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FT-IR analysis and correlation studies on the antioxidant activity, total phenolics and total flavonoids of Indian commercial teas (*Camellia sinensis* L.) - A novel approach

S.R. Senthilkumar[1], T. Sivakumar[1], K.T. Arulmozhi[2*] and N. Mythili[3]
[1]Department of Botany, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India
[2]Physics Wing (DDE), Annamalai University, Annamalainagar 608 002, Tamil Nadu, India
[3]Department of Physics, Mohamed Sathak Engineering College, Kilakarai-623806, Ramnad, India
arulsheelphy@gmail.com

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Abstract

Fourier Transform Infrared (FT-IR) spectra were recorded to analyse the phytochemicals in six commercial green tea (Camellia sinensis L.) samples acquired from diverse locations in India. Main focus was made on the absorbance (%) of selected prominent vibrational bands relevant to the chemical compounds with antioxidant properties (polyphenols, flavonoids). The colorimetric data of the green tea samples such as antioxidant activity assayed by 1,1-diphenylpicrylhydrazyl (DPPH) – radical scavenging activity (RSA) and ferric reducing antioxidant power (FRAP) assay and total phenolic content (TPC) assayed by Folin-ciocalteu method and total flavonoids (TF) assayed by aluminium chloride method were used in the present study (Authors' work communicated elsewhere). A novel attempt has been made to correlate the FT-IR data and the colorimetric data of the green tea samples without using advanced chemometric procedures. Very interestingly excellent correlations were observed among the studied parameters with higher values of correlation coefficients (R^2 > 0.9). This implies that FT-IR analysis may be used in a simple way to rapidly estimate antioxidant potentials of plant extracts.

Keywords: FT-IR, Green tea, Antioxidant activity, Total phenolics, Total flavonoids, Correlation analysis.

Introduction

Next to water, tea (*Camellia sinensis*) is the most preferred and favourite beverage all over the world. The popular causes for this usage are its flavour and taste. In recent years tea has gained more attention due its beneficial health effects. Tea is generally consumed in different forms and are categorized according to the degree of fermentation adopted during the production process. Green tea is unfermented (0%), while black tea is fully fermented (100%). Partial fermentation results in white tea (20 ~ 30%) and Oolong tea (30 ~ 60%). This makes different compositions of phytochemicals present in them and as such different teas exhibit different chemical reactivities. There are hundreds of phytochemical compounds in tea. The general composition of tea is, fresh leaves containing 75 ~ 80% of water and 20 ~ 25% of stem compounds. The commonly known phytochemicals in tea are: polyphenols, proteins and amino acids, alkaloids (caffemine), vitamins (C, E), mineral compounds (potassium, fluoride, aluminium, etc.), trace elements (zinc, magnesium, folic acid), fats, pigments, fragrance elements[1].

The polyphenols are primarily flavonoids, with flavonols as the most abundant subcategory within these. The flavonols are commonly known as catechins which are comprised of aromatic rings and hydroxyl groups possessing strong antioxidant groups possessing strong antioxidant activities[2]. Flavonoids are polyphenols with a 15 carbon skeletal unit and the flavonol in its subgroup. Thus catechins are flavonol flavonoid polyphenols. Based on their chemical structure catechins that contain three hydroxyl groups in the bring (position 3′, 4′, 5′) are called gallocatechins while gallic acid substitution in position 3 of the ring is characteristic of catechin gallate[3].

Sidduraja and Manian[4] have suggested that the scavenging potential of phenolic substances might be due to the active hydrogen donating ability of the hydroxyl substitution. Hagerman *et al*[5] have also explained that high molecular weight and the number and proximity of aromatic rings and nature of hydroxyl group substitution are more important for the radical scavenging activity rather than their specific functional groups[6]. Thus the antioxidant activity depends on the tea verity as well as the number of hydroxyl groups in the catechin. Further the values of antioxidant activity varied 2–3 times due to tea grades as well as cultivation, climatic conditions, production process and storage conditions. There exist several assays to evaluate the antioxidant activities, total phenolic content, total flavonoids[7–10]. The recent technique used to precisely analyse the catechins is the gas chromatography-mass spectrometer (GC-MS) method[11,12].

Several reports are available on correlation studies of antioxidant activity *versus* total phenolics and total flavonoids[1,10,13–17]. A linear correlation may exist between the antioxidant activity and the total phenolics and total flavonoids. Excellent correlation yields high value of correlation coefficient ($R^2 > 0.9$) while poor correlation means ($R^2 < 0.5$). When R^2 value is much smaller it implies that the phenolic compounds are not a major contribute to the antioxidant activity. Correlation and prediction of phytochemicals and antioxidant activity of different plant extracts using FT-IR data linked with chemometric models have been reported by several authors[18–24]. Even though the chemometric models are advantageous over the conventional colorimetric methods of estimating antioxidant activities, they require vast amount of input data and the use of advanced softwares like principal component analysis (PCA), artificial neural network (ANN) etc. In the present work, a novel attempt has been made to utilize only the FT-IR absorbance (%) of three selected vibrational bands of green tea samples to establish their correlation with the phytochemicals and antioxidant activities determined by conventional methods.

Materials and methods

About 10 g of dried green tea samples in each variety were finely ground separately in an electric blender and sieved with muslin cloth. The fine powder was dissolved in 100 mL of methanol and the extracts were obtained by maceration (48 h). The solvent was concentrated at temperature around 40°C and the resulting dry extracts, in fine powder form, were used for all studies[25]. The percentage yield was about 6% of the green tea material taken initially.

Characterization techniques: The FT-IR spectra of the green tea samples were recorded in Perkin Elmer Spectrum Version 10.03.09 using KBr pellete method. The antioxidant potentials were estimated through the DPPH assay. The total phenolic contents (TPC) were evaluated by the Folin-ciocalteu method and the total flavonoids (TF) by aluminium chloride method. Correlation studies were carried out using the statistical software SPSS Version 17.0.

Results and discussion

The FT-IR spectra of the six green tea samples are shown in Figures 1a–1f. The vibrational band assignment for the prominent peaks and the chemical compounds identified are provided in Table-1. The absorbance (%) of the selected vibrational bands derived from the FT-IR spectra of the green tea samples are presented in Table-2. In this table, the vibrational frequencies that are considered important, pertaining to the present study are given in bold numbers. Since the absorbance data corresponding to the bands around 1448 and 1113 cm^{-1} did not contain any useful information, they are not considered for correlations studies. For all the tea samples, the percentage of absorbance (100-T%) of the vibrational bands considered in the correlation analysis are provided in Table-3, along with the colorimetric data. These data are subjected to bivariate correlation analysis to establish the mutual correlation among them. The Pearson's linear correlation coefficient (R) matrix thus obtained is presented in Table-4.

Table-1: Infrared vibrational bands of six green tea samples.

Wave number (cm^{-1})	Vibration band/group	Chemical compound	Reference
3270 ~ 3320	O–H stretch, H–Bonded	Phenols, alcohols	26–29
2946	C–H stretch (asym.)	Alkanes	28
	O–H stretch	Carboxylic acid	30
2833	C–H stretch (sym.)	Alkanes	27,31
1629 ~ 1663	C=O stretch (carbonyls)	Flavonoids	32
		Polyphenols, catechins	33
	C=C stretch	Aromatics	34
1449	C–C stretch (in ring)	Aromatics	27,32,35
1239	C–N stretch	Aliphatic amines	32,36
1113	C–O stretch	Alcohols, esters, carboxylic acids	35
1014 ~ 1019	C–O stretch	Alcohols, esters, carboxylic acids	33,35
	C–N stretch	Aliphatic amines	36
	C–OH stretch	Secondary alcohols	37

Table-2: FT-IR absorbance (%) of selected vibrational bands of the green tea samples.

Vibrational band (cm^{-1})	Absorbance (%)					
	MOON	TAN	GT	TET	KOL	ASS
3270 ~ 3320*	34.11	32.23	27.56	29.25	31.34	24.20
1629 ~ 1663	22.36	19.18	12.34	12.05	10.68	4.52
1448 ~ 1450	12.39	12.20	15.48	13.52	15.81	15.46
1113 ~ 1148	12.71	13.02	12.32	11.31	9.83	12.10
1014 ~ 1019	23.06	38.71	52.61	52.53	70.67	75.62

*Data in bold numbers are used for correlation analysis.

Table-3: FT-IR and colorimetric data of the green tea samples.

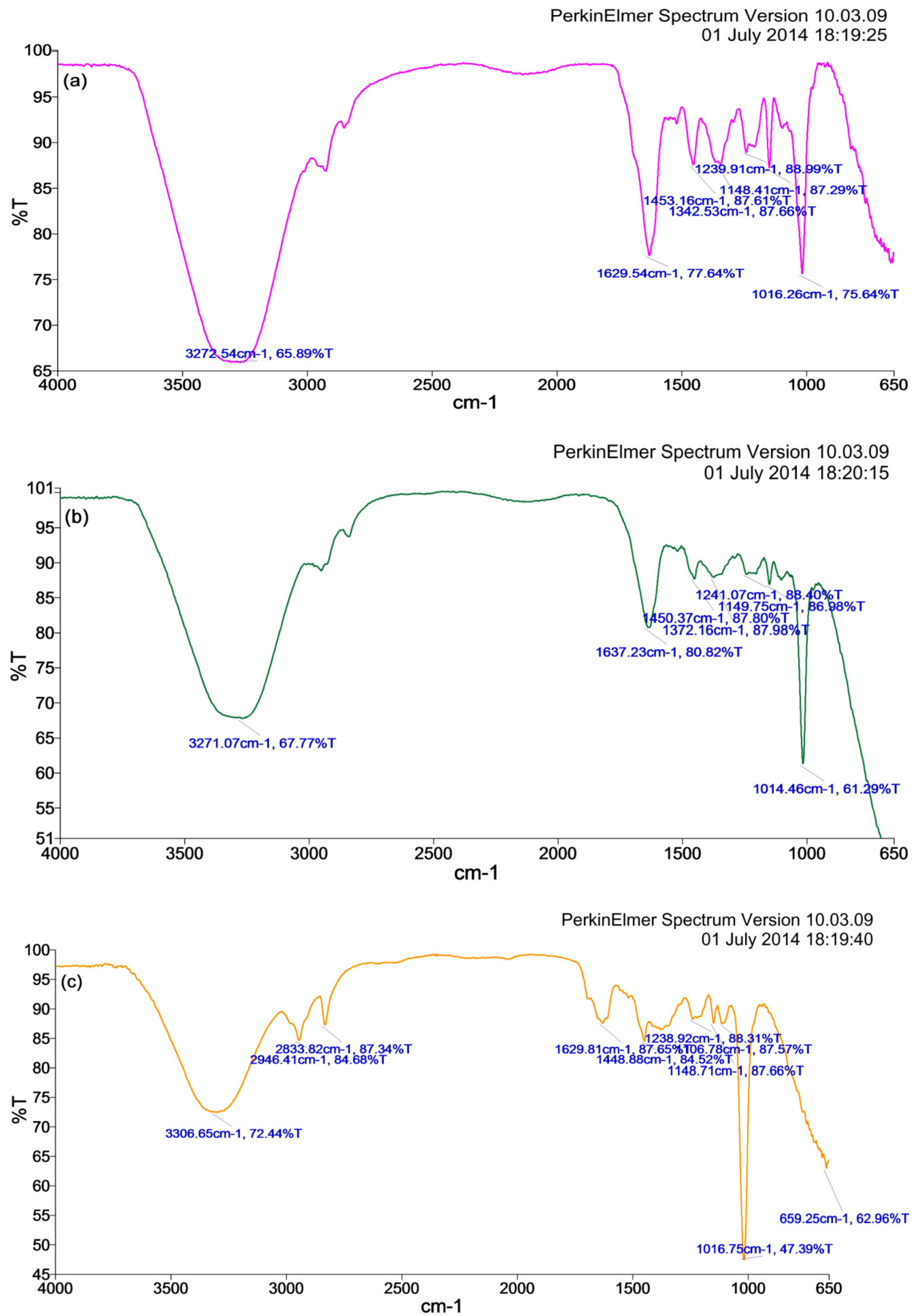
Green tea samples	FT-IR data (absorbance %)			Colorimetric data*			
	Polyphenols	Flavonoids	Alcohols/amines	DPPH-RSA (mg AE g^{-1})	FRAP (mg AE g^{-1})	TPC (mg GAE g^{-1})	TF (mg QE g^{-1})
MOON	34.13	22.40	24.40	651.00	731.00	267.00	27.40
TAN	32.20	19.10	18.70	610.00	683.00	238.00	24.20
GT	27.50	12.40	52.60	556.00	652.00	219.00	19.60
TET	29.20	12.00	52.50	521.00	424.00	191.00	15.40
KOL	31.30**	10.60	70.60	492.00	381.00	159.00	11.90
ASS	24.20	4.50	75.40	385.00	319.00	135.00	9.80

*Data from authors' work communicated elsewhere. **Case omitted in correlation studies. Ascorbic acid equivalent: AE; gallic acid equivalent: GAE; quercetin equivalent: QE.

Table-4: Pearson's linear correlation coefficient (R) matrix analysis between FT-IR and colorimetric data of green tea samples.

Variables		FT-IR data			Colorimetric data			
		Polyphenols	Flavonoids	Alcohols / amines	DPPH-RSA	FRAP	TPC	TF
FT-IR data	Polyphenols	1						
	Flavonoids	0.985**	1					
	Alcohols/amines	–0.953*	–0.940**	1				
Colorimetric data	DPPH-RSA	0.940*	0.977**	–0.914*	1			
	FRAP	0.805	0.886*	–0.888*	0.925**	1		
	TPC	0.924*	0.953**	–0.930**	0.974**	0.966**	1	
	TF	0.932*	0.958**	–0.954**	0.958**	0.969**	0.991**	1

**Correlation is significant at 0.01 level. *Correlation is significant at 0.05 level.


PerkinElmer Spectrum Version 10.03.09
01 July 2014 18:19:25
(a)
%T
cm-1
3272.54cm-1, 65.89%T
1629.54cm-1, 77.64%T
1453.16cm-1, 87.61%T
1342.53cm-1, 87.66%T
1239.91cm-1, 88.99%T
1148.41cm-1, 87.29%T
1016.26cm-1, 75.64%T
PerkinElmer Spectrum Version 10.03.09
01 July 2014 18:20:15
(b)
%T
cm-1
3271.07cm-1, 67.77%T
1637.23cm-1, 80.82%T
1450.37cm-1, 87.80%T
1372.16cm-1, 87.98%T
1241.07cm-1, 88.40%T
1149.75cm-1, 86.98%T
1014.46cm-1, 61.29%T
PerkinElmer Spectrum Version 10.03.09
01 July 2014 18:19:40
(c)
%T
cm-1
3306.65cm-1, 72.44%T
2946.41cm-1, 84.68%T
2833.82cm-1, 87.34%T
1629.81cm-1, 87.65%T
1448.88cm-1, 84.52%T
1238.92cm-1, 88.31%T
1106.78cm-1, 87.57%T
1148.71cm-1, 87.66%T
1016.75cm-1, 47.39%T
659.25cm-1, 62.96%T

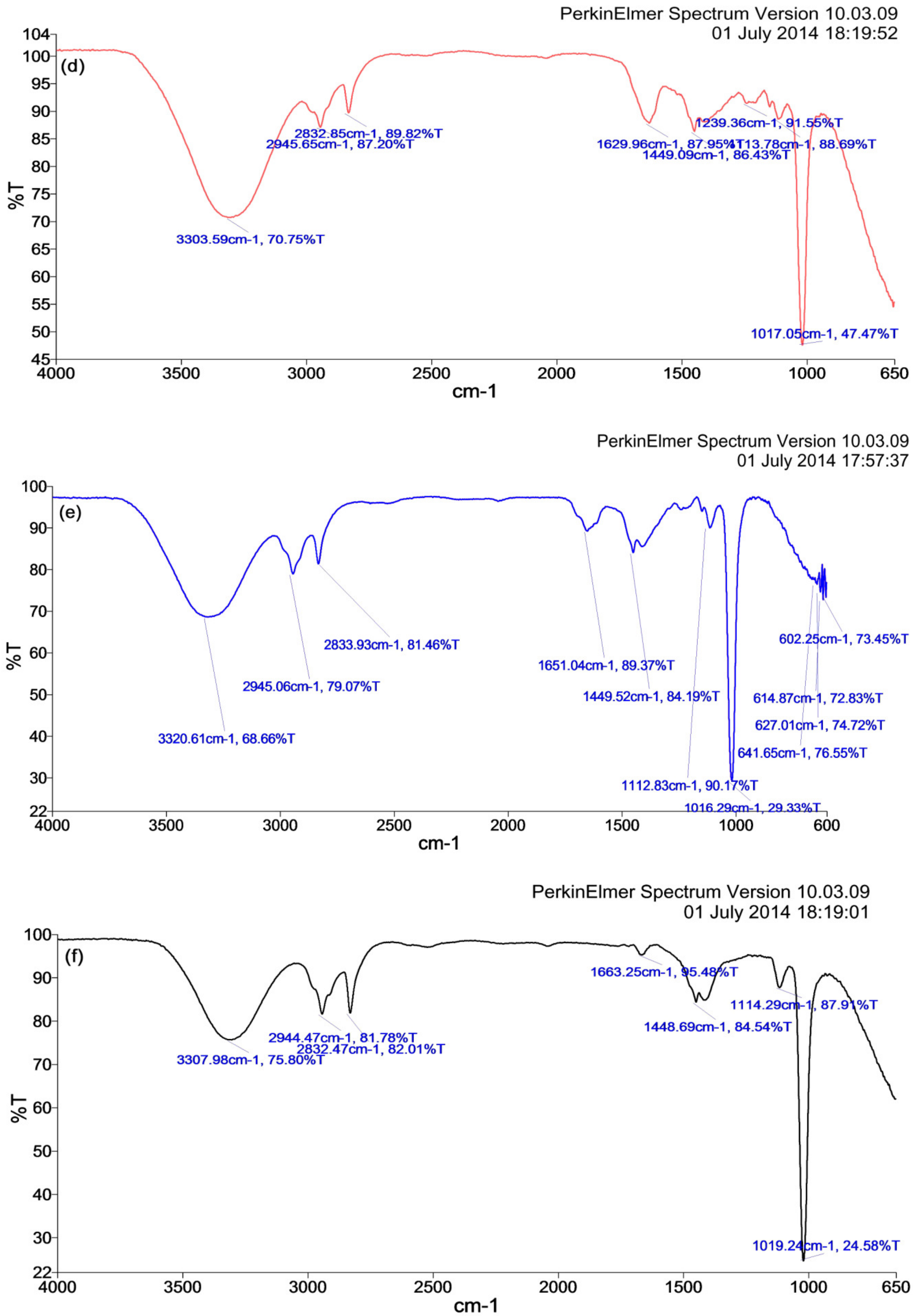


Figure-1: FT-IR spectra of green tea samples: (a) MOON, (b) TAN, (c) GT, (d) TET, (e) KOL and (f) ASS.

It can be observed from Table-4 that there exists excellent correlation between the FT-IR data and colorimetric data. In all cases the correlation coefficients are higher ($R^2 > 0.9$) except for $R^2 = 0.805$ to 0.888 for the FRAP assay with the FT-IR data. Such high values of correlation coefficients between antioxidant activity and the TPC and TF have been reported by Karori *et al*[1]. At the same time, even though the correlation coefficient is higher, the absorbance (%) of the ~1016 cm^{-1} band showed negative correlation with the remaining parameters. The possible reason for this may be due to the fact that, increase in absorbance is associated with corresponding decrease in absorbance (%) of other compounds with antioxidant potential. Further the compounds responsible for this peak had less significant effect on the antioxidant activity. Orcic *et al*[26] have reported that the antioxidant activity can be attributed to flavonoid glycosides and phenolic acids while compounds lacking polyphenolic structure or lacking catechol moiety have no significant antioxidant activity.

Conclusion

FT-IR spectra of six Indian commercial green tea samples were recorded to assess the presence of chemical compounds with antioxidant potentials. The absorbance (%) values obtained from the spectra, representing polyphenol and flavonoid contents were correlated to the DPPH-RSA, FRAP, TPC and TF values determined by colorimetric assays. Amazingly excellent linear correlations ($R^2 > 0.9$) were found among the FT-IR and colorimetric data. This opens up a fast and reliable approach to utilize the FT-IR data for estimating antioxidant potentials of plant extracts without the nee for advanced chemometric procedures.

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