



## ANTIOXIDANT ACTIVITY OF DIFFERENT TEA SAMPLES

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Article Received on 21/06/2017

Article Revised on 11/07/2017

Article Accepted on 31/07/2017

### ABSTRACT

Tea, *Camellia sinensis* is one of the most popular beverages consumed worldwide as green, black or Oolong tea. The present study was carried out to evaluate the antioxidant potential of commercially available seven different tea samples, green tea plain and formulated teas *viz.* green tea lemon, green tea tulsi, green tea mint, branded black tea, unbranded black tea and green tea of Chinese origin. Aqueous extracts of the all the tea samples were evaluated for the qualitative phytochemical screening and assayed for their antioxidant activities *viz.* DPPH, Nitric Oxide radical scavenging assay, Reducing power and Total Antioxidant capacity. The results of qualitative phytochemical screening revealed the presence of several bioactive compounds. The antioxidant activities of all the seven tea samples showed the different tea extracts manifesting varying antioxidant potentials. Further, among the extracts under study green tea tulsi (GTT) and green tea plain (GTP) has exhibited comparatively higher antioxidant activity followed by GTL, GTM, CGT, BT and UBBT. The results on the antioxidant potentiality of different tea samples are discussed.

**KEYWORDS:** Tea, Phytochemicals, Antioxidant activity.

### INTRODUCTION

Reactive oxygen species are formed during normal cellular metabolism, but when present in high concentration they become toxic. Mammalian cells possess intracellular defences such as superoxide dismutase, catalase or glutathione peroxidase, in order to protect the cells against excessive levels of free radicals. Also exogenous addition of compounds such as vitamins (A, E, b-carotene), minerals (selenium, zinc), or proteins (transferrin, ceruloplasmin, albumin) can provide additional protection.<sup>[1]</sup> These natural antioxidants or other compounds that can neutralize free radicals may be of central importance in the prevention of vascular diseases and some forms of cancer.<sup>[2,3]</sup>

Tea is one of the most popular beverages consumed worldwide. Tea, from the plant *Camellia sinensis*, belonging to the family *Theaceae* is consumed in different parts of the world as green, black, or Oolong tea. These teas are differed in processing methods and chemical composition.<sup>[4,5]</sup> The chemical composition of tea leaves has been well documented. The main constituents of tea leaves are polyphenols.<sup>[4]</sup> The fresh tea leaves contain caffeine (approximately 3.5% of the total dry weight), theobromine (0.15–0.2%), theophylline (0.02–0.04%) and other methylxanthines, lignin (6.5%),

organic acids (1.5%), chlorophyll (0.5%) and other pigments, theanine (4%) and free amino acids (1–5.5%), and numerous flavour compounds.<sup>[6]</sup> In addition, a wide variety of other components exists, including, flavones, phenolic acids and carbohydrates, alkaloids, minerals, vitamins and enzymes.<sup>[7]</sup> The flavanols of tea mainly includes quercetin, kaempferol, myricetin and their glycosides.

Several epidemiological studies and clinical trials showed that green tea may reduce the risk of many chronic diseases. This beneficial effect has been attributed to the presence of high amounts of polyphenols which are potent antioxidants. In particular, green tea may lower blood pressure and thus reduce the risk of stroke and coronary heart disease. Some animal's studies have suggested that green tea might protect against the development of coronary heart disease by reducing blood glucose levels and body weight.<sup>[8]</sup> Currently, the tea companies are marketing tea in combination with various formulations such as lemon, mint, honey, ashwagandha, ginger, aloe vera, tulsi *etc.* in order to supplement health benefits of tea and some of these have become popular choice among the consumers. Therefore the present investigation was aimed to

evaluate antioxidant potential of commercially available tea and their formulations.

## MATERIALS AND METHODS

### Materials

Seven different commercially available tea samples were selected. Among them commercially available green tea with infusions and black tea were purchased from Bigbazaar, Shivamogga, Karnataka which were having the same manufacturing month and year. Another tea sample which is of unbranded black tea is purchased from the local market of Shivamoga, Karnataka.

The tea samples comprised of green tea plain (GTP), green tea lemon (GTL), green tea Tulsi (GTT), green tea mint (GTM), black tea (BT), unbranded black tea (UBBT) and China green tea (CGT) procured from China.

### Methods

#### Extraction

The commercially available tea powder samples were extracted by using water. Tea water extract were prepared by adding 20g of ground tea leaves to 400ml of hot water (80°C) for 1h. The mixture was then cool to room temperature, filtered through muslin cloth at first and then through a Whatman No.1 filter paper.<sup>[9]</sup> The infusion was concentrated on water bath for 7days and then in the desiccators. The yield of the crude extracts so obtained is noted, stored in the refrigerator at 4°C for further use.

#### Qualitative phytochemical investigation

The preliminary qualitative phytochemical analysis was performed for testing the different chemical groups present in aqueous extracts of seven different Tea samples.<sup>[10,11,12]</sup>

#### Evaluation of *in vitro* antioxidant activities

##### General chemicals

All chemicals and solvents used in the study were of analytical grade. 2, 2-Diphenyl-1-Picryl Hydrazyl (DPPH), Trichloro acetic acid (TCA), Sodium Nitroprusside are purchased from Himedia, India. Ascorbic acid, monobasic and dibasic sodium phosphate, potassium ferric cyanide, ferric chloride, sulphuric acid, sodium phosphate, ammonium molybdate, sodium chloride, potassium chloride is procured from Sd Fine chem. Ltd, India.

##### Preparation of stock solutions of extracts

The stock solutions of extracts were prepared by dissolving 100mg of dried extracts in 100ml of distilled water to make a stock solution of 1mg/ml. Aliquots from this stock solution were further diluted with distilled water to get the required concentrations of the extract.

#### 2, 2-Diphenyl 1-Picryl Hydrazyl (DPPH) radical scavenging activity

The hydrogen atom or electron donating abilities of the aqueous extracts of tea samples were measured from the bleaching of the purple-coloured ethanolic solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent.<sup>[13]</sup> The different concentrations of each of the extracts were prepared in double distilled water and were added to 3ml of 0.1mM ethanolic solution of DPPH. After 30min incubation at room temperature, the absorbance was read against a blank at 517nm. Effective concentration of free radical DPPH in percent (E%) was calculated by,

$$\text{Percentage Effect} = ((A_0 - A_1) / A_0 \times 100)$$

Where,  $A_0$  is the absorbance of the control reaction (containing all reagents except the test compound) and  $A_1$  is the absorbance of the test extract.

Ascorbic acid was used as the standard. The values of inhibition were calculated in triplicate for the various concentrations of extract. Results have also been reported as  $EC_{50}$ , which is the amount of antioxidant necessary to decrease the initial DPPH• concentration by 50%.

#### Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat, 1964.<sup>[14]</sup> Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction.<sup>[15]</sup> 2ml of 10mM Sodium nitroprusside in 0.5ml phosphate buffer saline (pH 7.4) was mixed with various concentrations of the extracts and the mixture was incubated at 25°C for 150min. From the incubated mixture 0.5ml was taken out and added into 1ml sulfanilic acid reagent (0.33% in glacial acetic acid) and incubated at room temperature for 5min. Finally, 1ml of N-(1Naphthyl) ethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30min. The absorbance at 540nm was measured with a spectrophotometer. The Nitric oxide radicals scavenging activity was calculated as explained previously.

#### Reducing power assay

The reducing power of the compound was evaluated according to Oyaizu, 1986.<sup>[16]</sup> The reductive capability of the extract was quantified by taking 1ml of aqueous extract, mixed with 2.5ml of 0.2M phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide [ $K_3Fe(CN)_6$ ]. The mixture was incubated at 50°C for 20min and reaction was terminated by adding 2.5ml of 10% trichloroacetic acid. Then it was centrifuged at 3000rpm for 10min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and 0.5ml of 0.1%  $FeCl_3$ . The absorbance was measured at 700nm

against a blank sample. Ascorbic acid is used as standard. Increased absorbance of the reaction mixture indicated greater reducing power. EC<sub>50</sub> value ( $\mu\text{g}$  of extract/ml) is the concentration at which the absorbance was 0.5 for reducing power. All the tests were performed in triplicates and the results were recorded.

#### Total antioxidant capacity assay

The antioxidant activity of samples was evaluated by the green Phosphomolybdenum complex formation according to the method of Prieto, 1999.<sup>[17]</sup> The tubes containing different concentrations of aqueous extract is mixed with 4ml of distilled water, 1ml of phosphomolybdenum reagent (28mM of sodium

phosphate and 4mM ammonium molybdate and 0.6M sulphuric acid). The tubes were incubated at 95°C for 90mins. The mixtures are cooled to room temperature and absorbance is measured at 695nm against blank. The experiment was conducted in triplicates and the values are expressed as equivalents of Ascorbic Acid in  $\mu\text{g}/\text{mg}$  of extract.

## RESULTS

### Extraction Yield

Aqueous extracts of seven tea samples were weighed and the yields obtained were noted. GTL extract showed 11.09g of yield per 20g whereas BT extract showed least, 6.33g/20g of yield (Table 1).

**Table 1: Yield of seven different tea samples**

SL No.	Extracts	Yield g/20g
1	GTL	11.09
2	GTP	9.86
3	GTM	9.61
4	GTT	7.43
5	CGT	7.61
6	UBBT	8.36
7	BT	6.33

### Preliminary phytochemical analysis

The preliminary qualitative phytochemical analysis revealed that aqueous extracts of seven tea samples showed the presence of carbohydrates, proteins, amino

acids, steroids, saponins, flavanoids, alkaloids, tannins and polyphenols. The results of the analysis are shown in Table 2.

**Table 2: Results of Qualitative analysis for Phytoconstituents in seven tea samples**

Sl no	TESTS	Green tea				Black Tea		China tea
		GTP	GTL	GTT	GTM	BT	UBBT	CGT
1	Carbohydrates	+	+	+	+	+	+	+
2	Proteins	+	+	+	+	+	+	+
3	Amino acids	+	+	+	+	+	+	+
4	Steroids	+	+	+	+	+	+	+
5	Triterpenoids	+	+	+	+	+	+	+
6	Saponins	+	+	+	+	+	+	+
7	Flavanoids	+	+	+	+	+	+	+
8	Alkaloids	+	+	+	+	+	+	+
9	Tannins and Polyphenols	+	+	+	+	+	+	+

### Evaluation of *in vitro* antioxidant activities

#### DPPH radical scavenging activity

DPPH radical scavenging activity of the seven tea samples at varying concentrations were measured along with standard, Ascorbic acid. The aqueous extract of tea samples exhibited significant DPPH radical scavenging activity, with 50% effective concentration (EC<sub>50</sub>) at concentrations of 9.611, 9.66, 15.855, 37.309, 39.689, 52.866 and 59.369 $\mu\text{g}/\text{ml}$ , for GTP, GTT, GTL, GTM,

CGT, BT and UBBT respectively. The EC<sub>50</sub> value of standard Ascorbic acid is 9.44 $\mu\text{g}/\text{ml}$  (Fig 1). It is evident from the results that GTP and GTT aqueous extracts have the strongest radical scavenging activity as per with the standard. It is interesting to note that excepting green tea plain, that all the formulated teas have registered better antioxidant potential versus non formulated teas for the said parameter.

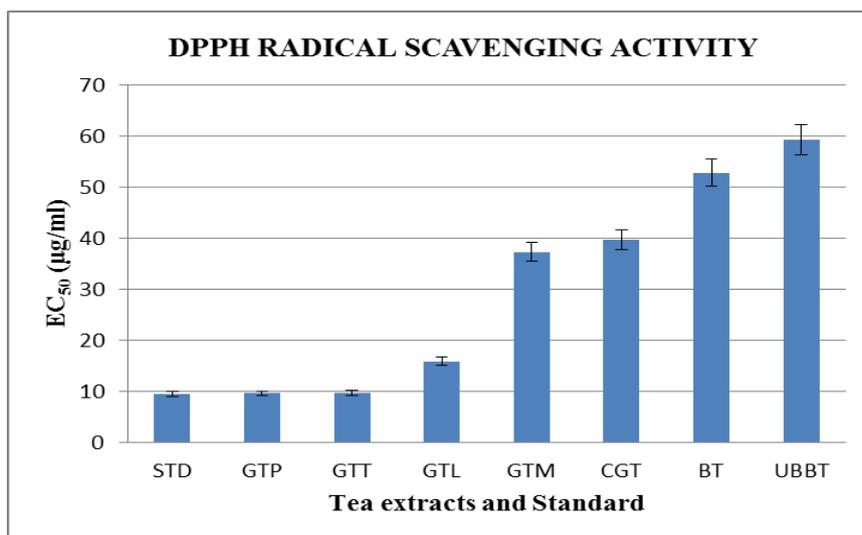


Fig 1: DPPH radical scavenging activity (EC<sub>50</sub>) of seven aqueous tea extracts

#### Nitric Oxide radical scavenging activity

The Aqueous extracts of seven tea samples showed a better activity in competing with oxygen to react with nitric oxide and thus the inhibition of anions. The extracts exhibited an EC<sub>50</sub> values of 45.270, 46.848, 48.320, 60.46, 60.805, 67.468 and 68.665 µg/ml for GTT,

GTL, GTM, GTP, CGT, BT and UBBT respectively whereas ascorbic acid has lower EC<sub>50</sub> value of 44.740 µg/ml (Fig 2). It is evident from the results that formulated teas *viz.* GTT, GTL and GTM possess the strongest radical scavenging activity compared to the other green tea and black tea extracts.

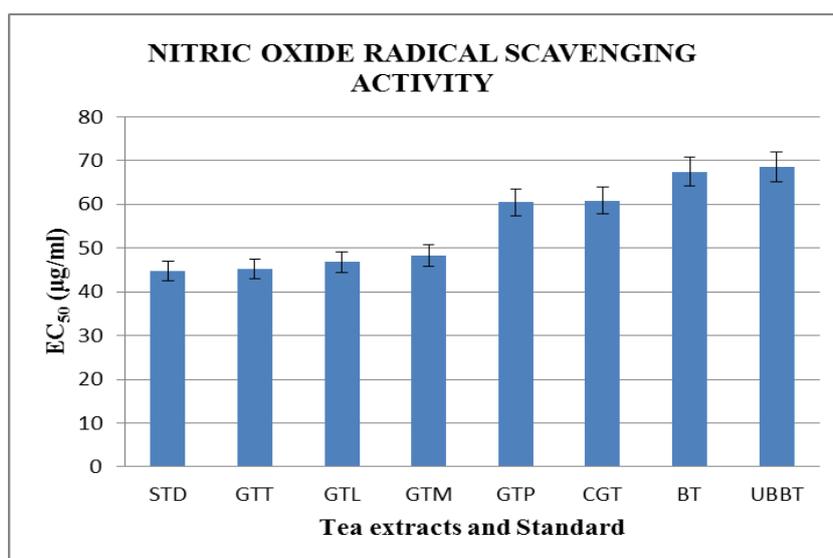


Fig 2: Nitric oxide radical scavenging activity (EC<sub>50</sub>) of seven tea extracts.

#### Reducing power assay

The results revealed that among the seven tea samples, the reducing power was found to be high in GTT with an effective concentration (EC<sub>50</sub>) of 105.93 µg/ml followed by GTM (112.86 µg/ml), CGT (133.21 µg/ml), GTL

(208.76 µg/ml), GTP (211.86 µg/ml), BT (250.5 µg/ml) and UBBT (280.26 µg/ml). The reductive capabilities of all the extracts were compared with standards ascorbic acid which is recorded an EC<sub>50</sub> value of 35.71 µg/ml (Fig 3).

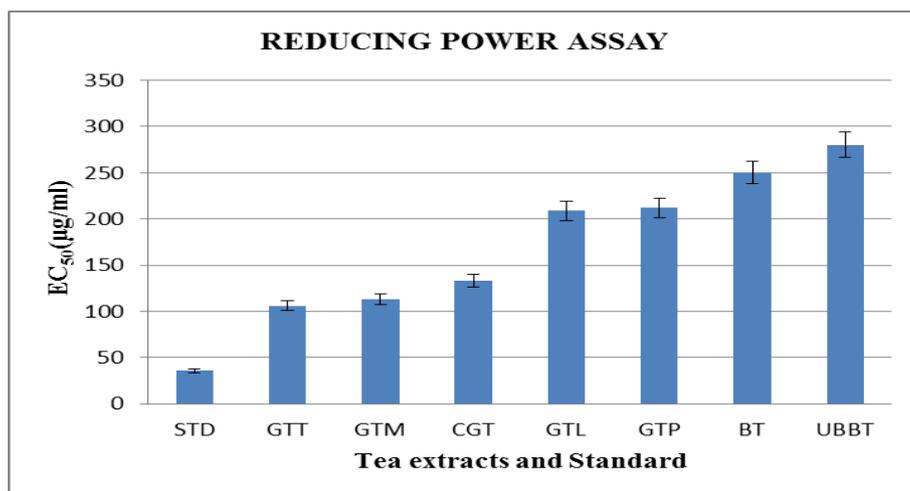


Fig 3: Reducing power assay of seven aqueous tea extracts

**Total antioxidant capacity**

The aqueous test extracts showed very potent total antioxidant capacity. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid (µg/ mg of extract). In the aqueous tea extracts, total

antioxidant capacity was found to be highest in GTP and GTL, followed by GTM, GTT, CGT, BT and UBBT and the values were 156.36, 156.14, 155.08, 149.94, 143.75, 93.68 and 84.705 µg of ascorbic acid / mg of extract respectively (Fig 4a and 4b).

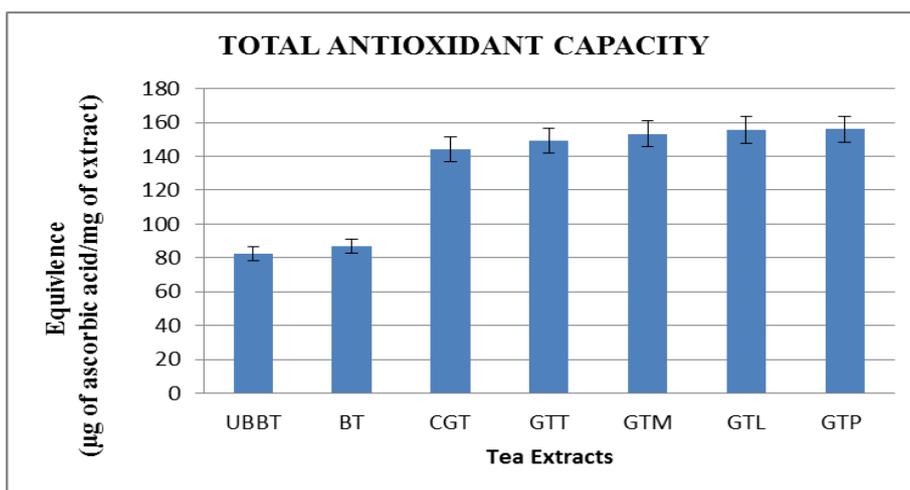


Fig 4a: Total antioxidant capacity of seven teas extracts (Equivalents of ascorbic acid)

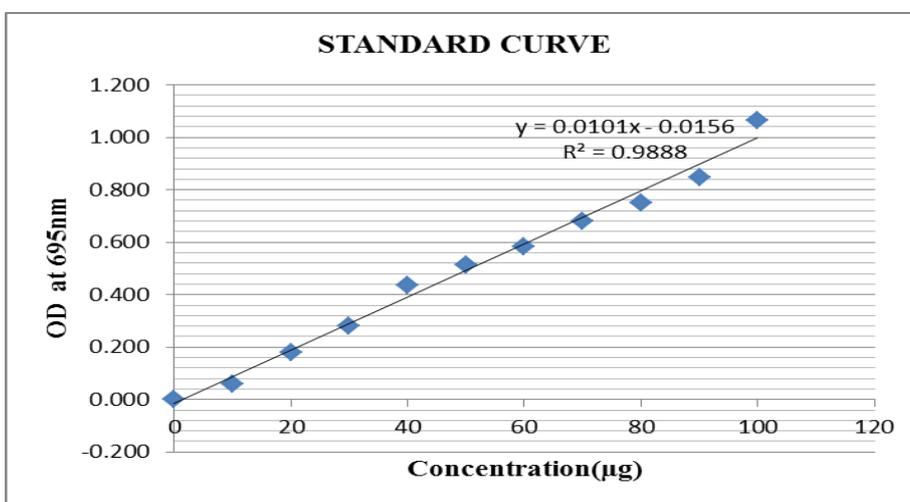


Fig 4b: Total antioxidant capacity of standard Ascorbic acid

## DISCUSSION

In recent years, potent free radical scavengers have attracted a tremendous interest as possible therapeutics against free radical mediated diseases. Free radicals are constantly generated in cells, either as accidental by-products of metabolism or deliberately.<sup>[18]</sup> When an imbalance between free radical generation and body defence mechanisms occurs, oxidative damage will spread over all the cell targets (DNA, lipids and proteins). It has been reported that a series of human illness such as cancer, atherosclerosis, cardio and cerebrovascular diseases, diabetes, immune system impairment, neurodegenerative diseases can be linked to the damaging action of extremely reactive free radicals.<sup>[19,20]</sup> There is increasing evidence suggesting that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and currently the research is focused on exploring the protective biochemical functions of natural antioxidants contained in spices, herbs and beverages.<sup>[21,22]</sup>

In the present study, tea extracts were tested for different phytochemical analysis and further screened to test their antioxidant potentials. The result of qualitative phytochemical analysis revealed that, aqueous tea extracts are bestowed with the presence of several bioactive compounds *viz.* glycosides, alkaloids, flavonoids, tannins and polyphenols which therefore encourages further antioxidant studies. From the results of four antioxidant assays performed in this investigation excepting plain green tea, the formulations of green tea with tulsi, lemon and mint were found to be superior over Chinese green tea and other black tea tested in all the assays wherein tulsi formulated tea was particularly better among the other formulations revealing its potential advantage. Among the other non formulated green tea extracts, green tea plain was found to be better in terms of antioxidant effect versus Chinese green tea. Further it is clear from the results that the green tea irrespective of formulations was clearly better over the black tea *viz.* branded black tea and unbranded black tea.

Plant tissues are naturally rich in nutritive or therapeutically active products of plant secondary metabolism. In the present study teas with different formulations have shown variable antioxidant potency, wherein Green tea with different formulations like Tulsi, Lemon and Mint exhibiting higher antioxidant effects could be ascribed to different bioactive constituents with different antioxidant activities. It may be due to synergistic, additive or antagonistic interaction among different compounds. The principal aim of this research is to identify the synergistic mixtures. As antioxidant potential depends upon the presence of phenolic compounds and flavonoids thus the higher antioxidant potential of GT Tulsi can be attributed to the high amounts of polyphenols like eugenol, cardinene, cubenol and flavon-3-ols, catechin, epicatechin, epicatechin gallate and their fermentative products—theoflavins, thearubigin dissolvable in water. Further the presence of

vitamins A, B, C, and E in the tea extracts which are considered as potent scavengers of free radicals and reactive oxygen species may be responsible for the antioxidant activity. Many of these phytochemicals may help to protect cells against the oxidative damage caused by free radicals.<sup>[23]</sup> The most powerful antioxidant tea is green tea which is characterized by the presence of large amount of flavan-3-ols known as catechins. (-) Epigallocatechin-3-gallate (EGCG) is most abundant catechin in green tea and may occur up to 50% of the catechins by weight and black tea is fully oxidized. The result of catechins oxidation is the formation of catechins dimers, known as theaflavins. These compounds are responsible for the colour and taste and also a key factor in the antioxidant activity.<sup>[24]</sup>

Tulsi is a traditionally important medicinal local herb containing many potent and useful compounds.<sup>[25]</sup> The ancient systems of medicines including Ayurveda, Greek, Roman, Siddha and Unani have mentioned its therapeutic applications in cardiovascular disorders.<sup>[26]</sup> In traditional systems of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of tulsi have been recommended for the treatment of bronchitis, bronchial asthma, malaria, diarrhoea, dysentery, skin diseases, arthritis, chronic fever and insect bite *etc.* Tulsi has also been suggested to possess anti-fertility, anticancer, anti-diabetic, anti-fungal, anti-microbial, hepatoprotective, cardioprotective, analgesic and adaptogenic properties.<sup>[27]</sup> The present investigation suggesting higher antioxidant activity as a supplement in green tea therefore contributes to the extra value of the green tea.

Another important flavouring agent with tea is lemon which is having high antioxidant potency. The health benefits of lemon (*Citrus limon*) and radical scavenging activity of its peels and juices have also been attributed to the presence of bioactive compounds, such as phenolic compounds and vitamin C, all powerful antioxidants. Due to its special flavour, it has been used to prepare flavoured teas widely related to traditional and complementary healthcare approaches, such as reducing skin cancer or helping during cold and flu season.<sup>[28,29,30,31]</sup> Therefore the use of the lemon as an ingredient in green tea therefore could be complimentary.

*Mentha spicata* (Mint) have traditionally been used in folk medicine. The distinctive smell and flavour is a characteristic feature of Mint and it is due to the naturally occurring cyclic terpene alcohol called menthol. Menthol is prescribed as a medication for gastrointestinal disorders, common cold and musculoskeletal pain.<sup>[32]</sup> Infusion, decoction and distilled water of the aerial parts of Mint species have been used for centuries as tonics, carminative, digestive, stomachic, antispasmodic and anti-inflammatory agents in Iranian Traditional Medicine.<sup>[33,34]</sup> Due to its spectacular ability of smell, flavour and rich antioxidant

activity, its use as an additive with green tea is therefore justified.

Therefore the present study reveals that green tea with different formulations enhances the good antioxidant potential. Among the seven tea samples, GTT has showed the highest antioxidant activity followed by the GTP, GTL, GTM, CGT, BT and UBBT. Since the green tea formulated with various additives of tulsi, mint lemon *etc.*, each of which has other therapeutic benefits for various ailments of man, the authors are of the opinion that such formulated add on teas should serve as an value added but customised therapeutic approach and such products therefore in turn could be vital in the future of functional food or beverage industry.

### CONCLUSION

The present study suggest that all the seven aqueous Tea extracts possess potent antioxidant activity, even though extent potential varies from case to case, which might be helpful in preventing or slowing the progress of various oxidative stress- related diseases. From the results it was revealed that tea with flavours like Tulsi, Lemon and Mint manifested the higher antioxidant activity due to the presence of rich polyphenols. Among different tea samples GTT has showed the best antioxidant potential.

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