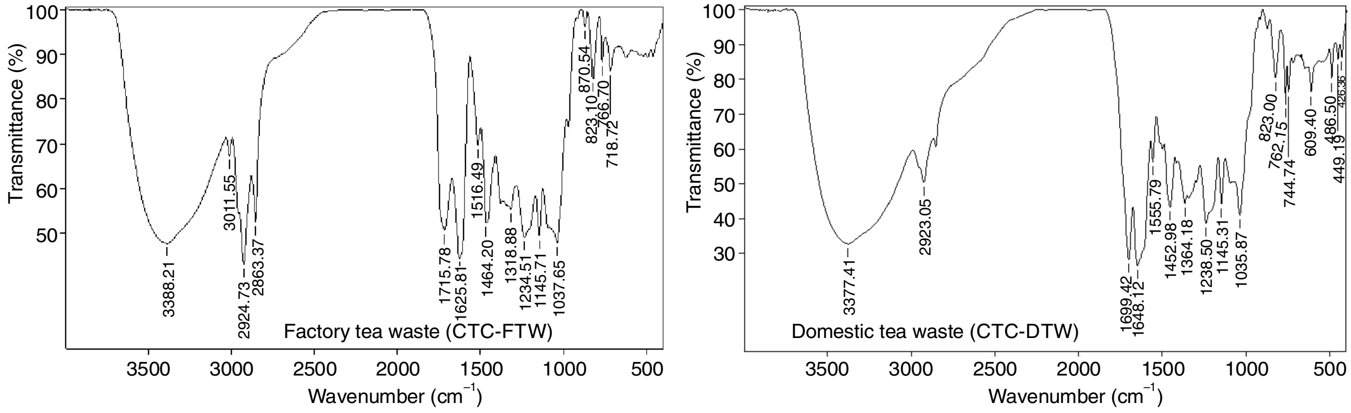


Fig. 3. FTIR spectra of tea waste samples

and signals at δ 1.1 to 1.6 ppm are attributed for aliphatic protons. In contrast, DTW contains more methyl protons (CH_3 or α-protons of carbonyl) rather than methoxyl protons as indicated by the signals at lower ppm (2.0-2.7). In DTW, signal at 5.3 ppm may be attributed to alcoholic, phenolic or olefinic proton. All these contentions receive further support from the ^{13}C NMR. Signals at 127 ppm (in DTW) and 141 ppm (in FTW) clearly indicate the presence of aromatic protons. Signals at δ 76.7-77.3 ppm suggest the presence of carbons attached with oxygen atom. Presence of more aliphatic carbons in DTW is evidenced by the signals at lower value (14-31 ppm) than those in FTW (27-41 ppm). Moreover, all these results are supported by GC and HRMS spectral analysis (Fig. 4). These evidences lead us to suggest that both the tea wastes contain some organic molecules with bonded OH and/or NH_2, carbonyl and methoxyl functions and some aromatic rings.

Morphology: Fig. 5 shows the differential morphology of FTW and DTW samples as conducted by FESEM technique and appears like a large scale mesosphere, which contains multiple closely winded nanoslices.

Orbitrap-HRLCMS analysis: The MS experiments using high resolution Orbitrap liquid chromatography mass spectrometer were carried out to detect the analytes and are listed in Tables 3 and 4 for FTW and DTW samples, respectively.

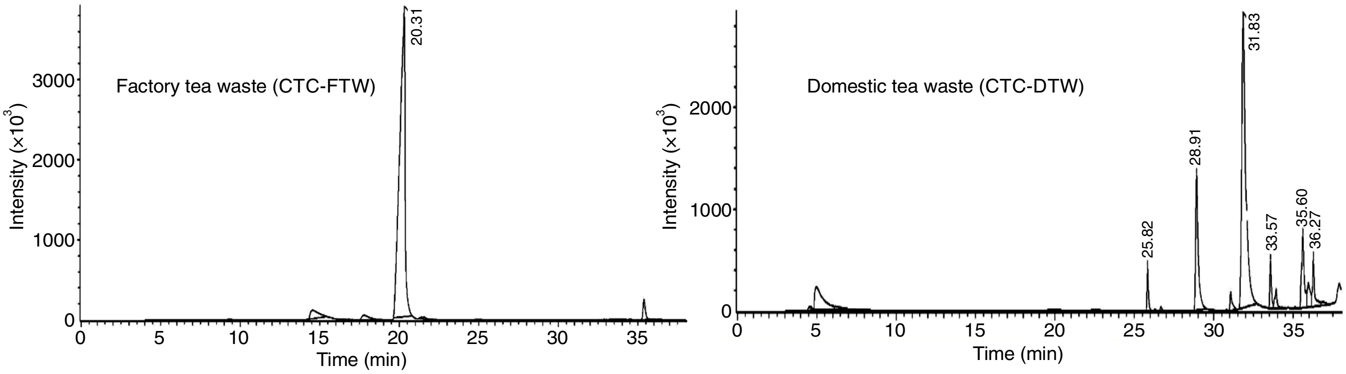
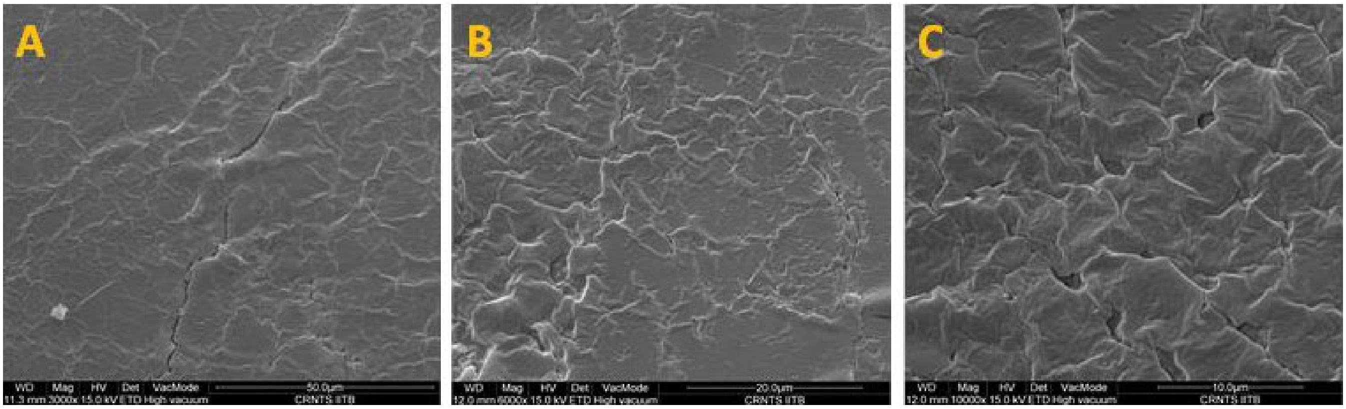
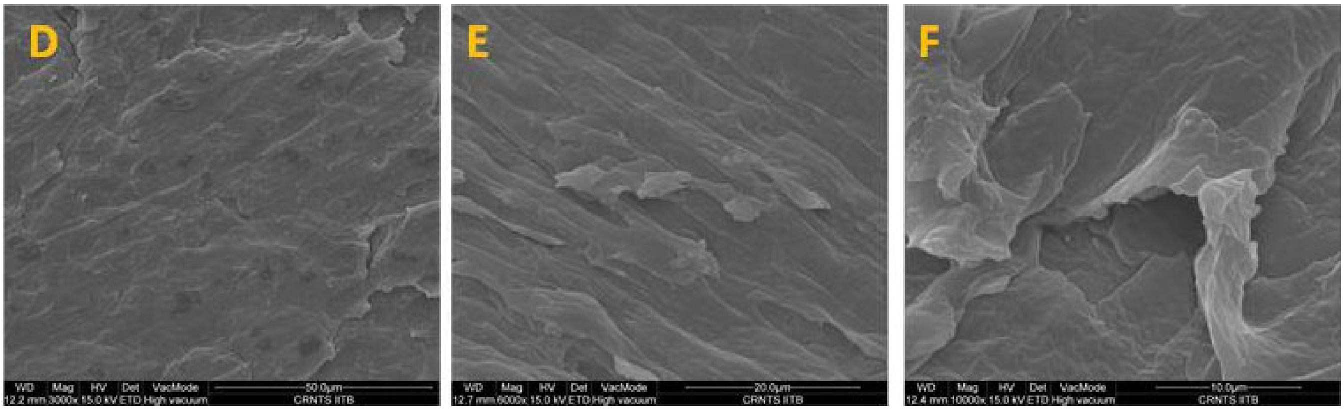


Fig. 4. GC-HRMS spectra of tea waste samples



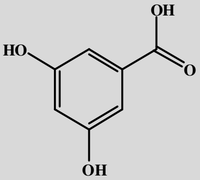
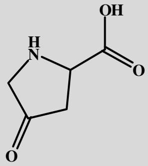
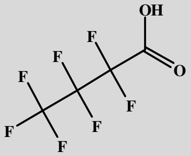
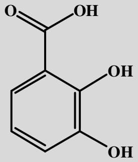
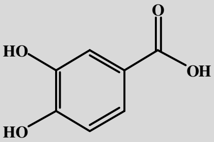
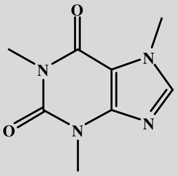
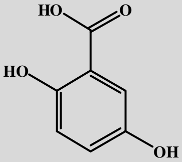
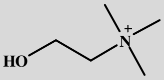
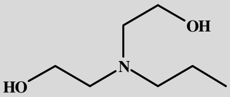
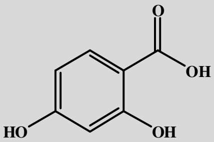
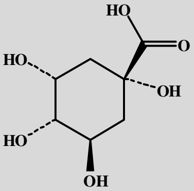
Factory tea waste (CTC-FTW)

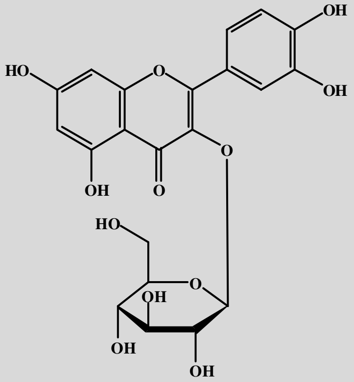
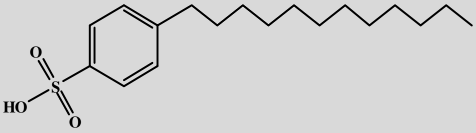
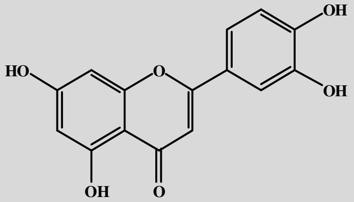
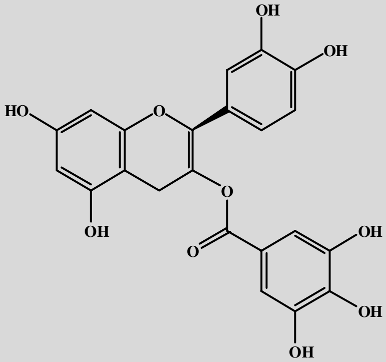
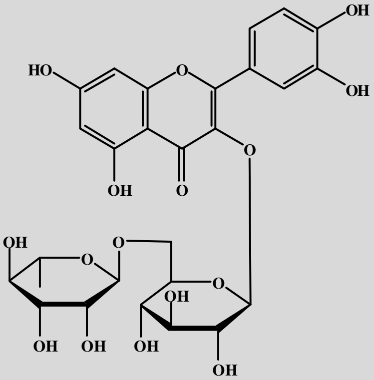
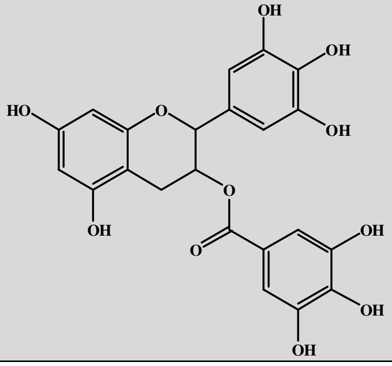


Domestic tea waste (CTC-DTW)

Fig. 5. FESEM images of tea waste samples

TABLE-3
ORBITRAP-HRLCMS DATA CTC-FTW COMPOUNDS

Structure	Name	m.f.	m.w.	Best match (%)
	3,5-Dihydroxybenzoic acid	$C_7H_6O_4$	154.02661	97.1
	4-Oxoproline	$C_5H_7NO_3$	129.04259	97.1
	Perfluorobutanoic acid	$C_4HF_7O_2$	213.98648	96.7
	2,3-Dihydroxybenzoic acid	$C_7H_6O_4$	154.02661	95.9
	Protocatechuic acid	$C_7H_6O_4$	154.02661	95.4
	Caffeine	$C_8H_{10}N_4O_2$	194.08038	94.7
	Gentisic acid	$C_7H_6O_4$	154.02661	94.3
	Choline	$C_5H_{13}NO$	103.09971	94.3
	Triethanolamine	$C_6H_{15}NO_3$	149.10519	94.0
	2,4-Dihydroxybenzoic acid	$C_7H_6O_4$	154.02661	93.9
	D-(-)-Quinic acid	$C_7H_{12}O_6$	192.06339	93.7

	Quercetin-3β–Dglucoside	$C_{21}H_{20}O_{12}$	464.09548	93.6
	4-Dodecylbenzenesulfonic acid	$C_{18}H_{30}O_3S$	326.19157	92.9
	Luteolin	$C_{15}H_{10}O_6$	286.04774	92.8
	Catechingallate	$C_{22}H_{18}O_{10}$	442.09000	91.7
	Rutin	$C_{27}H_{30}O_{16}$	610.15338	91.1
	Epigallocatechingallate	$C_{22}H_{18}O_{11}$	458.08491	91.1

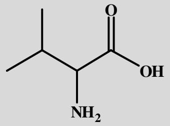
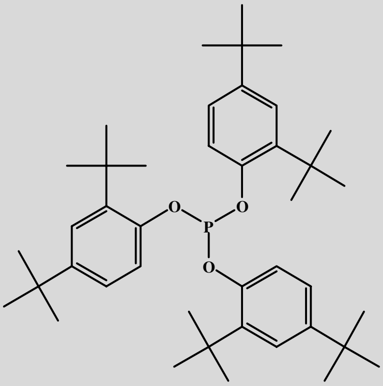
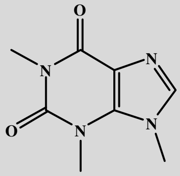
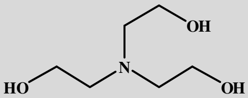
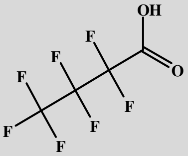
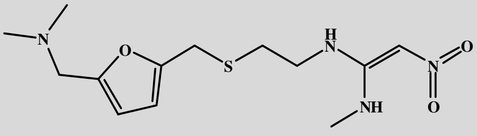
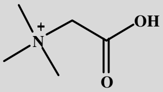
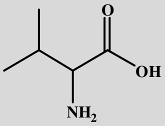
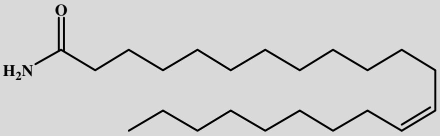
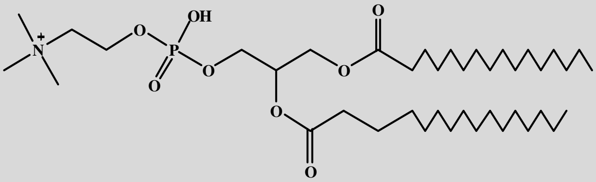
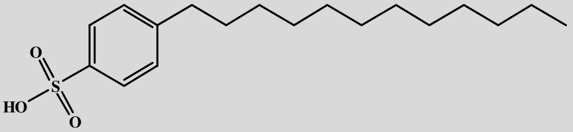
	Valine	$C_5H_{11}NO_2$	117.07898	90.9
	Irgafos 168	$C_{42}H_{63}O_3P$	646.45148	90.5
	Isocaffeine	$C_8H_{10}N_4O_2$	194.08038	90.5

TABLE-4
ORBITRAP-HRLCMS DATA CTC-DTW COMPOUNDS

Structure	Name	m.f.	m.w.	Best match (%)
	Triethanolamine	$C_6H_{15}NO_3$	149.10519	96.8
	Perfluorobutanoic acid	$C_4HF_7O_2$	213.98648	95.4
	Ranitidine	$C_{13}H_{22}N_4O_3S$	314.14126	93.3
	Betaine	$C_5H_{11}NO_2$	117.07898	92.9
	Valine	$C_5H_{11}NO_2$	117.07898	90.6
	Erucamide	$C_{22}H_{43}NO$	337.33447	90.3
	DL-Dipalmitoylphosphatidyl choline	$C_{40}H_{80}NO_8P$	733.56215	90.2

	4-Dodecylbenzene sulfonic acid	$C_{18}H_{30}O_3S$	326.19157	90.2

Conclusion

The chemical profiling of crush, tear, curl (CTC)-factory tea waste (FTW) as well as the domestic waste (DTW) have been documented by using elemental and spectroscopic methods. It appears that FTW sample contains greater amount of non-metal element such as sulfur, phosphorus and metal element potassium, calcium, iron along with some other elements in trace, whereas DTW sample contains non-metal element sulfur and, metal calcium and manganese in greater amount. UV-vis analysis of both the wastes indicates that these two wastes contain highly conjugated compounds. IR spectrum of DTW clearly indicates the presence of bonded OH function and/or NH_2 function. The ^1H NMR of FTW sample revealed the presence of proton attached to carbon frame, which is attached to hetero-atom like oxygen, nitrogen. Similarly DTW sample contains olefinic proton which is reflected from the ^1H NMR signal at δ 5.34 ppm. However, DTW mainly contains aliphatic proton. Since, the present work is the first report on the chemical profiling of CTC-FTW and CTC-DTW of eastern Sub-Himalayan region; thus, a systematic treatment from the large amount of tea waste could be a potential source of various bioactive ingredients such as raw material in caffeine industry, compost in horticulture and agricultural industries, dietary ingredients in poultry, fishery and piggery farms, *etc.*

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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