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Evaluation of antioxidant activity of locally available green teas in India

Yasser Hussein Issa Mohammed, Guruprasad N. and Shaukath Ara Khanum*

Department of Chemistry, Yuvaraja's, University of Mysore, Mysore, Karnataka, India

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ABSTRACT

Green Tea is one of the most ancient and popular therapeutic beverages consumed around the world. This product is made from the leaf of the plant called "Camellia sinensis". It can be prepared as a drink, which can have many systemic health effects or an "extract" can be made from the leaves to use as medicine. 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. The aim of recent study was achieved to compare the antioxidant properties and phenolic contents. In this view the antioxidant efficacy of green tea extract and the effectiveness compared to the standard antioxidant, ascorbic acid based on many methods have been demonstrated.

Keyword: Extract, Antioxidant, Green tea, Phenols, Flavonoids, *Camellia sinensis.*

INTRODUCTION

Green tea is one of the popular natural health drinks. Tea, from the plant Camellia sinensis, is consumed in different parts of the world as green, black, or Oolong tea. Among all of these, the most significant effects on human health have been observed with the consumption of green tea. [1-9] In recent years, the health benefits of consuming green tea, including the prevention of cancer and cardiovascular diseases, the anti-inflammatory, anti-arthritic, anti-bacterial, anti-angiogenic, anti-oxidative, anti-viral, neuroprotective and cholesterol-lowering effects of green tea and isolated green tea constituents are under investigation.[10-19] Green tea consumption has also been linked to the prevention of many types of cancer, including lung, colon, esophagus, mouth, stomach, small intestine, kidney, pancreas, and mammary glands. Several epidemiological studies and clinical trials showed that green tea may reduce the risk of many chronic diseases. [20, 21] The flavonoids have potent scavenging reactive oxygen and chelating redox active. The aim of recent work was managed to compare the antioxidant properties of different commercial green. [22] Antioxidant activity of tea infusions was determined based on common synthetic free radical scavenger by 2,2-diphenyl-1-picrylhydrazyl (DPPH), total phenol and flavonoids content.[23]

Figure 1: Green Tea of Locally Available Green Teas In India

MATERIALS AND METHODS

Preparation of The Sample

The sample was prepared by weight 1g of the sample then added 10ml of 70% methanol, added magnetic bits shaking for 20 min, the solution placed to centrifugal tube, centrifuged at 4000 rpm for 20 min then collected superannuated, dried the superannuated in water bath at 40 ºC to get solid residue and added 1ml of 70% methanol to the solid residue, then collected the solution up&off tube for analysis.

1. In-vitro antioxidant assays

1.1. 1,1-Diphenyl-2-Picrylhydrazyl Radical Scavenging Activity (DPPH)

Stock solution of each mushroom extract (50mg/mL) was diluted to a concentration in the range of 0.1 to 50 mg/mL. For the test, 3.9mL of 0.06mM DPPH radical (Sigma) was added to 0.1mL of mushroom extract. Reaction mixture was vortexed and absorbance was measured at 515nm using a spectrophotometer with methanol as the blank.[24] The decrease in absorbance was monitored at 0min, 1min, 2min, and every 15min until the reaction has reached a plateau. The time taken to reach the steady state was determined by one-way analysis of variance (ANOVA). The DPPH free radical scavenging activity, expressed as percentage of radical scavenging activity, was calculated as follows:

$$\text{Radical scavenging activity (SA)} = \{(A0 - As)/ A0\} * 100$$

Where, A0 is the absorbance of 0.06mM methanolic DPPH only whereas as is the absorbance of the reaction mixture.

1.2. Fe^{2+} Metal-chelating Ability

Mushroom extract (1mg/mL) was mixed with 3.7 mL of distilled water. It was then reacted with a solution containing 0.1 mL 2 mM $Fecl_2$ and 0.2 mL of 5 mM ferrozine. After 10 min, the absorbance of the reaction mixture was measured at 562 nm.[25]

$$\% \text{ Metal-chelating ability} = [1-(\text{O.D. of the sample}/ \text{O.D. of the control})]*100$$

1.3. Reducing Power Ability

The reducing power can be determined by the method of 1.0 ml extract is mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM) and incubated at 50°C for 20 min. [26] Thereafter, 2.5 ml of trichloroacetic acid (600 mM) is added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) is mixed with 2.5 ml of distilled water and 0.5 ml of FeCl3 (6 mM) and absorbance is measured at 700 nm. Ascorbic acid, butylated hydroxyanisole (BHA), _-tocopherol, trolox (Oyaizu, 1986) or butylated hydroxytoluene (BHT) (Jayaprakasha et al., 2001) can be used as positive control.

2. Determination of Total Phenolic compounds

Total phenolic compounds in the ethanol extracts were determined using Folin–Ciocalteu method. 1mL of the extract was added to 10.0 mL distilled water and 2.0 mL of Folin-Ciocalteu phenol reagent. The mixture was allowed to stand at room temperature for 5 min and then 2.0 mL of 20% sodium carbonate was added to the mixture. The resulting blue complex was then measured at 680 nm. Catechin was used as a standard for the calibration curve.

The phenol compound was calibrated using the linear equation based on the calibration curve. The contents of the phenolic compound were expressed as mg catechin equivalent/g dry weight. As shown in table 1

3. Determination of Total Flavonoid concentration

The $AlCl_3$ method was used for determination of the total flavonoid content of the sample extracts. Aliquots of 1.5 mL of extracts were added to equal volumes of a solution of 2% $AlCl_3$ (2 g in 100 mL methanol). [27-29] The mixture was vigorously shaken, and absorbance at 367 nm was read after 10 min of incubation. Quercetin was used as a standard for the calibration curve. The flavonoid compound was calibrated using the linear equation based on the calibration curve. The contents of the flavonoid compound were expressed as mg Quercetin equivalent/g dry weight. As shown table 2

RESULTS AND DISCUSSION

DPPH scavenging activity: DPPH is nitrogen cantered free radical that shows strong absorbance at 517 nm. Deep violet colured of DPPH solution changes to yellow in presence of DPPH radical scavengers. DPPH radical accepts an electron to become a stable diamagnetic molecule. Extent of DPPH radical scavenged was determined by the decrease in intensity of violet colour in the form of IC_{50} values. Lower IC_{50} value represents higher antioxidant activity. To evaluate the free radical scavenging activity, the mushroom extracts were subjected to scavenge DPPH by donating an electron. Results confess that scavenging activity was increased with increasing concentration of green tea extract. The results of DPPH radical scavenging activity revealed that the Ellachi flavor shown potent antioxidant activity with IC_{50} $115.85^{\pm}15$ compare to others extracts. all extracts were increased with increasing concentration. (Results shown in figure 1.1)

Metal chelating efficiency

Iron is known to generate free radicals through the Fenton and Haber–Weiss reaction (Halliwell and Gutteridge 1990). Metal ion chelating capacity plays a significant role in antioxidant mechanism since it reduces the concentration of the catalysing transition metal in lipid peroxidation (Duh *et al*., 1999). It is reported that chelating agents, which form *s*-bonds with a metal, are effective as secondary antioxidants since they reduce the redox potential thereby stabilizing the oxidized form of the metal ion (Gordon, 1990). Metal chelating efficiency of the extracts was estimated over a wide range of concentration (0-500ppm) and also compared with EDTA, the standard chelating agent. EDTA showed 98% chelation at ≥400ppm. The results of Fe^{2+} chelating ability revealed that % inhibition increased with increasing concentration. (Results shown in figure 1.2)

Reducing power ability

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Reducing power was 46.84 mg Ascorbic acid equivalent / g of green tea sample. The reducing power of the green tea extract increases with the increase in concentration. All the methods have proven the effectiveness of green tea extract compared to the standard antioxidant, ascorbic acid. (Results shown in figure 1.3).

RESULTS

Figure 1.1: DPPH radical scavenging activity of the green tea flavor

Types of green tea flavor	IC_{50}
Lemon flavor	173.92±28
Masala flavor	148±17
Ginger flavor	230±21
Ellachi flavor	115.85±15
GML flavor	168.87±22

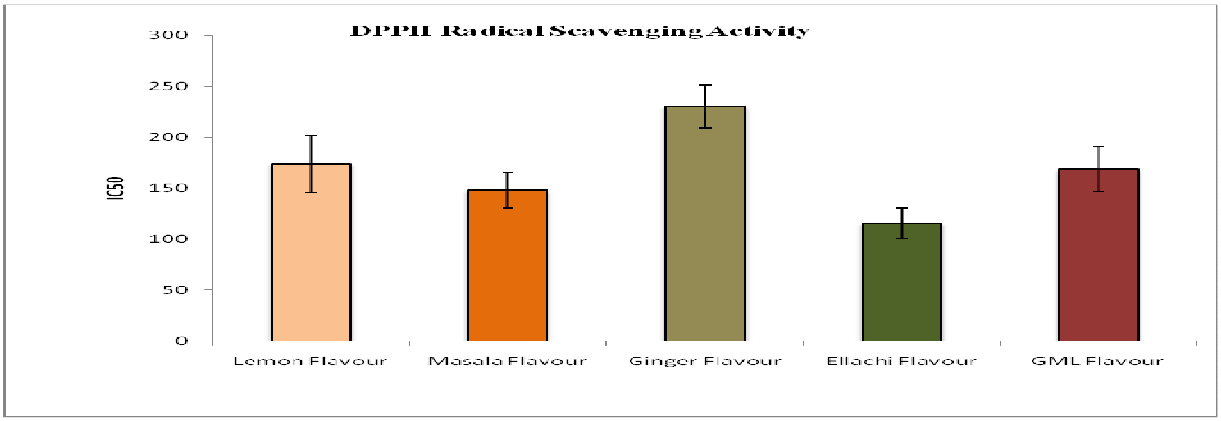


FIGURE 1.2: Metal chelating efficiency of green tea flavors

Types of green tea flavor	IC50
Lemon flavor	1149±57
Masala flavor	842±34
Ginger flavor	489±37
Ellachi flavor	345±24
GML flavour	720±48

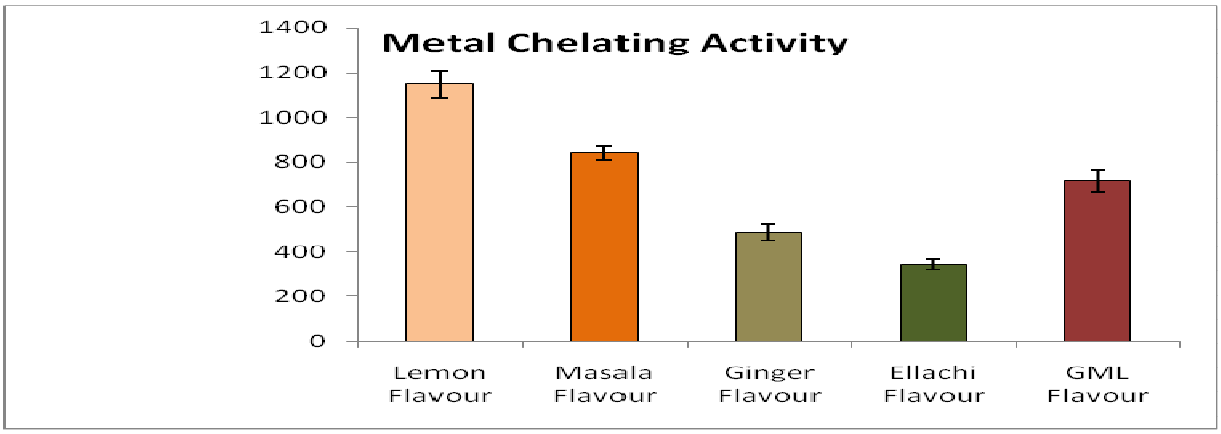
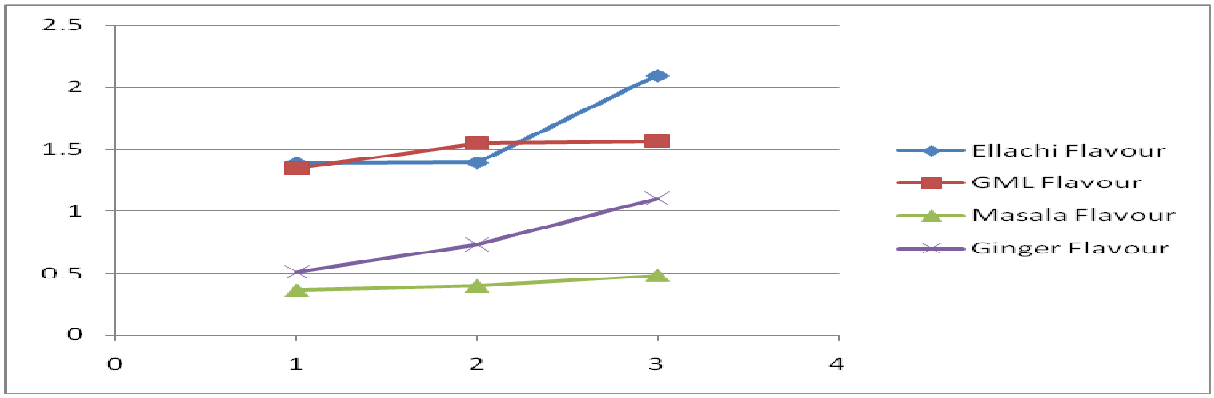


Figure 1.3: Reducing ability increases with the increase in concentration.

Types of green tea flavours	Absorbance		
	Reading 1	**Reading 2**	**Reading 3**
Ellachi flavour	1.38732	1.39131	2.093793
GML flavour	1.348783	1.553658	1.56475
Masala flavour	0.362897	0.397959	0.485048
Ginger flavour	0.5113	0.7336	1.1041



Polyphenols

Table 1 shown amount of Gallic acid in sample

Types of green tea flavours	Amount of Gallic acid in mg
Lemon Flavour	127.6 mg GAE/g Sample
Masala Flavour	81.2 mg GAE/g Sample
Ginger Flavour	188.1 mg GAE/g Sample
Ellachi Flavour	167.5 mg GAE/g Sample
GML Flavour	28.7 mg GAE/g Sample

Flavonoids

Table 2: 1 shown amount of quercetin in sample

Types of green tea flavours	Amount of quercetin /g sample
Lemon Flavour	52.8mg quercetin/g sample
Masala Flavour	27.68mg quercetin/g sample
Ginger Flavour	78.8mg quercetin/g sample
Ellachi Flavour	55.5mg quercetin/g sample
GML Flavour	17.7mg quercetin/g sample

CONCLUSION

Natural antioxidants have great impact on the safety and acceptability of the food system and will continue to do so. Not only do they keep the food stable against oxidation but can also be effective in controlling microbial growth. Although all organism possess antioxidant system for protection from oxidative damage, this system are insuffient to prevent all possible damage. That is why the interest towards the inclusion of nontoxic combination of nutritional and medicinal benefits may determine green tea extract as a functional food.

The properties of the green tea extract under the study compared with the synthetic antioxidant determine its potential as a natural preservative applicable in the food and pharmaceutical industrial practice of adding antioxidant during processing can still play a very important role since the added compounds have the potential for enhancing the activity of inherent antioxidant system. More work is needed to define the optimum dietary combination for obtaining the greatest stability in the resultant product. This will eventually require sophisticated feed formulations better understanding of the nutrient impact of the by products that are traditionally used as food. Green tea is an indispensable component of Indian culinary. With regard to antioxidant properties of green tea extract established in this work, can be successfully used reducing strees and obesity and other medical field as an antioxidant.

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