

Original Research Article

Antioxidant activity of *Camellia sinensis* leaves

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A B S T R A C T

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Antioxidants are substances found in the medicinal plants which may have a protective role to play in certain conditions such as heart disease, stroke and some cancers. By relaying on these benefits we have traced out the presence of antioxidant in *Camellia sinensis* leaves. The bioactive compound analysis showed the presence of steroids, alkaloids, tannins and flavonoids by changing the colour of the reaction mixture when treated with respective reagents. While the absence of terpenoids, saponins and glycosides was shown by no colour change in the reaction mixture. The total phenolic content was 0.8 grams in per gram of leaf extract while the flavonoid content was 16mg per gram of leaf extract. Reducing power of *Camellia sinensis* was 0.13grams in per gram of leaf extract. The antioxidant activity was 70% in per gram of leaf extract. The methanol extract of *Camellia sinensis* showed highest antioxidant activity 325.76 ± 0.14 mg than the ascorbic acid with 298.98 ± 0.14 mg.

Introduction

All beneficial effects of tea have been attributed to the strong anti-oxidative activity due to phenolic compounds catechins which protect the body from damage caused by free radical-induced oxidative stress (Jang *et al.*, 2007). Carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols are some of the antioxidants produced by the plant for their sustenance (Bajpai *et al.*, 2005). The oxidization process modifies the type and

total level of flavonoids present but not their overall antioxidant activity (Kaneria *et al.*, 2009). Antioxidants found in both green and black tea may have a protective role to play in certain conditions such as in cancer, cardiovascular disease, neurodegenerative diseases and in the aging process (Sarkar 2001; Zhong, 2001). Antioxidant defense systems consist of enzymatic superoxide dismutase, catalase, glutathione, peroxidase (Thangapazham *et al.*, 2007).

The ascorbic acid, glutathione and α -tocopherol compounds can maintain the balance between reactive oxygen species generation and protect from damage done by oxygen reactive species (Astley 2003). Antioxidants provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and concomitant lipid peroxidation, protein damage and DNA strand breaking (Srinivasan *et al.*, 2007). Antioxidant compounds like Phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Kumaraswamy *et al.*, 2008). Natural antioxidants tend to be safer and also possess anti-viral, anti-inflammatory, anti-cancer, antimutagenic, antitumor and hepatoprotective properties (Baravalia *et al.*, 2009).

Materials and Methods

Collection of the Sample

The fresh leaves of *Camellia sinensis* were collected by following (Tariq and Reyaz, 2012) from the dense tea state garden at Ooty, Coimbatore district, Tamil Nadu South India.

Preparation of the Extract

The leaves of fresh samples were cleaned and washed under running tap water (Tariq and Reyaz, 2012). The samples were dried in the oven at 37°C for 6 days. After drying the samples were weighed and blended with warring blender and soaked with methanol [in ratio methanol: plant (6:1)] for 2 days and filtered using Whatman No. 1 paper. The methanol was completely removed by vacuum evaporator at 50°C to give viscous mass. The crude extracts were weighed and

stored at 4°C before analysis.

Determination of Total Phenolic Content

Determination of total phenolic content was carried out according to Marinova *et al.*, (2005) by taking 100 μ l methanol extract and dissolved in 1ml (1:1ratio) of the Folin–Ciocalteu reagent. The solutions were mixed and incubated at room temperature for 1 minute. After 1 minute, 1ml of 35% sodium carbonate solution was added and made up to 8ml with distilled water. The final mixture was shaken and then incubated for 90 minutes in the dark place at room temperature. The absorbances of all samples were measured at 725nm in ultra violet spectrophotometer by keeping Gallic acid as standard. The triplicate measurements were carried out and total phenolic content was expressed as milligram of gallic acid equivalents (GAE) per 100 gram of samples.

Determination of Total Flavonoids Content

The flavonoid content was estimated by Marinova *et al.*, (2005) in which 0.5 ml of the sample was added to a test tube containing 1.5 ml of methanol and mixed uniformly. Then followed by the addition of 0.3 ml of 5% sodium nitrite solution and allowed to stand for 5 min. To this 0.3 ml of 10% aluminium chloride was added and after 6 minutes 1ml of 1M sodium hydroxide was added then mixture was diluted with distilled water. The absorbance of the solution mixture was measured immediately at 510nm in ultra violet spectrophotometer by keeping Apigenin as standard. The flavonoid content was expressed as milligram catechin equivalents/g sample.

Assay of Reducing Power

The reductive capability of the extract was quantified by the method of Oyaizu (1986). One ml of methanolic extract was mixed with 2.5ml of 0.2M phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. The reaction was terminated by adding 2.5ml of 10% trichloroacetic acid. Then this was centrifuged at 3000rpm for 10 minutes. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and 0.5ml of 0.1% FeCl₃. Blank reagent is prepared as above without adding extract. The absorbance was measured at 700nm in ultra violet spectrophotometer against a blank sample. Ascorbic acid was used as standard. The increased absorbance of the reaction mixture indicated greater reducing power

Antioxidant Activity

Evaluation of Total Antioxidant Capacity by Phosphomolybdenum Method

The total antioxidant capacity of *Camellia sinensis* and its different fractions was evaluated by the method of Locatelli *et al.*, (2010). An aliquot of 1 ml of sample (1mg) solution was combined with 1ml of reagent (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution was measured at 695nm in ultra violet spectrophotometer against blank. The total antioxidant capacity of the medicinal plant *Camellia sinensis* was expressed as the number of gram equivalent of ascorbic acid.

Determination of Free Radical Scavenging by DPPH Method

The free radical scavenging capacity of the plant extracts were determined using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay as described by Braca *et al.*, (2001) with a slight modification. The tested sample was mixed with 95% methanol to prepare the stock solution (5mg/mL). A 1.0mL of DPPH (0.25mM) in methanol was taken in tubes and 2.0mL solution of various concentrations of plant extract was added. The reaction mixture was then allowed to stand at room temperature in a dark chamber for 30minutes. The change in colour from deep violet to light yellow was then measured at 518nm in ultra violet spectrophotometer. The test samples were measured in three replicates and Ascorbic acid was used as a reference standard. A 95% methanol was served as blank. Percentage of DPPH scavenging activity was calculated as % inhibition of DPPH = $[\text{Abs control} - \text{Abs sample} / \text{Abs control}] \times 100$.

Result and Discussion

Identification of Leaves

The leaves belonged to the Kingdom-Plantae, Order-Ericales, Family-Theaceae, Genus-*Camellia*, Species -*sinensis*.

Qualitative Analysis of Phytochemicals

The extract showed the presence of phytochemicals namely alkaloids, flavonoids, steroids, gallic tannins and catecholic tannin by changing the colour of the solution to yellow, white, green bluish, blue, green black respectively. While indicated the absence of terpenoid, saponins, and glycosides as there was not colour change in the solution with respect to them.

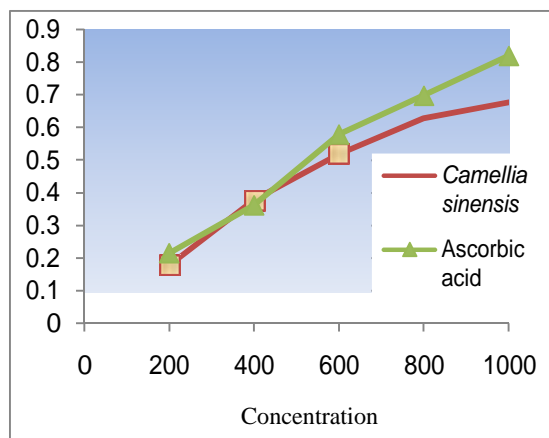
Total Phenolic and Flavonoid Content

The total phenolic content of the medicinal plant *Camellia sinensis* was estimated and found 3.015 ± 0.171 g of gallic acid per 100g DW while the total flavonoid content of the medicinal plant *Camellia sinensis* was 0.324 ± 0.007 g CE per 100g DW.

Reducing Power Assay

The reducing power of extract for *Camellia sinensis* was observed to rise as the concentration of the extract gradually increased (Figure 1).

Figure. 1 *Camellia sinensis* leaf extract showing the antioxidant activity (12mg/gram) by phosphomolybdenum method. Where B is a control; S1, S2, S3, S4 and S5 are ascorbic standards; and T1 and T2 are test samples.



Total Antioxidant Activity

The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximum absorption at 695nm in ultra violet spectrophotometer (Figure 2). The methanol extract of *Camellia sinensis* has higher antioxidant



Figure 2. Phosphomolybdenum method showed that *Camellia sinensis* leave extract showed the antioxidant activity of 12mg/gram where B-Control, S1, S2, S3, S4 and S5 are ascorbic acid standards. The T1 and T2 are test samples

capacity 325.76 ± 0.14 mg than to ascorbic acid with 298.98 ± 0.14 mg.

DPPH Radical Scavenging Activity

The DPPH free radicals scavenging activity of the compounds was measured by the decolorizing effect following the trapping of the unpaired electrons of DPPH (Figure 3). The plant extract of *Camellia sinensis* exhibited antioxidant activity with an IC_{50} value of $70.25 \pm 2.85 \mu\text{g/mL}$ when compared to the IC_{50} value of ascorbic acid $54.72 \pm 2.19 \mu\text{g/ml}$.

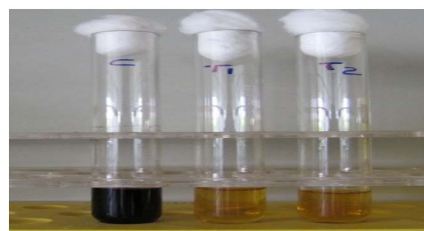


Figure 3. DPPH assay in which 200ml of DPPH mixed well, dissolved in 0.8 ml of methanol and optical density values were taken at 510nm and found containing 70% antioxidant activity Where C-control. T1 and T2 are test samples of *Camellia sinensis*

Tea (*Camellia sinensis*) is consumed worldwide and is second only to water in

its popularity as a beverage (Atoui *et al.*, 2005). Medicinal plants are important source for the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents. The presence of phytochemical namely alkaloids, flavonoids, steroids, gallic tannins, catecholic tannin plays the vital role in the plant defense mechanisms to counteract reactive oxygen species in order to survive and prevent molecular damage and caused by microorganisms, insects, and herbivores (Akowuah *et al.*, 2005). In this work 1gram of *Camellia sinensis* leaves extract contained 0.7gram of phenolic compounds (Bajpai *et al.*, 2005). The reduction of DPPH in the presence of a radical scavenger or hydrogen donors leads to the formation of non-radical form of DPPH-H.

The antioxidant activity of *Camellia sinensis* values was found 12 μ g/ml (Nooman *et al.*, 2008; Yang *et al.*, 2002). There was no correlation between total phenolic content and antioxidant capacities of a number of medicinal plants extracts (Setiawan 2001). Reducing power has been used as an antioxidant indicator for the medicinal herbs (Hsu *et al.*, 2003). Reducing ability of compound generally depends on the presence of reductants which exhibit antioxidant activity by breaking the free radical chain through donation of a hydrogen atom (Rathee *et al.*, 2009). In the reducing power assay, the presence of antioxidants in the sample resulted in the reduction of Fe³⁺ to Fe²⁺ by donating an electron. Increasing absorbance indicates an increase in reductive ability. In this work, 1gram of *Camellia sinensis* leaves extract carries 0.11g of reducing power (Astley 2003). The methanolic extract of dry *Camellia sinensis* had high reducing power that

showed statistically significant correlation between reducing power and amount of total phenolic compounds in plant extracts ability (Thirugnanasampandan *et al.*, 2008). This variation expected in plant extracts due to their constituents as well as the type of the phenolic and this differ considerably between the genotypes of the same plant and from species to species (Leenen *et al.*, 2000).

It is here concluded by this research study that *Camellia sinensis* leaves showed good antioxidant activity which provides the protection to living organisms from the damage caused by the uncontrolled production of reactive oxygen species. The beneficial effects of *Camellia sinensis* would play the protective role to control over overseas diseases.

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