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Research Article

COMPARATIVE ANALYSIS OF EXTRACTION AND ESTIMATION OF TEA POLYPHENOLS, FLAVONOIDS AND ANTIOXIDANT IN COMMERCIALLY AVAILABLE TEA POWDERS

***Karishma Rajbhar, Himanshu Dawda and Usha Mukundan**
Plant Biotechnology Laboratory, Ramniranjan Jhunjhunwala College, Ghatkopar, Mumbai 400086, India
Author for Correspondence

ABSTRACT
Particle size, heating time and heating method are important factors affecting the yield of an extraction process. This study aims to determine the total concentrations of phenolic compounds and flavonoids as well as their antioxidant activity in 37 samples (12 loose and 25 commercial branded tea powders) by different extraction methods. Our results show that among the different extraction methods used for polyphenols and flavonoids, the microwave assisted extraction (MAE) method gives the best results. In 1 min, MAE shows significantly higher extraction than water-bath extraction for 15 min, ultrasound-assisted extraction for 15 min and brewing extraction for 5 min. Microwave extraction shows high efficiency in extraction while using a minimum amount of solvent, shorter extraction time, ease of maintenance of extraction vessels and the enhancement of recovery and repeatability. During the study we found that extraction of polyphenols and flavonoids from the tea powder has two important limiting factors which affect its estimation namely, the particle size and the time for which heat is supplied. Tea powder samples were divided into five classes A, B, C, D and E using different Indian standard sieve meshes (4mm, 2mm, 1mm and 0.5mm) to vary particle size. The duration and method of supplying heat were varied. It was generally found that greater the surface area more is the extractability. In case of tea powder, however, the processes by which the tea sample have been prepared is also significantly affects the extraction.

Keywords: *Tea, Type of Extraction, Polyphenols, Flavonoids, Antioxidant Activity, Particle Size and Heating Time*

INTRODUCTION
Tea, the second most popular beverage after water, is known for its variety of flavours and aromas. It is prepared from the apical leaves and buds of *Camellia sinensis* (L.) Kuntze. Tea infusion is widely used as a medicine by tribal people throughout India and China. It is also extensively used in various indigenous system of medicine like Ayurveda (Parmar *et al.*, 2012).

Different types of teas, produced using various processes, are known all over the world. Six types of teas that are most widely known are black (regular), green, yellow, white, oolong and reprocessed tea. The processing of the six types of teas depends on the physical and biological characteristics of young tea shoots (Ruan, 2005).

Importance of Polyphenols in Food
Polyphenols, predominantly flavonoids and phenolic acids, that are present in tea in large quantities act as micronutrients in our diet and contribute significantly to the flavour and health-enhancing properties. Their importance in the prevention of degenerative diseases is rapidly gaining importance. Plant polyphenols comprise a great variety of compounds, among which flavonoids and several classes of non-flavonoids are usually distinguished.

Dietary polyphenols show an enormous assortment of structures, ranging from simple molecules (monomers and oligomers) to polymers (Lung *et al.*, 2013).

Plant phenolics are increasingly being shown to retard the oxidative degradation of lipids and thereby improve the quality and nutritional value of food.

The most abundant dietary flavonol, quercetin, is a potent antioxidant because it has the required structural features for free radical scavenging activity. The consumption of polyphenols may decrease the occurrence of oxidative-stress related diseases such as cancer, cardiovascular diseases, and aging.

A majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanins, coumarins lignans, catechins and isocatechins. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer.

Flavonoids are known to inhibit or kill many bacterial strains, to inhibit important viral enzymes such as reverse transcriptase, protease and also to destroy some pathogenic protozoans (El Gharras, 2009; Manach *et al.,* 2004 & Khalaf *et al.,* 2008).

Different Methods of Extraction

Polyphenols (including aglycones, glycosides, and oligomers) naturally have a strong affinity for water and tend to dissolve easily in water. They are extracted using water solvents depending on the solubility of the target polyphenols (Khoddami *et al.,* 2013).

The quality of tea mainly depends on the variety of leaf, growing environment, manufacturing conditions, leaf appearance, method used for preparation of tea, size of ground tea leaves (tea powder particles) and infusion preparation.

The quality is determined by liquor brightness, briskness, colour, aroma and flavour. The production of a majority of branded teas involves the blending of many varieties of tea to maintain quantity, particle size and the consistency of flavour. Changes in processing variables like, substrates, solutes, time, temperature, etc. may affect the polyphenols, flavonoids and antioxidant extraction efficiency in plant (Seetohul *et al.,* 2006).

Most of the current analysis protocols use classic extraction techniques, among which maceration, Soxhlet and refluxing extractions are the most commonly used. Due to the disadvantages that these techniques present, there is a need to replace them with other techniques that require less reagents, energy and time, while exhibiting similar efficiency.

Consequently, techniques such as microwave-assisted extraction (MAE), ultrasound assisted extraction (UAE) and conventional method of water bath assisted extraction (WAE) and brewing assisted extraction (BAE) have been implemented (Lung *et al.,* 2013).

MATERIALS AND METHODS

Considering that tea products have different ages and shelf lives, and that specific leaf selection and processes could differ, the products selected for the study were representatives of commercially available black and green teas.

A total of 37 tea samples of which 12 were loose tea powder samples while 25 were commercial branded tea powder samples were used and are listed below (Table 1):

Types and Method of Decoction Preparation

A decoction was made using 0.05 g of tea powder suspended in 5 ml of distilled water prepared in distilled water.

The material to liquor ratio was maintained at 1:100. Optical density (OD) of the reaction mixtures was read on Jasco V-530 spectrophotometer.

Chemicals Used

Folin-Ciocalteau reagent, sodium carbonate (Na_2CO_3), sodium nirite (NaNO2), methanol, sodium phosphate were obtained from Merck (India).

Gallic acid (monohydrate), rutin trihydrate and quercetin were obtained from HI-Media (India) and catechin LR (hydrate) from Research-Lab Line Chemical Industries (India);

Ascorbic acid, aluminium chloride ($AlCl_3$), sodium potassium tartarate tetrahydrate and ammonium molybdate were obtained from LOBA chemie laboratory reagents and fine chemicals.

Table 1: List of Commercial Tea Powder Samples

No.	Sample Name	Sample Code
1	Girnar Kesari	CBTP 1
2	Girnar pure n fresh 5 jumbo brands	CBTP 2
3	Girnar royal	CBTP 3
4	Golden Earl grey tea	CBTP 4
5	Golden Nilgiri tea	CBTP 5
6	Golden orange pekoe	CBTP 6
7	Lipton Darjeeling tea	CBTP 7
8	Lipton green tea	CBTP 8
9	Lipton yellow label tea	CBTP 9
10	Red label	CBTP 10
11	Red label - natural care	CBTP 11
12	Reliance premium royal blend tea	CBTP 12
13	Reliance select leaf	CBTP 13
14	Society tea	CBTP 14
15	Taj mahal	CBTP 15
16	Tata tea Agni	CBTP 16
17	Tata tea -gold	CBTP 17
18	Tetley ginger tea	CBTP 18
19	Tetley green tea	CBTP 19
20	Tulsi green organic tea	CBTP 20
21	Twinings assam tea	CBTP 21
22	Twinings Darjeeling tea	CBTP 22
23	Waghbakri-perfect	CBTP 23
24	Chado chamomile whole flower	CBTP 24
25	Chado nilgiri	CBTP 25
26	Assam CTC	LTP 1
27	Darjeeling tea	LTP 2
28	Golden CTC/D	LTP 3
29	Golden O.P	LTP 4
30	Green tea	LTP 5
31	Kesari CTC	LTP 6
32	Nilgiri CTC	LTP 7
33	Royal DUST	LTP 8
34	Special BOP	LTP 9
35	Special CTC	LTP 10
36	Special H. DUST	LTP 11
37	Super CTC	LTP 12

*It is a simple form of alphabetical nomenclature followed in both commercial and loose tea sample to facilitate understanding and data tabulation.

Table 2: Method of Decoction Preparation

Type of extraction	Instrument	Method of Extraction
Brewing Assisted Extraction (BAE)	Electric Kettle	Boiled distilled water from tea kettle was poured on the tea powder sample and was brewed for 5 minutes
Waterbath Assisted Extraction (WAE)	Equitron Waterbath	Tea powder with distilled water was kept in waterbath for 15 minute at 80°C – 90°C
Microwave Assisted Extraction (MAE)	LG Microwave Oven (I- wave)	Tea powder with distilled water was microwaved for 1 minute
Ultrasonicator Assisted Extraction (UAE)	Ultrasonicator	Tea powder with distilled water was kept in ultrasonicator for 15 min

Determination of Total Phenolic Content

The total phenolic content (TPC) of the tea extracts is determined using the method of Singleton *et al.*, (1999) using with slight modifications Folin-Ciocalteau method i.e. in the ratio of 1:1 dilution with distilled water. Test sample 0.1 ml, .0.8 ml distilled water for dilution; 0.1 ml Folin-ciocalteau (1:1) reagent are mixed and allowed to stand for 5 min at 37ºC. After 5 min, 3.0 ml of 2% of sodium carbonate is added. This mixture is incubated for 15 min in 80°C–90°C. After incubation the development of blue colour is observed. The absorbance of blue colour in different samples is measured at 720 nm for gallic acid equivalence and 745 nm for catechin equivalence using V-530 Jasco spectrophotometer. The phenolic content is calculated as gallic acid equivalents GAE/g and CE/g on the basis of standard curve of gallic acid and catechin. The quantity of polyphenol in 1 g of tea powder sample was calculated (Singleton, 1999).

Determination of Total Flavonoids Content

The total flavonoids content of tea extract is estimated by slight modification method described Aluminium chloride method by Zhishen *et al.* for quercetin equivalents and aluminium chloride method by Sultana et al. for rutin trihydrate equivalents. Sample 0.1 ml is diluted with methanol 0.9 ml; add 0.5 ml of sodium nitrite $NaNO_2$ solution (10%).

After 5 min, 0.5 ml $AlCl_3$ solution (10%) is added followed by 1.0 ml of NaOH solution (1%) to the mixture. After incubation of 15 min the mixture is centrifuged at 2500 rpm and absorbance is read at 400 nm. Standard curve of quercetin is prepared and the result is expressed as quercetin equivalents (mg quercetin/gm) (Sultana *et al*, 2012).

Rutin trihydrate equivalents are determined using sample 0.1 ml and the volume is made up to 2 ml with methanol. Then 0.5 ml Na-K tartarate tetrahydrate (0.1 M) is added followed by incubation for 5 min after which 0.5ml $AlCl_3$ (10%), is added. After incubation of 15 min, the mixture is centrifuged at 2500 rpm and the absorbance is read at 400 nm. The concentration of flavonoid in the test samples is calculated from the calibration plot and expressed as mg rutin trihydrate equivalent/g of sample (Khatiwora *et al.*, 2010).

Total Antioxidant Capacity Assay

The total antioxidant capacities of tea extracts are evaluated by the phosphomolybdenum method, described by Prieto *et al.*, with some modifications. The sample (0.1ml) is diluted with methanol (0.9 ml) is combined with reagent solution (0.5 ml of 0.6 M sulphuric acid) followed by incubation for 5 min, the addition of 4 mM ammonium molybdate (0.6 ml), incubation for 5 min, addition of 28 mM sodium phosphate (0.9 ml) and incubation for 15 min at 80°C–90°C. After cooling the mixture to room temperature, the absorbance is measured at 740 nm. The antioxidant capacity of the sample is expressed as equivalents of ascorbic acid (AAE), utilising a calibration curve of ascorbic acid (Vladimir-Knezevic *et al.*, 2011).

RESULTS AND DISCUSSION

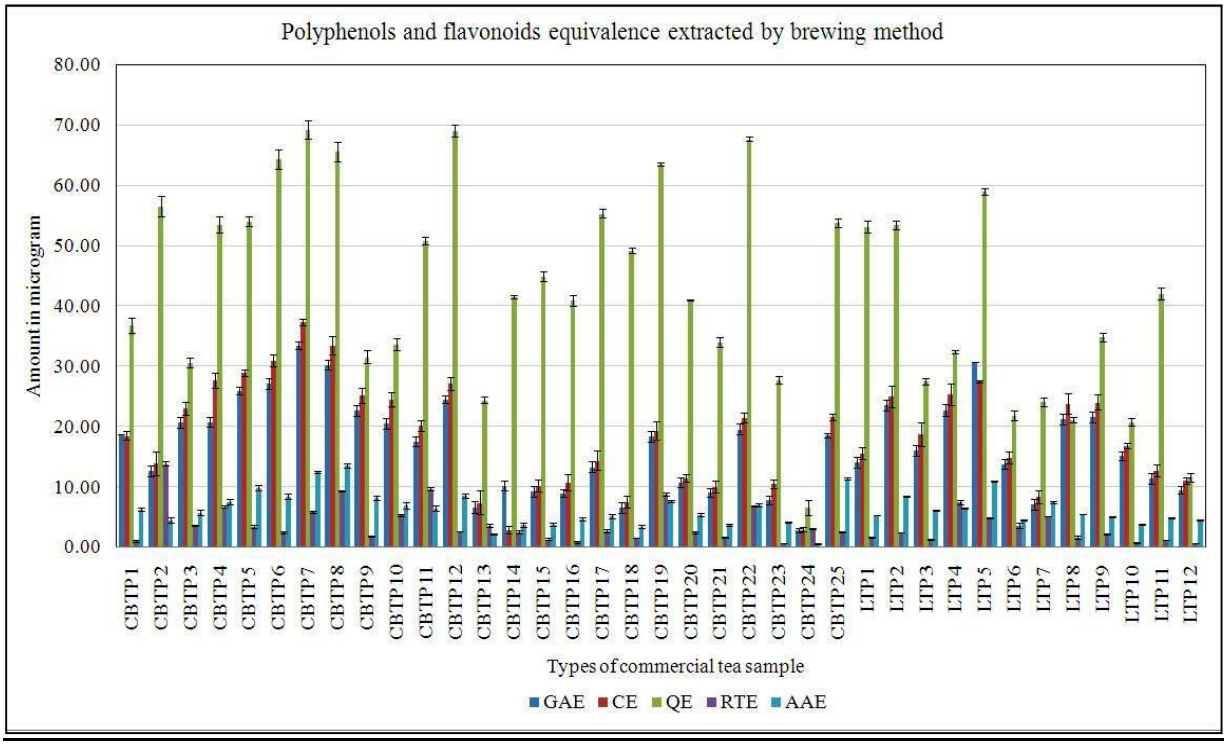


Figure 1: Polyphenols and Flavonoids Extracted by Brewing Assisted Method (BAE) from Commercial Samples

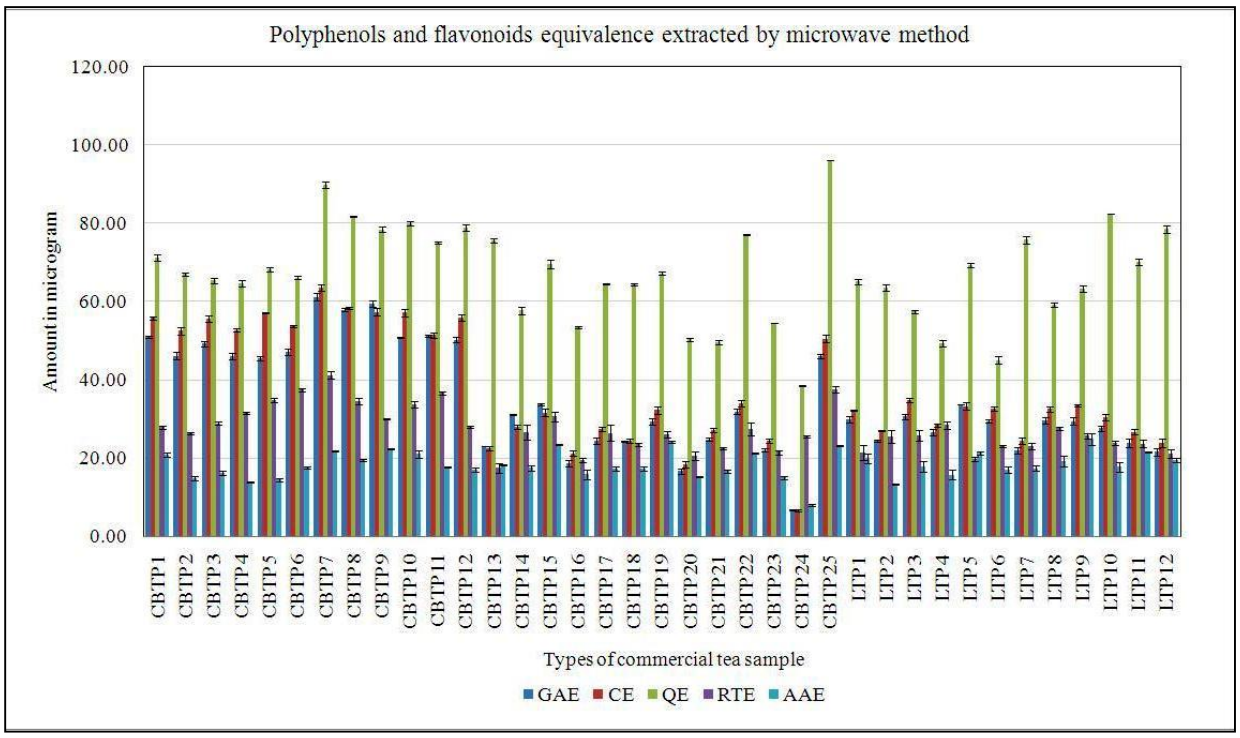


Figure 2: Polyphenols and Flavonoids Extracted by Microwave Assisted Method (MAE) from Commercial Samples

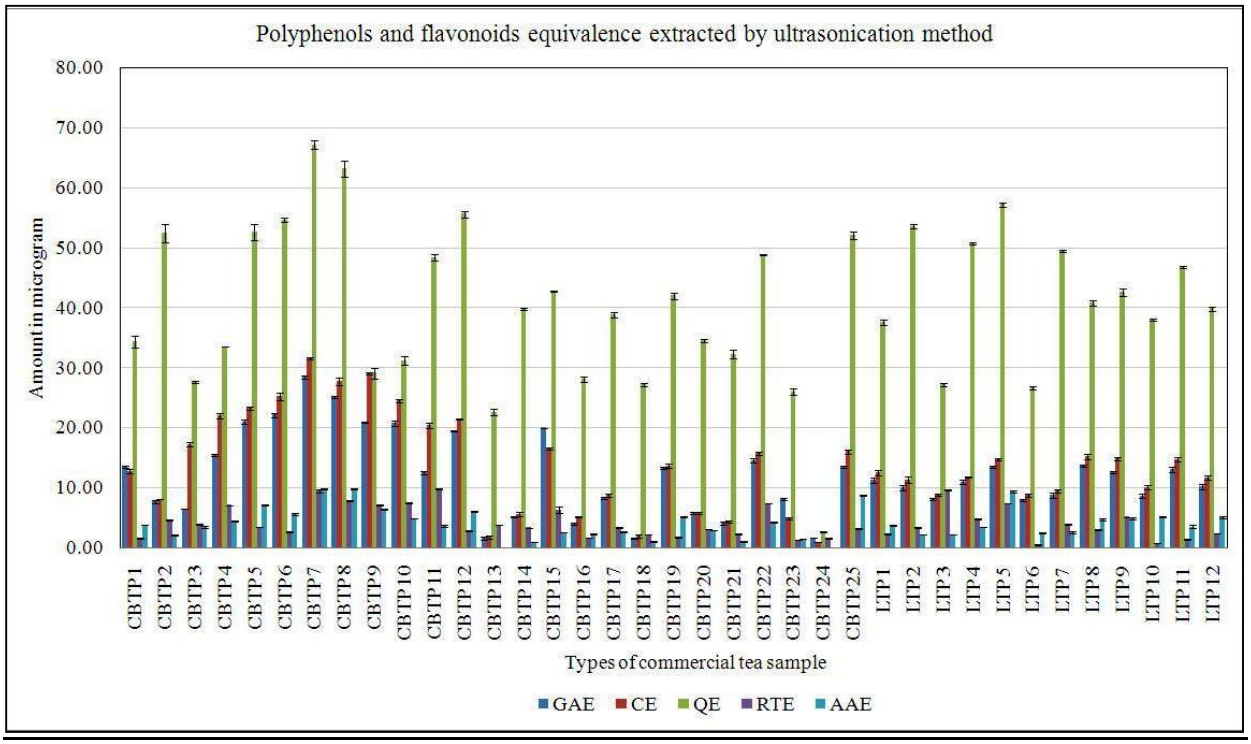


Figure 3: Polyphenols and Flavonoids Extracted by Ultrasonicator Assisted Method (UAE) from Commercial Samples

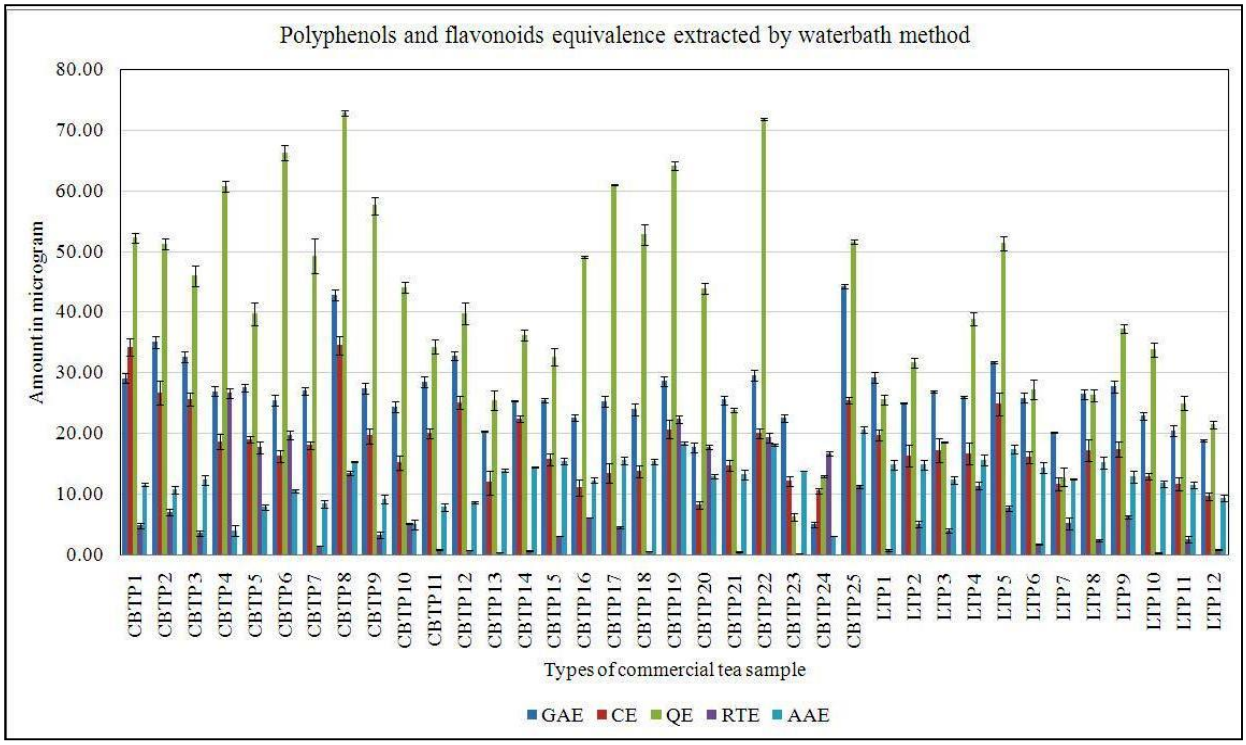


Figure 4: Polyphenols and Flavonoids Extracted by Waterbath Assisted Method (WAE) from Commercial Samples

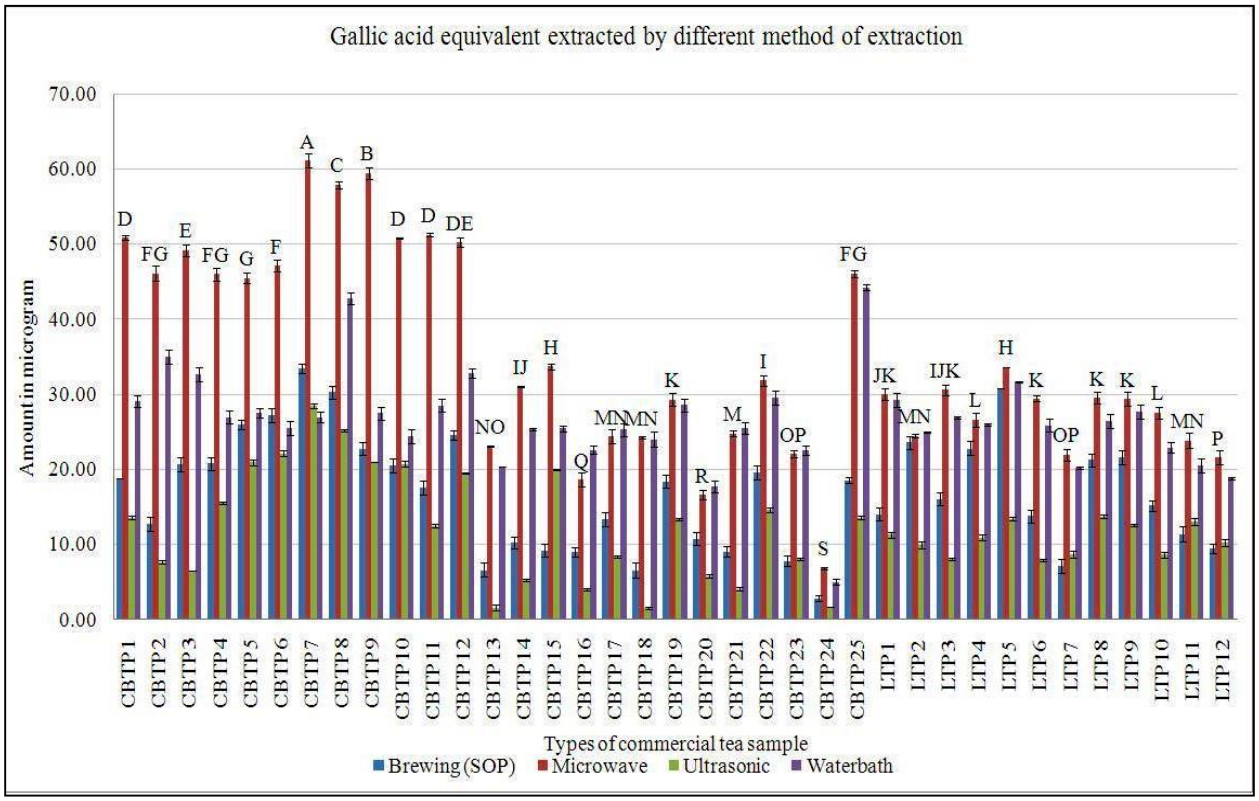


Figure 5: Gallic Acid Equivalent Extracted by Different Types of Method from Commercial Samples

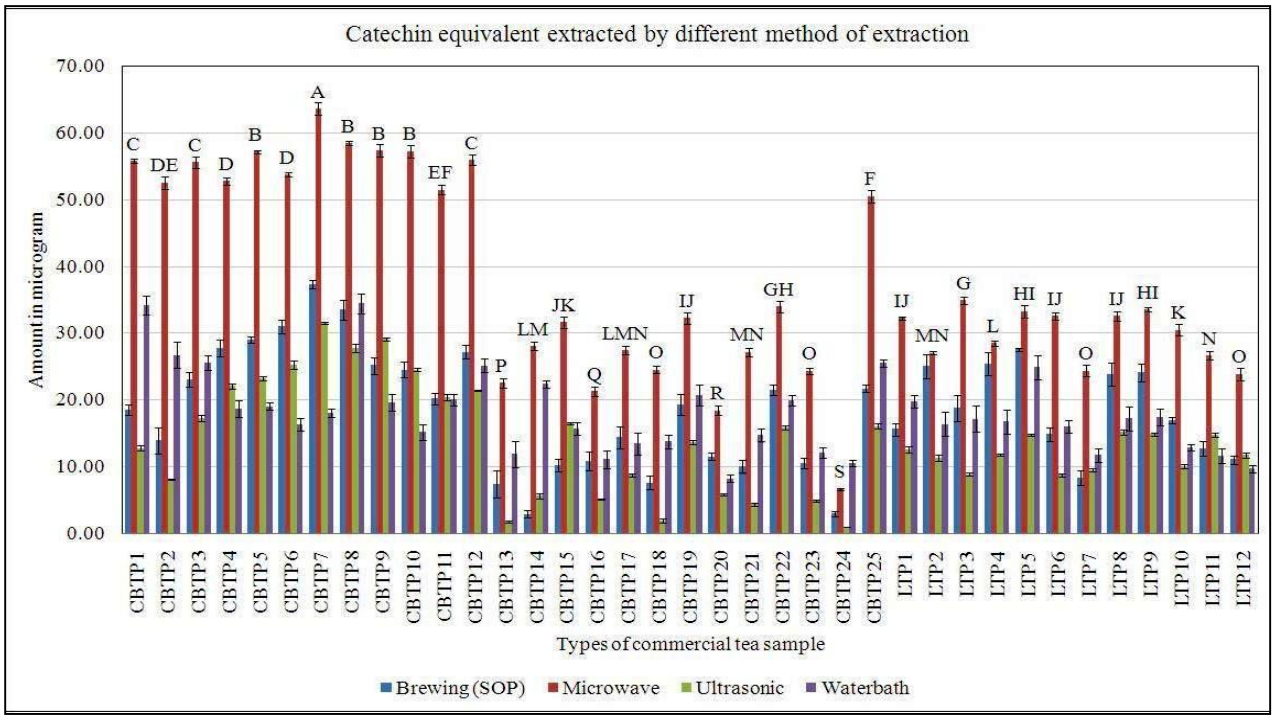


Figure 6: Catechin Equivalent Extracted by Different Types of Method from Commercials Sample

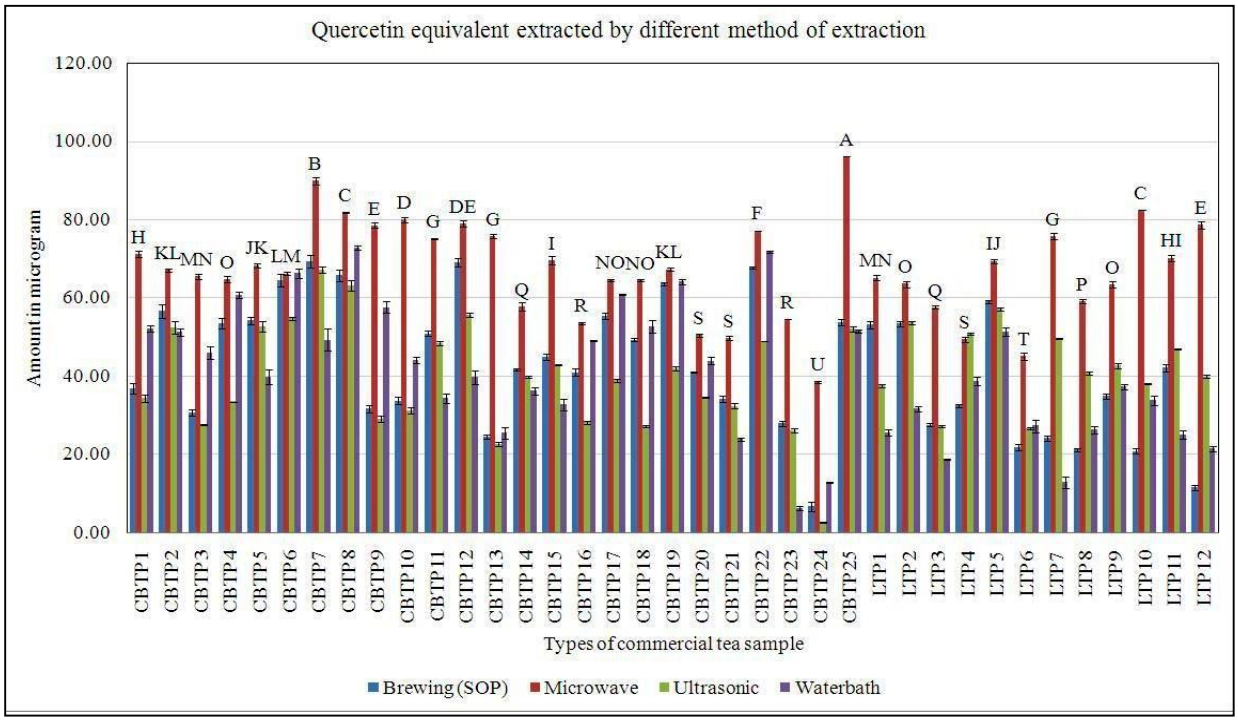


Figure 7: Quercetin Equivalent Extracted by Different Types of Method from Commercial Samples

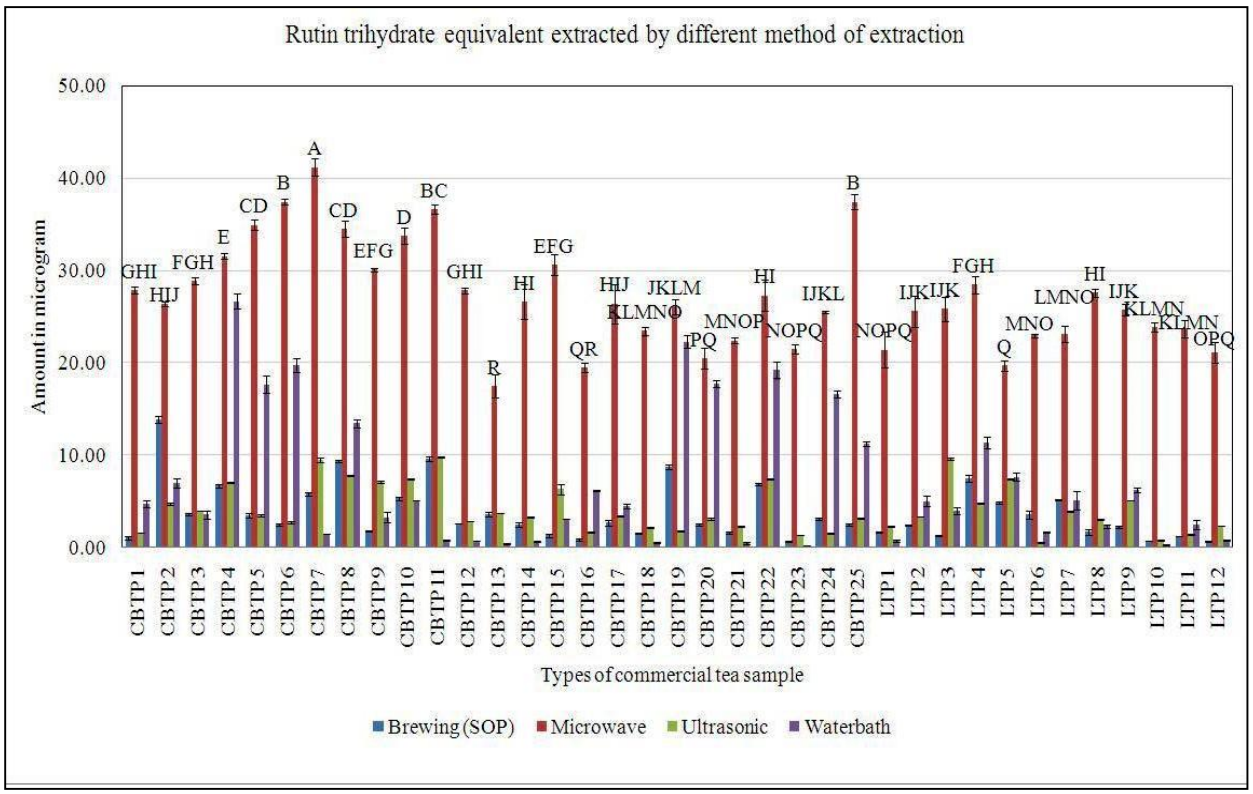


Figure 8: Rutin Trihydrate Equivalent Extracted by Different Types of Method from Commercial Samples

Research Article

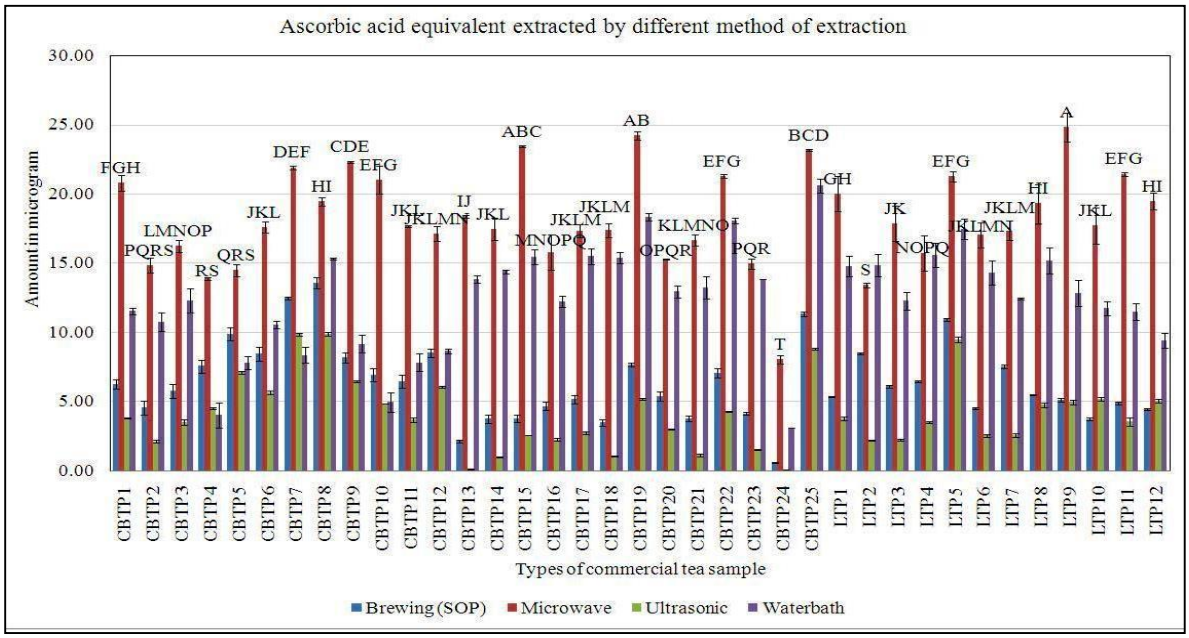


Figure 9: Ascorbic Acid Equivalent Extracted by Different Types of Method from the Commercial Sample

Statistical Analysis of Data

Statistical analysis of the data obtained from the studies was performed using SPSS version 19. The reported values are mean ±SD (n=3). The results of the analysis were obtained for $p<0.05$. In cases where ANOVA has been performed, multiple comparisons were made using Duncan's Multiple Range Test (DMRT). Gallic acid equivalents, catechin equivalents, quercetin equivalents, rutin trihydrate equivalents and ascorbic acid equivalents series have been assigned groups using upper case letters (A>B >C…) in graphs for microwave assisted extraction data while others extraction data is not shown. (* In a given series, mean assigned the same letter(s) are not significantly different from each other $p<0.05$).

Discussion

India is largest cultivator and consumer of tea in the world with three main well-known tea growing regions - Assam, Darjeeling and Nilgiri. Approximately 99% of the Indian tea produced is black. In India, these teas are picked during specific seasons or "flushes" The first flush is in spring followed by a second flush in summer. Assam, located in north-eastern India, cultivates hearty, robust teas that stand up to milk and sugar. In the south, Nilgiri grows tea not widely known in the West (Ruan, 2005 & Harbowy *et al.*, 1997).

The major types of tea, including black, oolong, green and white all originate from the *Camellia sinensis* tea bush. The differences among the teas result only from the way the plucked leaves are processed. Making black tea involves withering, rolling, oxidation and drying. Black tea undergoes a full fermentation stage before drying and steaming although the fermentation of black tea is oxidation. Oolong tea is derived when the fresh leaves are subjected to a partial fermentation stage before drying. As partially fermented tea, oolong can be thought as teas that are halfway between black and green tea and have a refined balance embodying the complexity of a black with the reinvigorating properties of green tea. Green tea differs from black tea in the fact that after plucking, fresh leaves are immediately steamed or pan-fired to stop any oxidation activity thus, no fermentation (i.e. oxidation) and often been marketed as a health beverage with an unsavoury, dull and bitter flavour. White tea is least processed of all tea, the youngest new buds are freshly plucked, and then air or steamed dried. It is also termed as herbal infusions which are caffeine free and also provide a variety of health benefits e.g. chamomile flowers are white tea noted for aiding digestion (Yuegang Zuo *et al*, 2002).

Table 3: Samples with the Highest and Lowest Concentration of Polyphenols, Flavonoids and Antioxidant Equivalents

Polyphenols, Flavonoids and Antioxidant Equivalent	Method of Extraction	Highest Concentration (mcg/g)	Lowest Concentration (mcg/g)
Gallic Acid Equivalent	Brewing	CBTP 7- 33.46±0.67 mcg	CBTP 18- 6.56±0.96 mcg
	Microwave	CBTP 7- 61.17±0.93 mcg	CBTP 24 - 6.82±0.14 mcg
	Ultrasonic	CBTP 7 - 28.40±0.30 mcg	CBTP 18 - 1.56±0.12 mcg
	Water bath	CBTP 25 - 44.25±0.38 mcg	CBTP 24 - 4.9±0.40 mcg
Catechin Equivalent	Brewing	CBTP 7- 37.32±0.59 mcg	CBTP 24 - 2.92±0.33 mcg
	Microwave	CBTP 7- 63.59±0.90 mcg	CBTP 24 - 6.64 ±0.19 mcg
	Ultrasonic	CBTP 7 - 31.56±0.16 mcg	CBTP 24 - 0.93±0.04 mcg
	Water bath	CBTP 8 - 34.47±1.49 mcg CBTP 1 - 34.21±1.44 mcg	CBTP 20 - 8.21±0.57 mcg
Quercetin Equivalent	Brewing	CBTP 12 - 69.07±1.00 mcg	CBTP 24 - 6.60±1.29 mcg
	Microwave	CBTP 25 - 96.13±0.01 mcg	CBTP 24 - 38.50±0.22 mcg
	Ultrasonic	CBTP 7 - 67.16±0.74 mcg	CBTP 24 - 2.61±0.07 mcg
	Water bath	CBTP 22 - 71.78±0.24 mcg	CBTP 23 - 6.18±0.57 mcg
Rutin Trihydrate Equivalent	Brewing	CBTP 2 - 13.86±0.38 mcg	CBTP 23 - 0.66±0.03 mcg LTP 10 - 0.75±0.03 mcg LTP 12 - 0.69±0.05 mcg
	Microwave	CBTP 7 - 41.21±0.94 mcg	CBTP 13 - 17.46±1.25 mcg
	Ultrasonic	CBTP 11 - 9.84±0.08 mcg	LTP 6 - 0.56±0.07 mcg LTP 10- 0.79±0.06 mcg
	Water bath	CBTP 4 - 26.67±0.82 mcg	CBTP 13 - 0.42±0.05 mcg CBTP 23 - 0.19±0.01 mcg LTP 10- 0.31±0.06 mcg
Ascorbic Acid Equivalent	Brewing	CBTP 8 - 13.55±0.39 mcg	CBTP 24 - 0.55±0.03 mcg
	Microwave	LTP 9- 24.81±1.03 mcg CBTP 19 - 24.22±0.29 mcg	CBTP 24 - 8.04±0.32 mcg
	Ultrasonic	CBTP 7 - 9.85±0.09 mcg CBTP 8 - 9.89±0.11 mcg	CBTP 24 - 0.07±0.03 mcg
	Water bath	CBTP 25 - 20.59±0.51 mcg	CBTP 24 - 3.11±0.02 mcg

Green and oolong tea leaves are generally not graded like most black teas. Whole leaf teas boast of a range of complex and subtle flavours whereas broken leaf teas produce a darker cup and infuse faster than whole leaf teas. A significant difference in commercial rates is seen not only due to grades or appearance of tea powder but the biochemical parameter also interfere as processing of tea leaves affects in every aspect when final outcome tea is considered. Black tea is the most prized form of tea the world over. Black teas are noted for their depth and complexity as they undergo an extended fermentation process as stated earlier. This extended fermentation makes the green leaf to a dark brown or black colour (Harbowy *et al.*, 1997).

The evaluation of the finest method of extraction was based on 5 representative indices, the polyphenol by gallic acid and catechin equivalent, flavonoids by quercetin and rutin trihydrate equivalent and total antioxidant content by ascorbic acid equivalent. The selected 37 samples were analysed and compared for their polyphenols, flavonoids and antioxidant contents. Extraction was performed using different simple and conventional methods.

The extraction method used was to enable complete extraction of the compounds of interest and must avoid chemical modification.

It appears, therefore, that different extraction methods or different mechanical pressure during decoction preparation may have a significant impact on the nature and concentration of the released compounds, and therefore particular emphasis should be given to the selection method of extraction (Zuo *et al.*, 2002). The contents of tea are related to the quality of tea leaves and the degree of fermentation during tea manufacturing. Thus, the variety of tea samples selected shows a wide range of polyphenols and flavonoids content

Brewing means pouring hot water on the sample and keeping it covered to prepare a decoction of that sample. Thus, the hot water acts as a force that extracts the inner contents of the cell. The contents released are mostly flavonoids due to their solubility in water. Flavonoids are polyphenolic molecules containing 15 carbon atoms and they show the presence of the phenyl ring. Flavonoids can be degraded by enzyme action in fresh plant material. Hence; it is advisable to use dry, lyophilised, or frozen samples (Harbowy *et al.*, 1997). Tea powders are usually made from dried and processed leaves, and this facilitates the extraction of flavonoids. Hence, in all the different types of extraction stated in the above graphs, flavonoids in quercetin equivalents and rutin trihydrate equivalents are second highest and third highest, respectively (Table 3 and figures 1, 5-9).

Microwave-assisted extraction (MAE) uses microwave energy to facilitate partition analytics and extracts various polyphenolic compounds from the tea powder samples matrix into the water. It is a simple technique that can be completed in a few minutes (Renoe, 1994). Microwave energy is applied to the tea sample suspended in distilled water. With a certain degree of heating, large numbers of molecules are extracted easily in a relatively less time. The advantage of this technique is reduced extraction time and solvent volume as compared to conventional extraction techniques. It has been used for the extraction of some small-molecule phenolic compounds such as phenolic acids including, gallic acid, catechin, quercetin and many more which were shown to be stable under microwave-assisted heating conditions at temperature up to 100°C for 20 min (Dai *et al.*, 2010). The extraction mechanism involves two types of physical phenomena: diffusion through the cell walls and washing out the cell's content once the walls are broken. Thus, the table 3 figure 2 and figure 5 - 9 shows the maximum extraction in microwave assisted method, as all the polyphenols and flavonoids are efficiently and easily extracted through the microwaves effect.

Under ultrasound-assisted extraction (UAE) the tea sample are affected by shear force created by implosion of cavitation bubbles upon the propagation of the acoustic waves in the kHz range. The collapse of bubbles can produce physical, chemical and mechanical effects, which resulted in the disruption of tea powders membranes to facilitate the release of extractable compounds and enhance the penetration of solvent into cellular materials of tea sample. The polyphenols are phenylpropanoid which need a particular pressure and force for their extraction in water, which is not much supplied by UAE (Dai *et al.*, 2010). Hence, a smaller amount of polyphenols are release as seen in table 3 and in figures 3 and 5-9 stated above concluding that it less favourable method of extraction.

Water bath is an indirect heating of tea sample in DW. It is slowly heated to enable maximum extraction as the temperature rises, the tea sample tissue starts releasing its inner content into the medium. The longer time of extraction gives better results but the bitterness of polyphenols results which is an undesirable effect as far as tea is concerned. An increase in temperature increases the efficiency of the extraction, since heat renders the cell walls permeable. This increases the solubility and diffusion coefficients of the compounds to be extracted (Dai *et al.*, 2010 and Lung *et al.*, 2013). Thus, its shows second highest method for extracting polyphenols and flavonoids as seen in table 3 and in graphs 5–9.

Conclusion

The comparison of the above extraction methods used for polyphenols and flavonoids shows that the microwave assisted extraction method gives the best results. MAE for 1 min gives significantly higher extraction than water bath extraction for 15 min, ultrasound-assisted extraction for 15 min and brewing extraction for 5 min. The advantage of microwave extraction is that high efficiency extraction can be performed using a minimum amount of solvent, while simultaneously reducing the extraction time as well as easy maintenance of the extraction vessels and the enhancement of recovery and repeatability. The

possibility of simultaneous extraction of multiple samples is also higher with MAE, than with other conventional extraction techniques. The extractions of polyphenols and flavonoids from the tea powder have a two important limiting factor which affects its estimation. First is the particle size of tea powder sample and second is time of heat supply which can be studied by kinetics of extraction. Tea powder sample was graded into five classes A, B, C, D and E by using different Indian standard sieve mesh of 4mm, 2mm, 1mm and 0.5mm, which is listed below:-

Sorting of Commercial Tea Powder Samples on the Basis of its Size in Millimetre (mm)

Table 4: Tea Powder Samples with Particle Size of 1mm to 0.5mm in Class A

Class A - 1mm – 0.5mm	
Tetley Ginger Tea	CBTP 18
Twinings Assam Tea	CBTP 21
Royal DUST	LTP 8
Special H. DUST	LTP 11

Table 5: Tea Powder Samples with Particle Size of 2mm to 1mm in Class B

Class B - 2mm – 1mm	
Lipton Green Tea	CBTP 8
Reliance Select Leaf	CBTP 13
Tata Tea Agni	CBTP 16
Tulsi Green Organic Tea	CBTP 20
Twinings Darjeeling Tea	CBTP 22
Assam CTC	LTP 1
Special BOP	LTP 9
Special CTC	LTP 10
Super CTC	LTP 12

Table 6: Tea Powder Samples with Particle Size of 4mm to 0.5mm in Class C

Class C - 4mm – 0.5mm	
Red Label	CBTP 10
Red Label - Natural Care	CBTP 11
Reliance Premium Royal Blend Tea	CBTP 12
Society Tea	CBTP 14
Taj Mahal	CBTP 15

Table 7: Tea Powder Samples with Particle Size of 4mm to 1mm in Class D

Class D - 4mm – 1mm	
Girnar Kesari	CBTP 1
Girnar Pure n Fresh 5 Jumbo Brands	CBTP 2
Girnar Royal	CBTP 3
Golden Earl Grey Tea	CBTP 4
Golden Nilgiri Tea	CBTP 5
Golden Orange Pekoe	CBTP 6
Lipton Yellow Label Tea	CBTP 9
Tata Tea -Gold	CBTP 17
Tetley Green Tea	CBTP 19
Waghbakri-Perfect	CBTP 23
Chado Chamomile Whole Flower	CBTP 24
Chado Nilgiri	CBTP 25
Darjeeling Tea	LTP 2
Golden O.P	LTP 4

Table 8: Tea Powder Samples with Particle Size of 4mm to 2mm in Class E

Class E - 4mm – 2mm	
Lipton Darjeeling Tea	CBTP 7
Golden CTC/D	LTP 3
Green Tea	LTP 5
Kesari CTC	LTP 6
Nilgiri CTC	LTP 7

Sorted tea powder sample group were analysed to see the trend of polyphenols, flavonoids and antioxidant amount in its different graded classes. Greater the surface area more is extractability but in case of tea powder extraction the quality of process by which the sample is passed is also a very significant. Data below state that even if the surface area is greater in class A the amount of polyphenols, flavonoids and antioxidant equivalents is more in class E, D and C respectively. Detailed report for microwave assisted extraction (due to better method than others) is tabulated below:-
* (Note: Minimum particle size greater the surface area)

Table 9: Samples with the Highest and Lowest Concentration of Polyphenols, Flavonoids and Antioxidant Equivalents Present in Microwave Assisted Extraction for Sorted Sample

Grade class	**Polyphenols, Flavonoids and Antioxidant Equivalent**	**Highest Concentration (mcg/g)**	**Lowest Concentration (mcg/g)**
A - 1mm – 0.5mm	GAE	LTP 8 - 29.53±0.80 mcg	LTP 11- 23.86±0.97 mcg
	CE	LTP 8 – 32.55±0.66 mcg	CBTP 18 – 24.48±0.56 mcg
	QE	LTP 11 – 70.18±0.75 mcg	CBTP 21 – 49.71±0.58 mcg
	RTE	LTP 8 – 27.63±0.43 mcg	CBTP 21 – 22.42±0.30 mcg
	AAE	LTP 11 – 21.43±0.11 mcg	CBTP 21 – 16.64±0.40 mcg
B - 2mm – 1mm	GAE	CBTP 8 - 57.93±0.50 mcg	CBTP 20 – 16.62±0.60 mcg
	CE	CBTP 8 - 58.37±0.31 mcg	CBTP 20 – 18.37±0.71 mcg
	QE	CBTP 8 – 81.77±0.13 mcg	CBTP 20 – 50.39±0.41 mcg
	RTE	CBTP 8 - 34.49±0.87 mcg	CBTP 13 – 17.46±1.25 mcg
	AAE	LTP 9 – 24.81±1.43 mcg	CBTP 20 – 15.28±0.04 mcg
C - 4mm – 0.5mm	GAE	CBTP 11 – 51.25±0.22 mcg	CBTP 14 – 31.08±0.09 mcg
	CE	CBTP 10 – 57.20±0.92 mcg	CBTP 14 – 28.05±0.59 mcg
	QE	CBTP 10 – 79.96±0.63 mcg	CBTP 14 – 57.73±0.99 mcg
	RTE	CBTP 11 – 36.66±0.45 mcg	CBTP 14 - 26.61±1.86 mcg
	AAE	CBTP 15 – 23.42±0.07 mcg	CBTP 12 – 17.10±0.54 mcg
D - 4mm – 1mm	GAE	CBTP 9 – 59.47±0.76 mcg	CBTP 24- 6.82±0.14 mcg
	CE	CBTP 9 – 57.35±0.90 mcg	CBTP 24- 6.64±0.19 mcg
	QE	CBTP 9 – 78.45±0.69 mcg	CBTP 24- 38.50±0.22 mcg
	RTE	CBTP 25 – 37.47±0.78 mcg	CBTP 23 – 21.51±0.53 mcg
	AAE	CBTP 19- 24.22±0.29 mcg	CBTP 24 – 8.04±0.32 mcg
E - 4mm – 2mm	GAE	CBTP 7 – 61.17±0.93 mcg	LTP 7 – 021.97±0.77 mcg
	CE	CBTP 7 – 63.59±0.90 mcg	LTP 7 – 24.34±0.83 mcg
	QE	CBTP 7 – 89.87±0.88 mcg	LTP 6 – 45.06±0.94 mcg
	RTE	CBTP 7 – 41.21±0.94 mcg	LTP 5- 19.72±0.54 mcg
	AAE	CBTP 7 – 21.90±0.13 mcg	LTP 6 – 17.02±0.93 mcg

Research Article

Validation of Extraction Procedure by Showing the Kinetic Study of Extraction Method for the Best Sample Lipton Darjeeling Tea Powder

Data of lipton darjeeling tea powder was selected (as its gave the best result) to study the kinetics of extraction process. MAE data shows that 1min best extraction time to amount of sample size selected as the amount of compound estimated shows the plateau as seen in graph figure 10 and in WAE,UAE and BAE expect QE all the same trend as seen in graph figure 11, 12, and 13. QE can be extracted for more time in this extraction process but there is quantity versus quality scenario. Hence, the data shows we have selected best time of heat supply by different extraction process. Others samples data is not provided in this manuscript.

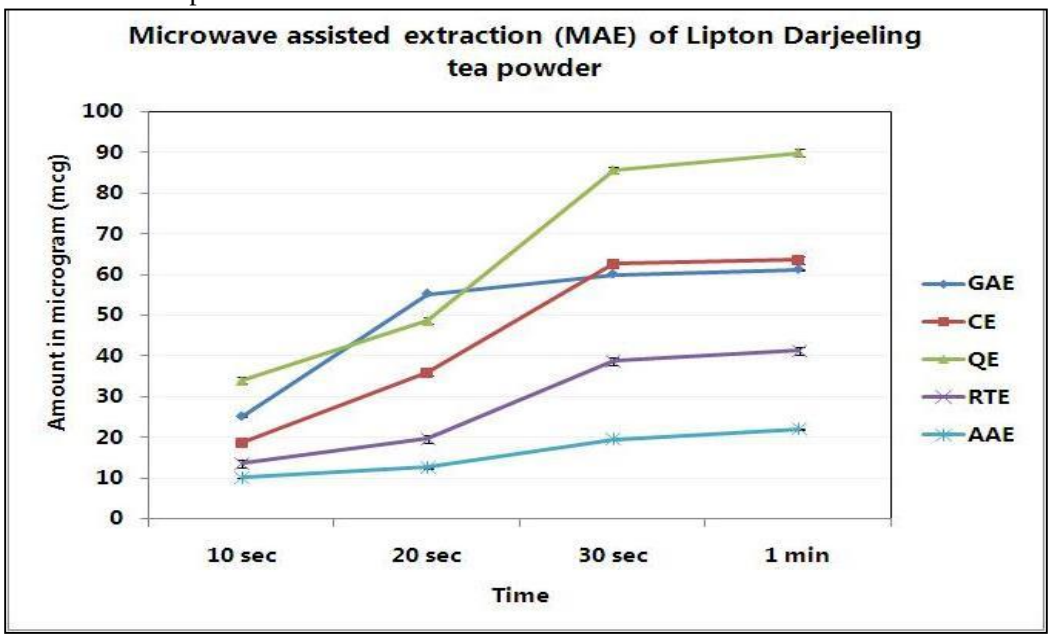


Figure 10: Polyphenols and Flavonoids Extracted by Microwave Assisted Method (MAE)

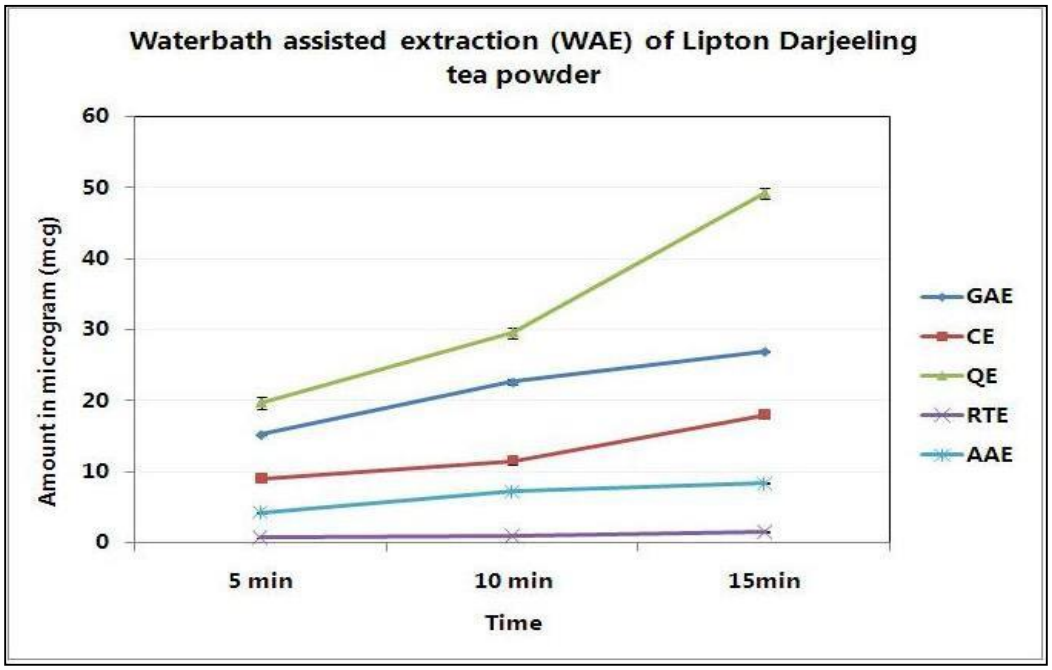


Figure 11: Polyphenols and Flavonoids Extracted by Waterbath Assisted Method (WAE)

Figure 12: Polyphenols and Flavonoids Extracted by Ultrasonic Assisted Method (UAE)

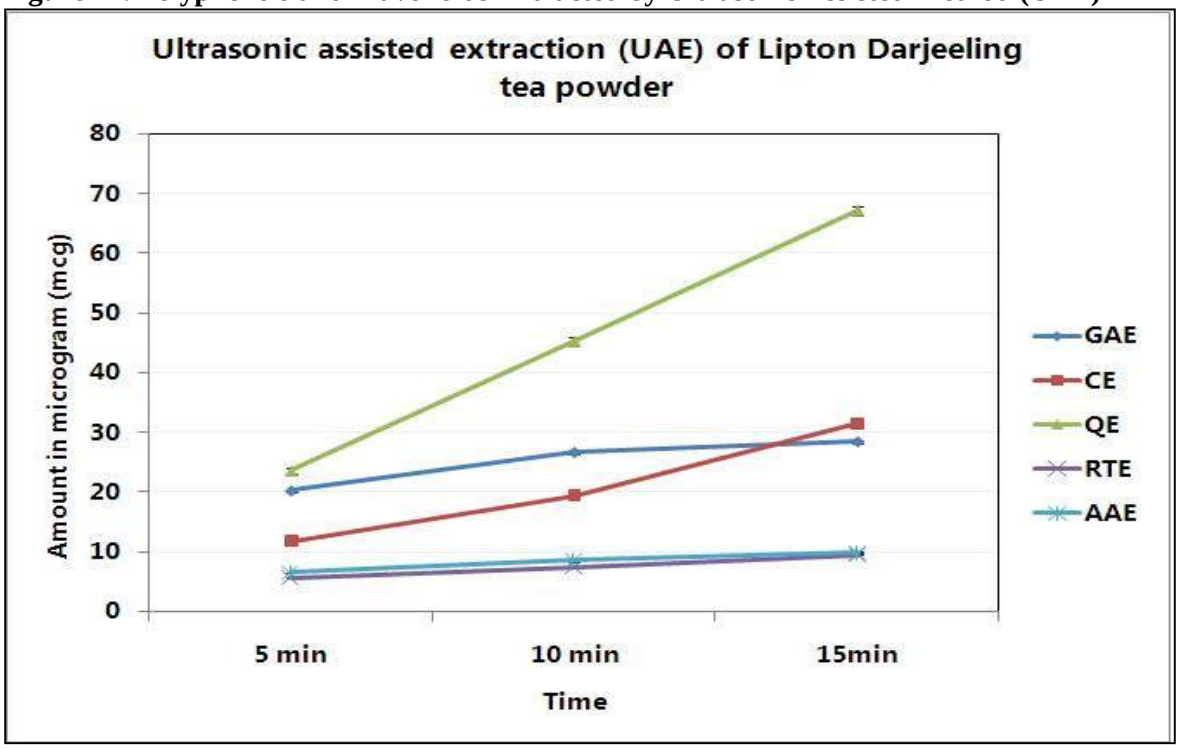
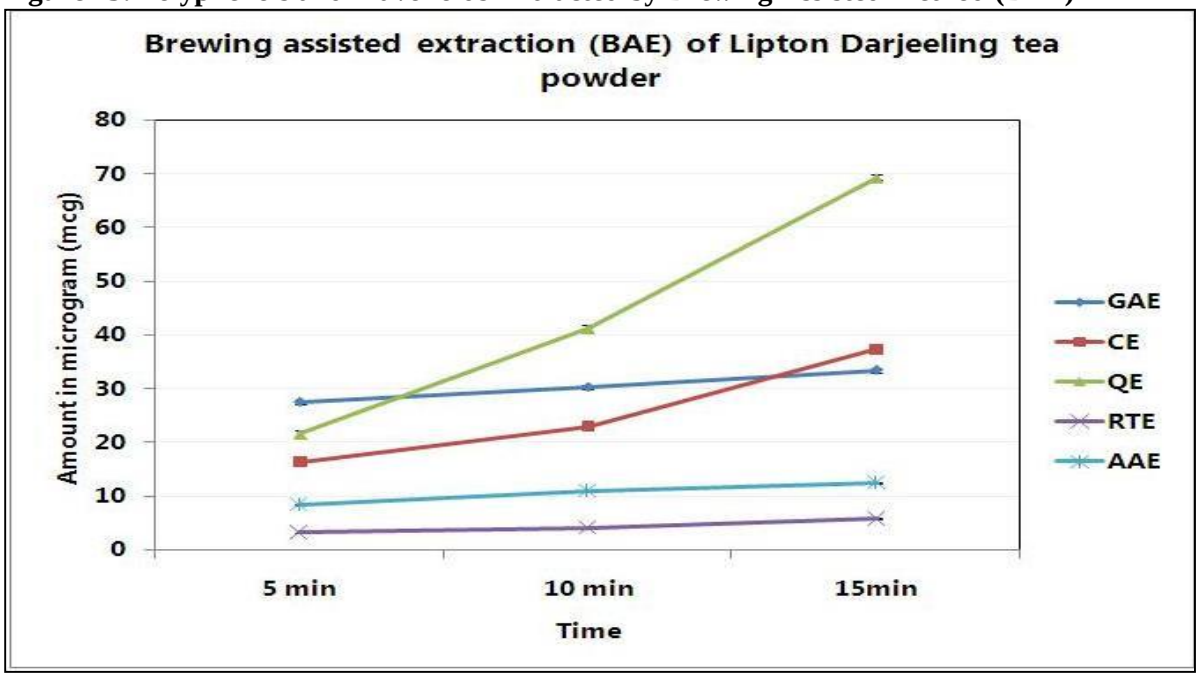


Figure 13: Polyphenols and Flavonoids Extracted by Brewing Assisted Method (BAE)



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