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Biochemical characterization of different tea

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ABSTRACT:
Tea is a kind of medicinal beverage. According to different types of manufacturing processes different types of teas are available like Green, Black, Oolong and White tea. The types of teas are differentiated according to the processing parameters (oxidation/fermentation)which can change its active metabolite content. To characterize the secondary active metabolic therapeutic values/components biochemical characterization of differentially manufactured teas are needed. Which could be done with different bioinstrumentation techniques. Our study includes various analytical techniques, like FTIR analysis, LC-MS analysis, thin-layer chromatography (TLC), Spectrophotometric and Spectrofluorimetric assays and scan to characterize the differences among these different kind of manufactured tea varieties. In some cases the active metabolite content may differ from the fresh leaves also. Mainly total polyphenol and flavonoids are the main secondary active metabolites which vary in different types of tea and could be correlated with main therapeutic action like antioxidant potential.

INRODUCTION:-
Tea (*Camellia sinensis*) is universally accepted beverage. The characteristics of the tea can vary depending on its region of origin.According to manufacturing processes mainly four different types of teas are there-white tea, green tea, oolong tea and black tea which are made from the leaves of the same species *Camellia sinensis* (Wang et al., 2022). The plant variety, the weather conditions, tea type and soil contribute to the final taste of the tea. The significant differences of tea type develop in the processing (varying levels of oxidation/fermentation) of the leaves. White and green teas are heated early on and not allowed to oxidise, oolong tea is part oxidised and black tea is oxidised (Salman et al., 2022). After white tea leaves are harvested, the leaves are withered and then dried immediately using natural sunlight, heat vents, or drying chambers. This helps prevents oxidization, giving the tea a light flavor and color and preserving some of the prized benefits of tea, such as antioxidants. For green tea, young leaves of the plant are harvested, withered, steamed, or pan-fried and then dried (Maulana et al., 2020).Oolong teas are bruised, briefly oxidised, pan fried and Black teas are rolled, fully oxidised, fired and stored. In all the cases the main secondary active metabolites like total polyphenol contents (catechins), flavonoids are changed. In White and green tea catechins like epigallocatechin gallate is prevalent but due to oxidation by polyphenol oxidase (PPO) the catechins are turned to tannins like theaflavin (TF) and thearubigins (TR) which are prevalent mostly in oolong and black tea (Musial et al., 2020).
Quercetin is also an important secondary metabolite and bioactive flavonoid which varies in different types of manufactured tea (Peng et al., 2015).All these secondary metabolites are responsible for antioxidant, antimicrobial and anticancer activities (therapeutic values) of all the tea. Biochemically different types of teas are also characterized by Marzuki et al., 2017 by spectrophotometric analysis; LC-MS and TLC by Ligor et al., 2008; FTIR analysis by Kokalj et al., 2014.
Our aim of work is to characterize the secondary active metabolic therapeutic values/components (biochemical characterization) of differentially manufactured teas with different bioinstrumentation techniques. Our study includes various analytical techniques,

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including FTIR analysis, LC-MS analysis, thin-layer chromatography (TLC), Spectrophotometric and Spectrofluorimetric assays to characterize the differences among these different kind of manufactured tea varieties.

MATERIAL AND METHODS:

All four types of tea like White, Black, Green and Oolong teas were acquired from Mahabodhi tea Company, Kolkata for the same garden of Castleton from where the fresh leaves were collected.

FTIR analysis: FTIR has been used to determine level of flavonoids in different types of tea leaves. The spectral scan was done between 200-500nm wavelength. The excitation wavelength for Quercetin was given at 360nm whereas the emission peak for Quercetin was observed at 460nm.The same trend was observed with different types of tea ensuring the presence of Quercetin, Kokalj et al., 2014(Fig 1 and Table 1).

LC –MS: It was done from NIPER, Kolkata according to the method of Shevchuk et al., 2018 (Fig 2 and Table 2)

Thin layer chromatography-TLC assay was done in silica plate with (by using solvents butanol: acetone: acetic acid-5:5:3 v/v) under UV radiation (UV light 254nm and UV 366 nm) and specific colorimetric agents like Iron chloride and ethanol reagent (Pramiastuti and Joraho, 2020) after that densitometric scan was done (Kamal et al., 2022)(Fig 3a. 3b, 3cand Table 3a and 3b)

GC-MS analysis: An HP 7890A GC instrument integrated with an Agilent 5975C MSD mass spectrometer The GC oven temperature was held at 50°C for 5 min, increased to 210°C at a rate of 3°C/min, maintained at 210°C for 3 min, and finally increased to 230°C at 15°C/min. The mass spectrometer conditions were- ionisation energy, 70 eV; ion source temperature, 230°C; quadrupole temperature, 150°C; quadrupole mass spectrometer scan range, 30–500 atomic mass units (amu); solvent delay time, 2.8 min (Lee et al., 2013)(Fig 4 and Table 4).

Spectrophotometric scan analysis:U-2900 Spectrophotometer was used for the spectral scan of tea samples with a specific standard that is quercetin. The spectral scan was run from 500 nm till 200 nm with the scan speed of 400nm/min, sampling interval of 0.5nm and slit width of 1.50nm.The absorption peaks are around 240nm and 440nm for each of the samples as well for the standard (Atomssa and Ghoslap, 2015) (Fig 5 and Table 5).

Spectrofluorimetric analysis: :F-7000 FL Spectrofluorimeter was used for the for different tea samples with a specific standard that is quercetin**.** 360nm was taken as the excitation wavelength for fluorimetric scan for each of the tea samples. The maximum emission peak of the standard came around 538nm whereas for the tea samples the maximum emission peaks were around 535nm (Khanchi et al., 2007. This trend ensures the presence of Quercetin in different types of tea (Fig 6 and Table 6)

Total Polyphenol content: The polyphenol content of tea samples was measured with 7%Na_2CO_3 and FC reagent according to Anesini et al.,2008.The % polyphenol content was measured spectrophotometrically at 715nm (Fig 7).

Total Flavonoid Content: : The flavonoid content of tea samples was measured with 75ul of 5% Na_2CO_3 ,150ul of 10% $AlCl_3$ and 750 ul of NaOH according to Zhisen et al.,1999. The % flavonoid content was measured spectrophotometrically at 510nm (Fig 8)

Antioxidant potential: Antioxidant potentials are calculated according to the reduction of 0.004% DPPH and recording the O.D at 517nm according to Shyam Choudhury et al., 2015(Fig 9)

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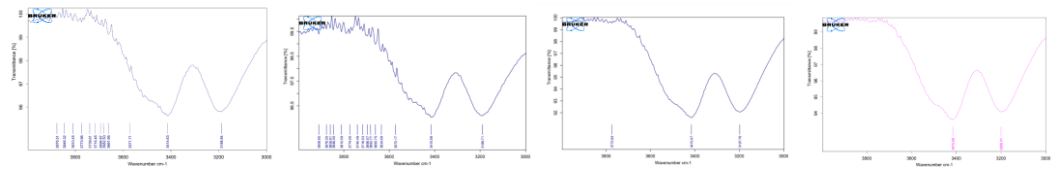
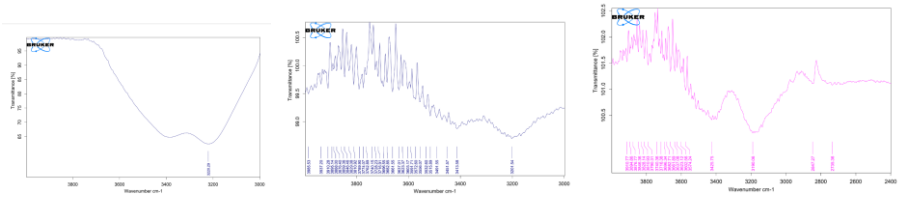
RESULTS AND DISCUSSION:



a.BLACK TEA b.OOLONG TEA c.GREEN TEA d. WHITE TEA



e. FRESH TEA LEAF f. QUERCETIN g.EPIGALLOCATECHIN GALLATE

Fig 1: FTIR analysis of different tea (a.Black tea, b.Oolong tea, c.Green tea, d.White tea,e.Fresh tea leaf, f.Quercetin.g.Epigallocatechin gallate

Table 1a.

Tea Type	Range (cm^{-1}) of wavelength	Transmittance (%)
White Tea	3400-3200	>99%
Black Tea	3414.63-3188.84	98%
Oolong Tea	3415.98-3189.71	>97.5%
Green Tea	3415.67-3197.76	95%
Quercetin	3600-3200	>99%
Fresh leaf (low altitude)	3221.21-3400	>95%
Fresh leaf (high altitude)	3414.54-3202.04	>100
Epigallocatechin Gallate	3425.75-3109.06	>100

Tea type	Transmittance wavelength (cm^{-1})	Transmittance (%)
Epogallo catechin gallate	3425.75	100.4
	3190.06	100
	2847.27	101
	2735.36	101.3
Black tea	3414.63	95.5
	3186.84	96

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White tea	3415.59	95.5
	3200.41	94.5
Green tea	3773.89	99.2
	3415.67	91.9
	3197.76	92.2
Oolong tea	3415.98	95.5
	3198.71	95.3
Fresh tea leaf	3220.29	63
Quercetin	3985.53	99.5
	3937.2	99.8
	3774.07	99.5
	3463.58	98.9
	3451.57	99
	3201.94	98.5

Table 1b.

Table 1a and 1b: FTIR analysis of all the tea varieties

Table 1a and Fig 1 shows that for white tea has broad higher transmittance within the frequency range from 3400-3200 nm showing stretching of O-H bonds, then black tea, oolong tea and then green tea, but specifically the phenolic bond stretching of the epigallocatechin gallate (polyphenol catechins) are almost similar for fresh tea leaves mainly in high altitude grown tea >100%. In case of black tea (Brza et al, 2020) and green tea extracts (Senthilkumar and Thirumal,2014) similar types of transmittance pattern of FTIR spectral scan were shown.

Table 1b indicates that within wavelength 3550 - 3200 cm^{-1} (indicates stretching of alcoholic/phenolic O-H group) which are lower in green tea (91.9% at 3415.67 cm^{-1}); at 3770 cm^{-1} stretching indicate sharp alcohol group stretching which is only available in green tea as quercetin, at around 3190 cm^{-1} weak alcohol group stretching is available in all tea varieties except white tea and fresh tea leaves, and highest in black tea 96% at 3186.84 cm^{-1}

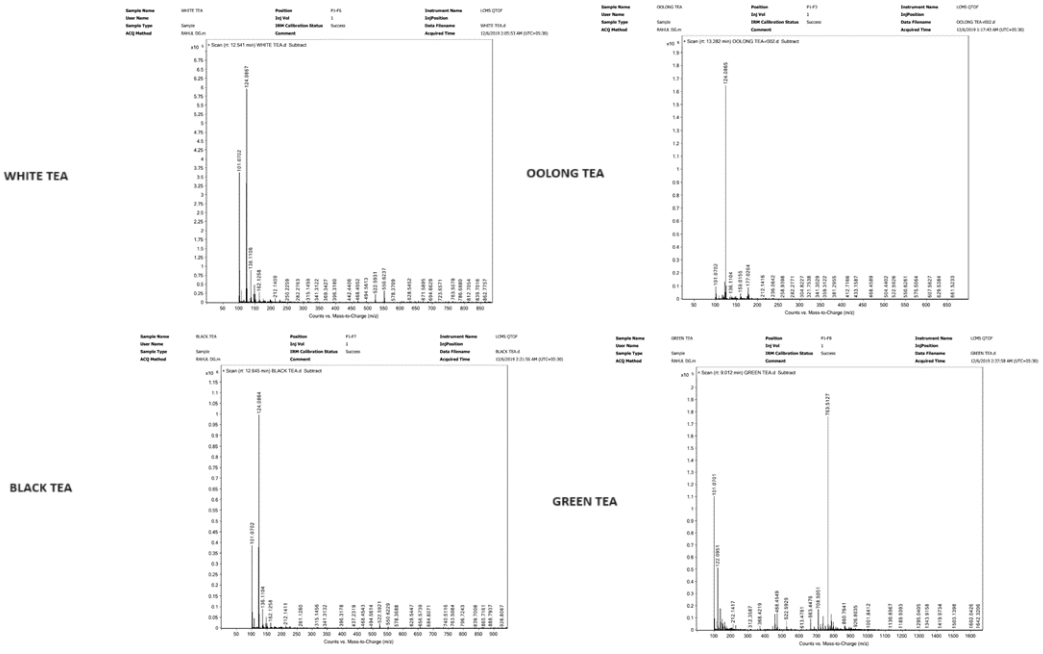

Fig 2: LC-MS analysis

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SL.NO.	Counts Range	Counts	m/z Ratio Range	Maximum m/z	
1.	0-6.75	6.5	100-850	124.0867	
2.	0-2.2	2.1	100-900	101.0701	
3,	0-1.15	1.1	100-900	124.0864	
4.	0-3.6	3.4	100-900	195.0869	

Table 2a LC-MS data analysis for White tea

SL.NO.	Counts Range	Counts	m/z Ratio Range	Maximum m/z
1.	0-6	1.58	50-990	104.1064
2.	0.05-1.25	1.1	50-990	195.0866
3.	0-8.25	7.25	50-990	124.0868
4.	0-1.15	1	50-990	124.0864

Table 2b LC-MS data analysis for Black tea

SL.NO.	Counts Range		Counts	m/z Ratio Range	Maximum m/z
1.	0-1.05		0.95	50-650	104.1066
2.	0-1.9		1.7	50-650	195.0870
3.	0-8		7	50-650	312.3609
4.	0-1.65		1.45	50-650	101.0702
5.	0-3		3	50-650	101.0703
6.	0-1.9		1.68	50-650	124.0865

Table 2c LC-MS data analysis for Oolong tea

SL.NO.	Counts Range	Counts	m/z Ratio Range	Maximum m/z
1.	0-3.8	3.4	100-1600	104.1060
2.	0-1.4	1.2	100-1600	195.0868
3.	0-9	8	100-1600	124.0866
4.	0-3.2	2.8	100-1600	124.0866
5.	0-2	1,8	100-1600	763.5127

Table 2d LC-MS data analysis for green tea

LC-MS data is shown in Table 2a, 2b, 2c and 2d and Fig 2 states that the characteristic m/z ratio of epigallocatechingallate at around 120 is found (124.08) in oolong tea that is 8 (Ungrala et al., 2020)

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Fig 3a. Thin layer Chromatography with Iron-chloride-Ethanol reagent coloring agent

LANE	VOLUME%	VOLUME	HEIGHT	AREA
A Epigallocatechin gallate	100	94329607	56075	3036
B Black tea	24.393	23009627	55945	660
C Oolong tea	76.134	71816654	56145	2295
D Green tea	153.896	145169246	57321	3480
E White tea	29.468	27796946	58006	504

Table 3a. Densitometric scan of TLC plate of Fig 3a

Table 3a and Fig 3a show that the catechin contents are highest in Green tea by comparing with the volume % of the standard epigallocatechin gallate with colorimetric spot analysis

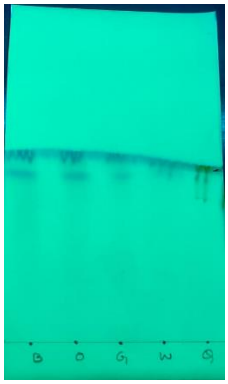

Fig 3b. Thin layer Chromatography under UV 366 nm

Fig 3c. Thin layer Chromatography under UV 254 nm

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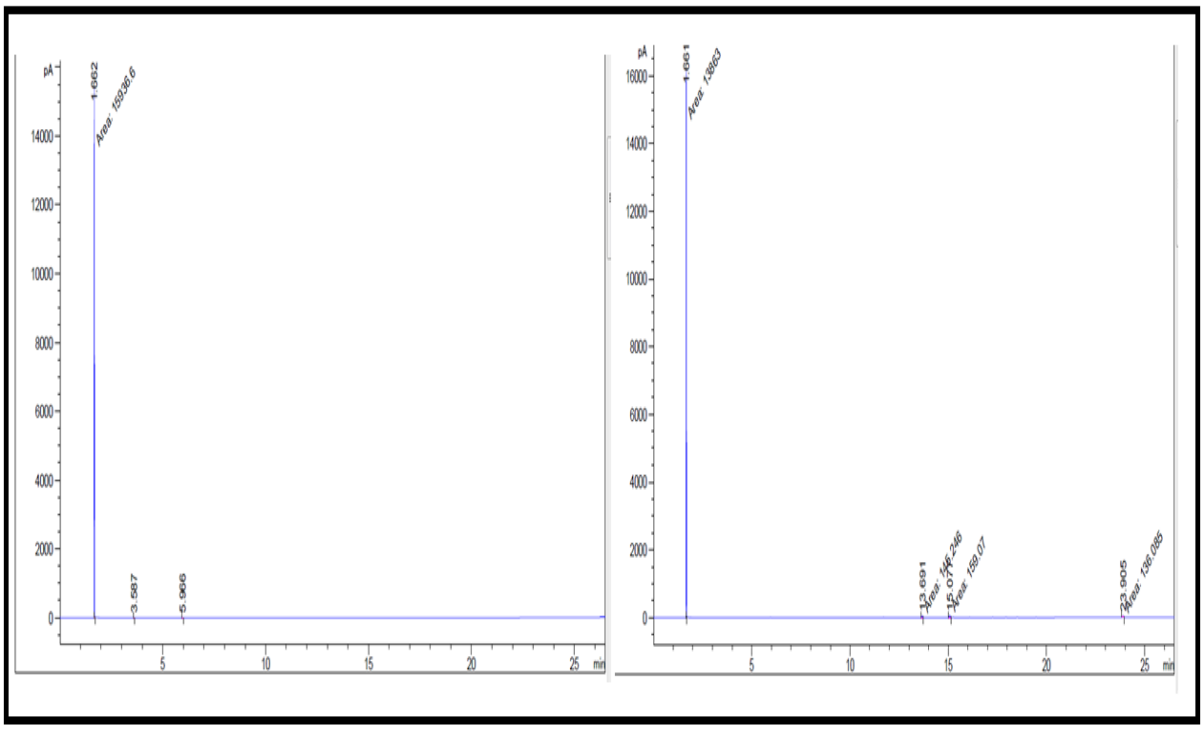
Table 3b.Rf value table

Samples	TLC Plate	Rf-I(cm)	Rf-II
Quercetin	Colour agent	0.81	
White Tea		0.81	
Green Tea		0.81	
Oolong Tea		0.81	
Black Tea		0.80	
Quercetin	UV 254	0.89	
White Tea		0.89	0.92
Green Tea		0.89	0.92
Oolong Tea		0.89	0.92
Black Tea		0.89	0.92
Quercetin	UV 366	0.44	
White Tea		0.44	
Green Tea		0.44	
Oolong Tea		0.44	
Black Tea		0.44	

Table 3a and 3b and Fig 3a and 3b shows that green tea shows higher quercetin content and R_f values indicate that all the characteristics bands are present in all the tea varieties.Wang et al., 2008 have established the same types of data of catechins with black and green tea samples.



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.661	MM	0.0143	1.38630e4	1.61970e4	96.92101
2	13.691	MM	0.0577	145.24640	41.95574	1.01546
3	15.077	MM	0.0549	159.07043	48.25845	1.11211
4	23.905	MM	0.0543	136.08499	41.78861	0.95141
Totals :				1.43034e4	1.63290e4	

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.662	MM	0.0171	1.59366e4	1.54967e4	99.98710
2	3.587	BV	0.0199	4.64272e-1	3.52554e-1	0.00291
3	5.966	BB	0.0290	1.59111	8.25933e-1	0.00998
Totals :				1.59386e4	1.54979e4	

Fig 4

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Table 4 : Analysis of Gas Chromatography

Tea Variety	Peak Amplitude (pA)	Area %
Green Tea	18936.6	99.98
Black Tea	13863	96.921

Fig 4 and Table 4 shows that Green tea has higher volatile contents (99.98%). Lee et al., 2013 had documented such volatile compounds with Gas chromatographic technique.

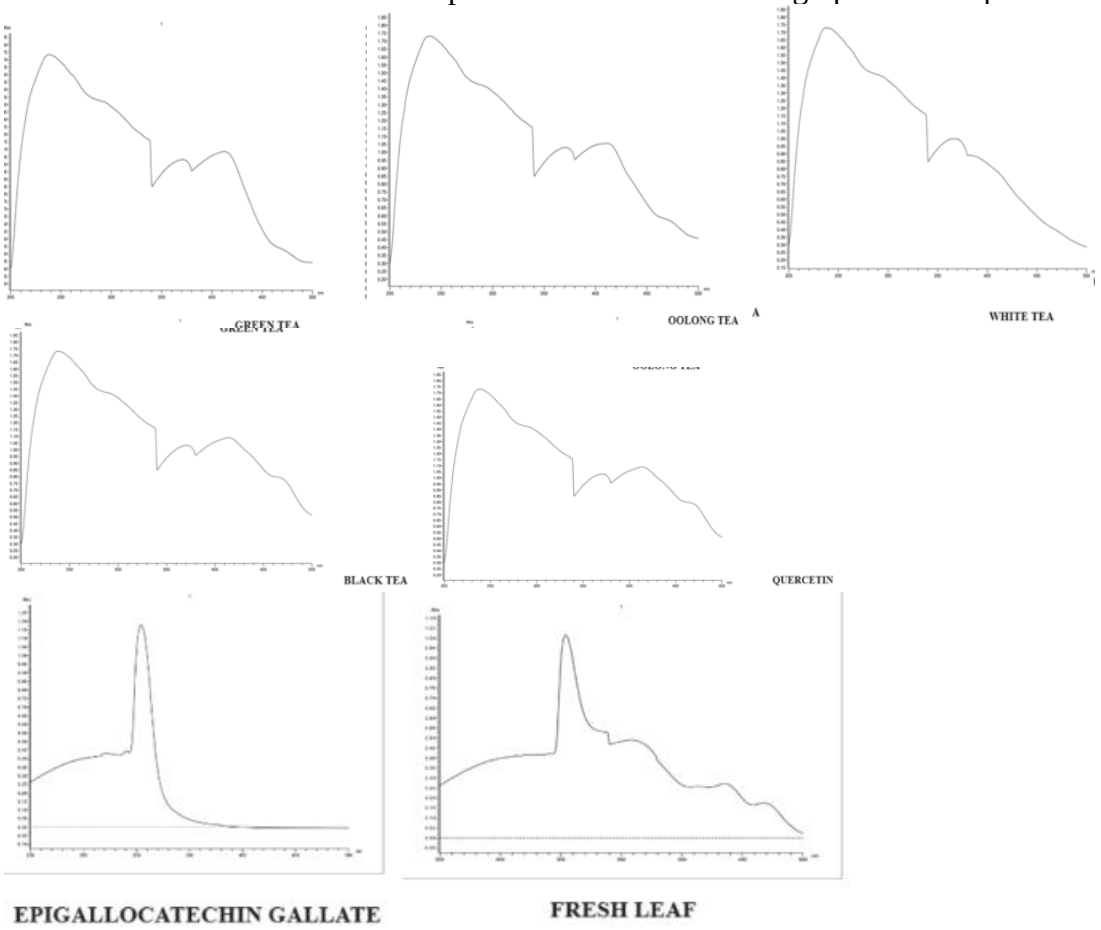

Fig 5. Spectrophotometric scan from 200 to 500 nm

Table 5: Analysis of Spectrophotometric Scan

Quercetin (standard)	Sl.no.	0.D	Wavelength (nm)
	I	1.71	250nm
	II	1.0	360nm
	III	1.12	425nm
White Tea			
	I	1.71	250
	II	0.91	360
Black tea			
	I	1.71	250nm
	II	1.4	310nm
	III	1.0	360nm
	IV	1.06	415nm
	V	0.70	470nm
Oolong Tea			

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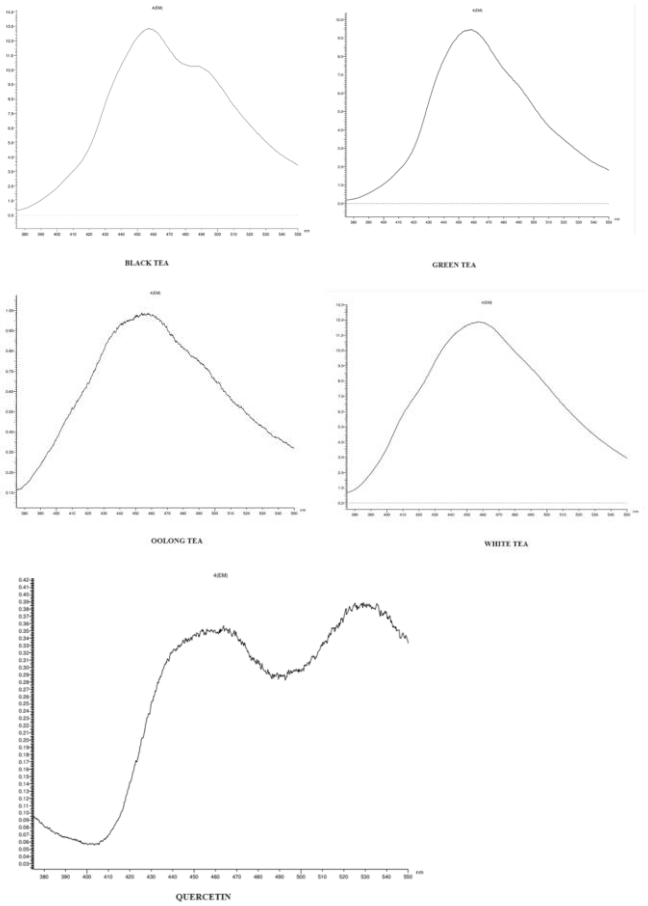
	I	1.71	250nm
	II	1.11	330nm
	III	0.94	360nm
	IV	0.97	410nm
	V	0.54	450nm
Green Tea			
	I	1.72	250nm
	II	1.15	340nm
	III	0.96	360nm
	IV	1.05	410nm
	I	1.17	300nm
Fresh leaf	I	1.00	300
	II	0.5	360

Fig 5 and Table 5 states that the characteristic band at 250 nm of quercetin is present in all the tea varieties except fresh tea leaf and almost similar that is 1.71-1.72; but 360 nm characteristic wavelength of absorption is lowest in fresh leaf that is around 0.5



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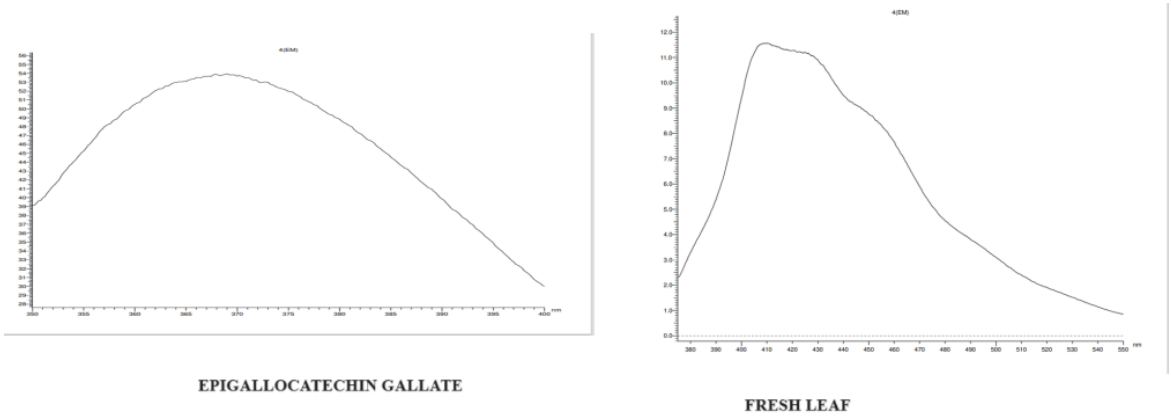

Fig 6 Spectrofluorimetric scan

Table 6: Analysis of spectrofluorimetric scan

SAMPLE	WAVELENGTH (nm)	Absorbance x 10^{-1}
Quercetin	I-455	0.345
	II-528	0.38
Epigallocatechin gallate	I-370	54.0
Fresh leaf	I-410	11.5
	II-430	11
Oolong tea	I-452	0.95
Black tea	I-460	12.5
	II-495	10
Green tea	460	9
White tea	455	11.5

Fig 6 and Table 6 show that the higher content of quercetin similar bands are available for white tea which is around 11.5

Spectrophotometric and spectrofluorimetric scan of Iranian teas were done by Khanchi et al., 2007.

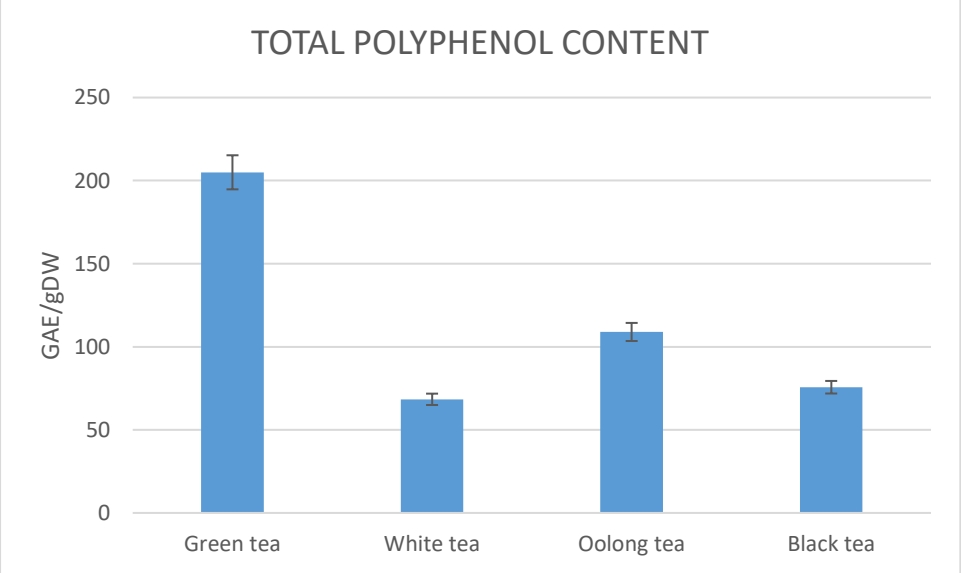

Fig 7: Total polyphenol content

Fig 7 shows the highest total polyphenols in green tea (210 ±0.01mg GAE/gDW), then Oolong tea (108.1±0.01mg GAE/gDW), black tea (65.2±0.01mg GAE/gDW) and at last white tea

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(24.3±0.01mg GAE/gDW)-same type of polyphenol content pattern was reported by Zhao et al., 2019.

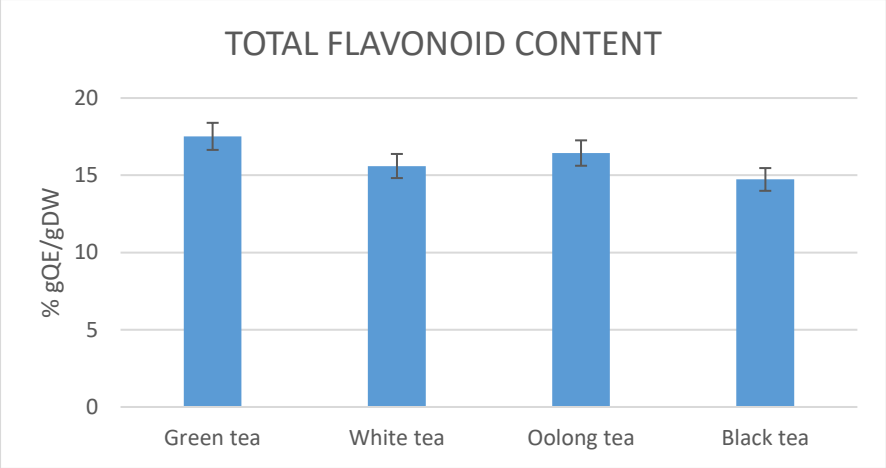

Fig 8: Total flavonoid content

Fig 8 also shows highest flavonoid content in green tea (18.1±0.05% gQE/gDW) but lowest in black tea (14.2±0.05% gQE/gDW),the similar trend was also reported by Lohadi and Putri, 2019.

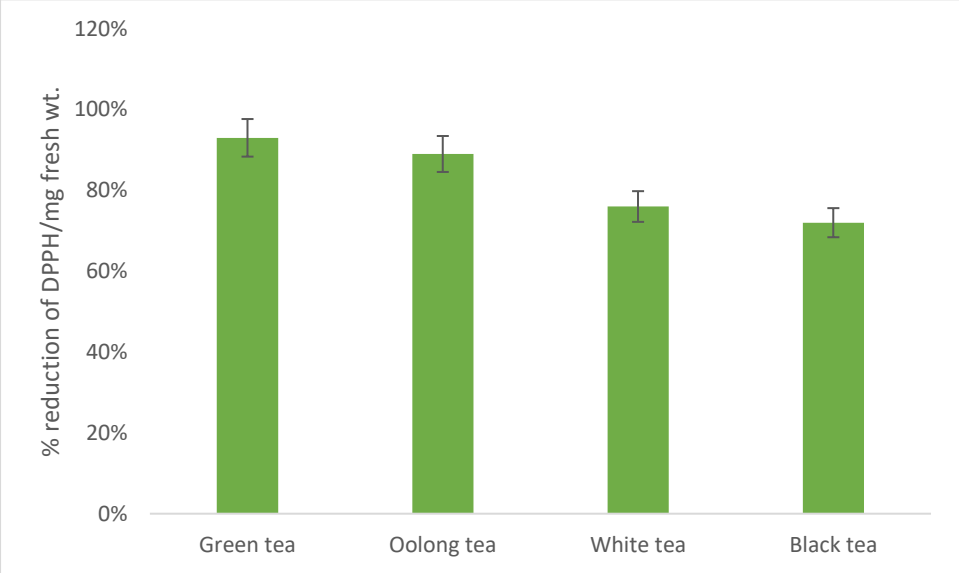

Fig 9. Antioxidant potential of all tea varieties

Fig 9 corroborates the flavonoid and polyphenol data (Fig 7, Fig 8) and other data (Fig 3a, Table 3a, Fig 4 and Table 4)that they contribute for the highest antioxidant potential of green tea that 93.1% which is highest among all. Same type of trend also was followed by Shyam Choudhury et al., 2015

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