

## **Phytochemical screening, *invitro* antioxidant and anti-inflammatory activity of polyphenolic fraction extracted from *Camellia sinensis* bud extract**

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### **Abstract**

*Tea is one of the most widely consumed beverages in the world today, second only to water, well ahead of coffee, beer, wine and carbonated soft drinks. The tea plant, *Camellia sinensis*, is a member of the Theaceae family and black, oolong, white and green tea are produced from its leaf and buds. Tea beverage is an infusion of the dried leaves of *Camellia sinensis*. The present study aims to screen the phytochemicals screening, in vitro antioxidant and anti-inflammatory activity of polyphenolic compound extracted from *Camellia sinensis* bud extract. The qualitative phytochemical results revealed the presence of important phytochemicals like alkaloids, flavonoids, phenols, and tannins. The *C. sinensis* buds could be a rich source of antioxidants. The extract showed significant antioxidant and anti-inflammatory activity. Total phenolic content of the bud's extract was found to be 123 mg/GAE. The extract possesses a significant antioxidant and anti-inflammatory activity.*

### **Keywords**

Phytochemicals, Antioxidant, Anti-inflammatory, column chromatography, Total phenolics.

### **Introduction**

Tea is a popular nutraceutical as an antioxidant. Increasing evidence has expanded the role of white tea from a traditional beverage to a source of pharmacologically active molecules with diverse health benefits. Phytochemicals residing in plants give organoleptic properties and colour to the plant. In many places, as a dietary accessory they are comfortably approachable but dormant health advantages of phytochemicals are only reachable from the utilization of whole plant [1]. Phytochemicals are beneficial to boost up immunomodulatory responses and also provide immunity against many diseases. Some phytochemicals are known to reveal medicinal and physiological activities which are phenols, tannins, flavonoids, saponins, carbohydrates, alkaloids, phytosterols etc. [2]

Antioxidants or inhibitors of oxidation are compounds which retard or prevent the oxidation and in general prolong the life of the oxidizable matter. Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals. The free radicals (oxidants) are species with very short half-life, high reactivity and damaging activity towards macromolecules like proteins, DNA and lipids. In general, the reactive oxygen species circulating in the body tend to react with the electron of other molecules in the body and these also effect various enzyme systems and cause damage which may further contribute to conditions such as cancer, ischemia, aging, adult respiratory distress syndromes, rheumatoid arthritis etc. [3]

Aromatic plants are widely used also as nutritional supplements. Of special interest is their value as a source of natural antioxidants. Due to increasing safety concerns involved with consumption of synthetic antioxidants, exploitation of cheaper and safer sources of antioxidants from natural origins, and especially from plants, is of interest nowadays. The major plant compounds characterized by antioxidant activity are polyphenols. The antioxidant activity of polyphenols is accredited to their redox properties [4] and anti-inflammatory activities (5).

## Materials and Methods

### Plant sample

The buds of *Camellia sinensis* was collected from the local area of Nilgiris and were authenticated by Botanical Survey of India, Southern Regional Centre, Coimbatore.

### Sample preparation

The collected buds were washed and cleaned with water, then shade dried for about 15 days. The dried *Camellia sinensis* bud sample were crushed to coarse powder and then grinded using mortar and pestle. 10g of powdered sample was suspended in 100ml of Hydro ethanol and kept in a shaker for about 24 hours and then filtered.

### Qualitative phytochemical analysis

Test for alkaloids: To 1 ml of the extract, add 2 ml of Wagner's reagent (iodine in potassium iodide). The formation of reddish-brown precipitate confirms the presence of alkaloids.[6]

Test for phenols: To 1ml of extract added 2ml of distilled water followed by few drops of 10%  $\text{FeCl}_3$ . The appearance of blue or black colour indicates presence of phenols. [7]

Test for Saponins: About 2ml of extract and 2ml of distilled water was added in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam for few seconds. The presence of foam confirms the presence of saponins. [6]

Test for Flavonoids: To about 2ml of extract, few drops of 10% ferric chloride solution were added. The formation of green or blue colour indicates the presence of flavonoids.[8]

Test for Phytosterols: 1ml of the extract was treated with 2 ml of chloroform and few drops of acetic anhydride to which equal amount of concentrated sulphuric acid was added. The formation of bluish green colour indicates the presence of phytosterols. [11]

Test for amino acids and proteins: To few ml of the extract added small amount of Ninhydrin reagent. After mixing it well, the solution was boiled in water bath for 2-3 minutes. A bluish or blackish colour indicated the presence of proteins. [9]

Test for steroids: To 1ml of extract dissolved 2ml chloroform and 0.2ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added. The formation of red colour precipitate indicates the presence of steroids. [7]

Test for tannin: To:1ml of extract added few drops of dilute ferric chloride solution. The presence of tannin is indicated by the formation of bluish black or greenish black precipitate.[7]

Test for cardiac glycosides: To 1ml of plant extract added few ml of concentrated sulphuric acid. Formation of red colour indicates the presence of glycosides. [12]

Test for carbohydrates: To 0.5ml of the extract, added 4-5 drops of Benedict's reagent and heated in a boiling water bath for 5 minutes. The appearance of blue colour indicates the presence of carbohydrates. [10]

#### Quantitative phytochemical analysis

##### Determination of Total Phenol

1.0 ml of plant extract was added to 2ml of Folin – ciocalteu's reagent and 1.0ml of 20% Sodium carbonate solution. The mixture was incubated at 45°C for 45 minutes. Then absorbance of the sample was read at 765 nm. The control was set up using 2ml of Folin's reagent and 1.0 ml of Sodium carbonate solution. A calibration curve was constructed using Gallic acid dilutions as standard and total phenolic content of the extract were identified [13].

##### Isolation of polyphenol from hydro ethanolic bud extract

##### Column chromatography

A cylinder-shaped glass column (32 x 2.5cm) containing stationary phase (silica gel- G) is added slowly from the top with a liquid solvent (mobile phase that flows down the column with the help gravity or external pressure applied. This technique is used for the purification of compounds. Once the column is ready, the sample (40ml and 20ml of Ethanol) is loaded inside the top of the column. The mobile solvent is then allowed to flow down through the column. The compounds in mixture have different interaction's ability with stationary phase (silica gel), and mobile phase, thereby will flow

along the mobile phase at different time intervals or degrees. In this way, the separation of compounds from the mixture is achieved. The individual compounds are collected as fractions and analysed further for structure elucidation.

A suitable size long cylindrical glass column should be stand firm on a column-chromatography stand. Hydroethanolic plant extract should be placed on the top of the pre-packed silica column and sample should be covered with a layer of cotton. Then solvents of different polarities were passed through column at uniform rate under gravity to fractionate the plant extract. Each fraction was collected separately to the capillary tubes and numbered consecutively for further analysis. [14]

#### *In vitro* Antioxidant assay

##### Total antioxidant activity

The total antioxidant capacity of the fractions was determined by phosphomolybdate method using ascorbic acid as a standard. An aliquot of 0.5 ml of *Camellia sinensis* bud extract was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 50°C for 90 min. After the sample had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. A typical blank contained 1 ml of the reagent solution and the appropriate volume of the solvent and incubated under the same conditions. The antioxidant capacity was estimated using following formula. [15]

$$\text{Percentage inhibition} = (\text{Abs Control} - \text{Abs Sample}) / \text{Abs Control} \times 100$$

##### DPPH assay

The antioxidant activity of the hydroethanolic extract of *Camellia sinensis* were determined using 1,1 - diphenyl-2-picryl-hydrazil (DPPH). To a test tube added 0.1ml of DPPH reagent and 0.4ml of Tris HCL. This serves as a control. To 0.5ml plant extract, added 0.1ml of DPPH. Then mix well and incubate for 5 minutes at room temperature, and added 0.4ml of Tris HCL. The test tube was incubated in dark at room temperature for about 30minutes. The absorbance was read at 517nm in a spectrophotometer and the readings were recorded. [16]

$$\text{Percentage inhibition} = (\text{Abs Control} - \text{Abs Sample}) / \text{Abs Control} \times 100$$

##### Determination of reducing/antioxidant power (FRAP)

The ferric ions ( $\text{Fe}^{3+}$ ) reducing antioxidant power (FRAP) method was used to measure the reducing capacity of the plant extracts with a slight modification, which involves the presence of extracts to reduce the ferricyanide complex to the ferrous form. 1.0ml of *Camellia sinensis* bud extract from the stock solutions and the standard (ascorbic acid) were mixed with 1.0 ml of phosphate buffer

(0.2 M, pH 6.6) and 1.0 ml of potassium ferricyanide (0.1% w/v). The mixture was incubated at 50°C for 20 min. Then 1.0 ml of trichloroacetic acid (10% w/v) was added to the reaction mixture. Afterwards, it was centrifuged at 5000rpm for 10 min. The upper layer of the solution (1.0 ml) was mixed with distilled water (1.0 ml) and ferric chloride (0.5 ml 0.1% w/v). The absorbance was measured at 700 nm at the reaction time of 30 min. [17]

$$\text{Percentage inhibition} = (\text{Abs Control} - \text{Abs Sample}) / \text{Abs Control} \times 100$$

#### Anti-inflammatory activity

##### Trypsin (Proteinase) inhibitory method

To 1 ml of *Camellia sinensis* bud extract added the reaction mixture. The reaction mixture (2.0 ml) contains 0.06 mg trypsin, 1.0 ml. 20 mM tris-HCl buffer (pH 7.4). The mixture was incubated at 37°C for 5 minutes, then 1.0 ml of 0.8% (w/v) casein was added. The mixtures were incubated for an additional 20 minutes. Then added 2.0 ml of 70% (v/v) perchloric acid to terminate the reaction. The cloudy suspension was centrifuged at 5000 rpm and absorbance of the supernatant was read at 210 nm against blank.[18]

$$\text{Percentage inhibition} = (\text{O.D. of control} - \text{O.D. of product test}) \times 100 / \text{O.D. of Control}$$

##### Protein denaturation inhibition method

To 0.2ml of egg albumin added 2.8ml phosphate buffer. To this solution 1ml of the *Camellia sinensis* bud extract was added. For the control, distilled water was added instead of test extract. The reaction mixture was incubated at 37°C for 20 minutes and afterwards heated to 90°C for 5 minutes. The absorbance was taken at 660nm, after cooling. Diclofenac sodium was taken as a standard. The protein denaturation inhibition percentage was determined by following formula. [19]

$$\text{Percentage inhibition} = (\text{O.D. of control} - \text{O.D. of test}) \times 100 / \text{O.D. of Control}$$

## Results and discussion

The phytochemical analysis of hydroethanolic bud extract of *Camellia sinensis* revealed the presence of alkaloids, flavonoids, saponins, steroids, glycosides, phenols, tannins, phytosterols and carbohydrates

**Table 1. Phytochemical analysis of *C.sinensis* buds extract**

Phytochemicals	Hydro ethanol
Alkaloids	+
Flavonoids	+
Saponin	+

Phenol	+
Phytosterol	+
Aminoacids & proteins	-
Steroids	+
Tannin	+
Carbohydrates	+

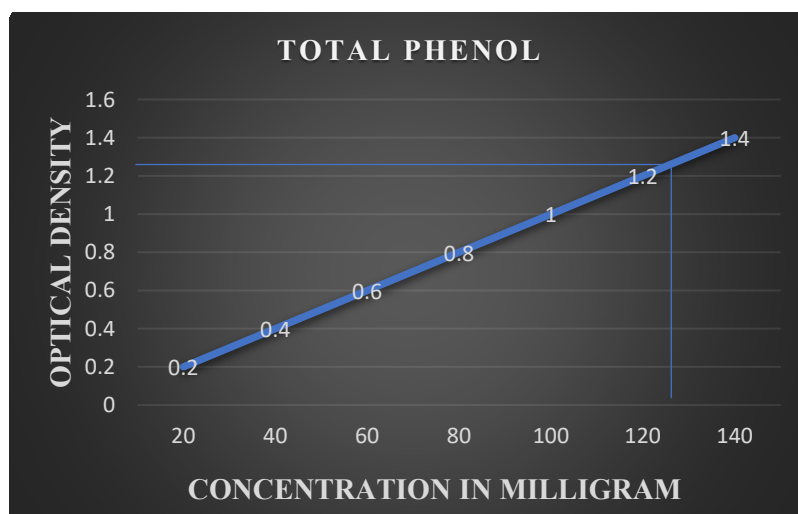
(+) = presence of phytochemicals (-) = absence of phytochemicals

In accordance with our studies Ibrahim *et al* (2020) also observed the presence of alkaloids, saponins, tannins, flavonoids, phenols and sterols in methanolic extract of green tea leaves.[20]

#### Quantitative determination of total phenol

The determination of the amount of total phenolic content of *C.sinensis* bud extract was performed by the Folin Ciocalteau's method. Gallic acid was used as standard for total phenolic acid. Total phenolic content of the plant extract was 123 mg/GAE for *C. sinensis*.

**Figure 1: Total phenol**



Amandeep *et al.*, 2019 observed the total phenolic content of different types of *Camellia sinensis* extract white (Silver Needle), Green and Black Tea. The results exhibited the following order: white tea (133.30mg/g) > green tea (118.37mg/g) > black tea (101.8mg/g). A strong correlation was observed between the total phenolic content and antioxidant activity of different types of Tea extracts. [21]

#### Column chromatography

The fractions obtained from silica gel -G column chromatography of *Camellia sinensis* bud extract was centrifuged at 5000rpm for 10mins and optical density values were determined using

spectrophotometer. The phytochemicals showing the same OD values were pooled into a single fraction and used for further analysis.

Raksha *et al* 2017 reported that the aqueous extraction of Green Tea polyphenols was performed at three different temperatures 40°C, 50°C and 60°C and at three corresponding time 15 minutes, 30 minutes and 60 minutes and observed that maximum polyphenols content was obtained at 40°C for 30 minutes. (22)

#### *In vitro* anti-oxidant activity

The result of the antioxidant study of Hydroethanolic bud extract *C. sinensis* was represented in table and figure

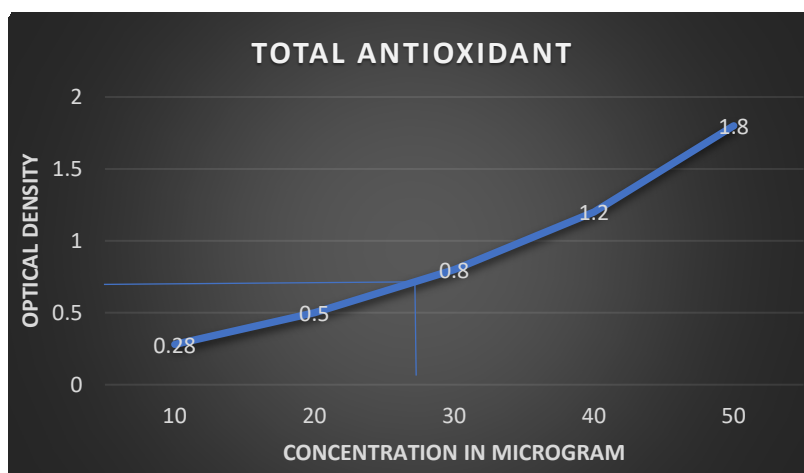
**Table 2: Antioxidant activity of *C. sinensis* bud extract**

S. No	Assays	Concentration in $\mu\text{g/ml}$
1	Total antioxidant	28
2	DPPH	66.3
3	FRAP	240

#### Total antioxidant assay

The total anti-oxidant capacity of hydroethanolic bud extract of *Camellia sinensis* was found to be 28  $\mu\text{g/g}$  at a concentration of 500  $\mu\text{g/ml}$ .

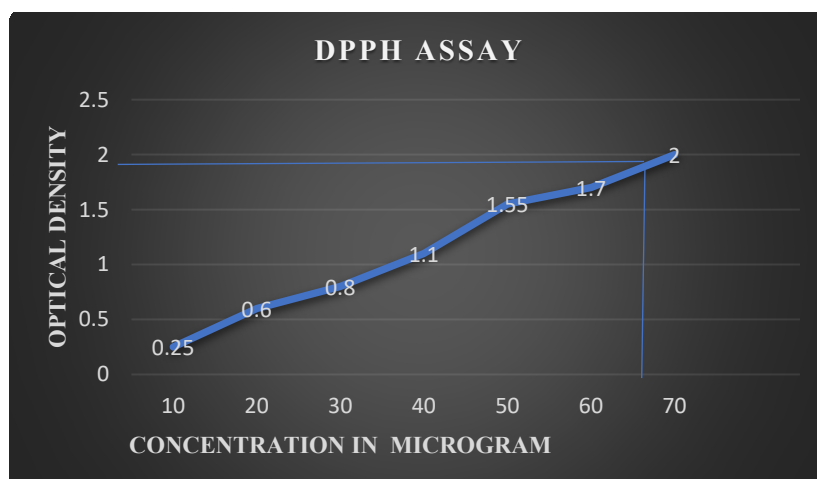
**Figure 2: Total antioxidant assay**



### DPPH assay

The present study shows that the DPPH activity of hydroethanolic bud extract of *Camellia sinensis* was found 66.3  $\mu\text{g/g}$  at a concentration of 500  $\mu\text{g/ml}$ .

**Figure 3: DPPH assay**

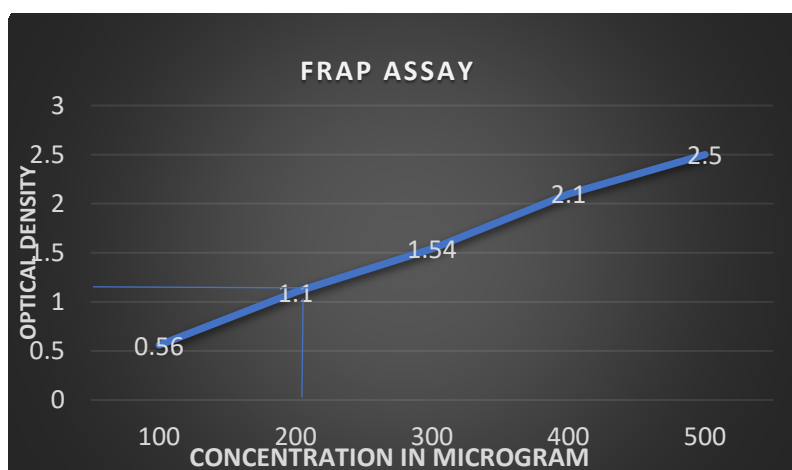


Rohadi *et al.*, 2018 observed the antioxidant capacity of dried white tea (*Camelia Sinensis*) Extract compared to green, oolong and black tea. The results showed that the significant differences in the radical scavenging DPPH among samples ( $p < 0.05$ ). The highest radical scavenging was 93.61% obtained on the white tea extract (1500  $\mu\text{g/ml}$ ), then the lowest was 72.07% on the black tea extract.[23]

### FRAP assay

The FRAP assay hydroethanolic bud extract of *Camellia sinensis* was found 240  $\mu\text{g/g}$  at a concentration of 1000  $\mu\text{g/ml}$

**Figure 4: FRAP assay**





Shagufta et al.,2015 determined the antioxidant activity of crude methanol extracts (*Murraya koenigii* (curry leaves), *Laurus nobilis* (bay leaves), and *Camellia sinensis* (green and black tea powder) including DPPH, FRAP, and ICA. The results showed that *C.sinensis* (green) exhibited the highest antioxidant potential whereas *M. koenigii* showed the lowest (*C. sinensis* (green) > (black) > *L. nobilis* > *M. koenigii*). The numerical values obtained for *C. sinensis* (green and black), *L. nobilis*, and *M. koenigii* were < 20mg/100 µl, 20-30 µg/100 µl, 150 mg/100 µl respectively.[24]

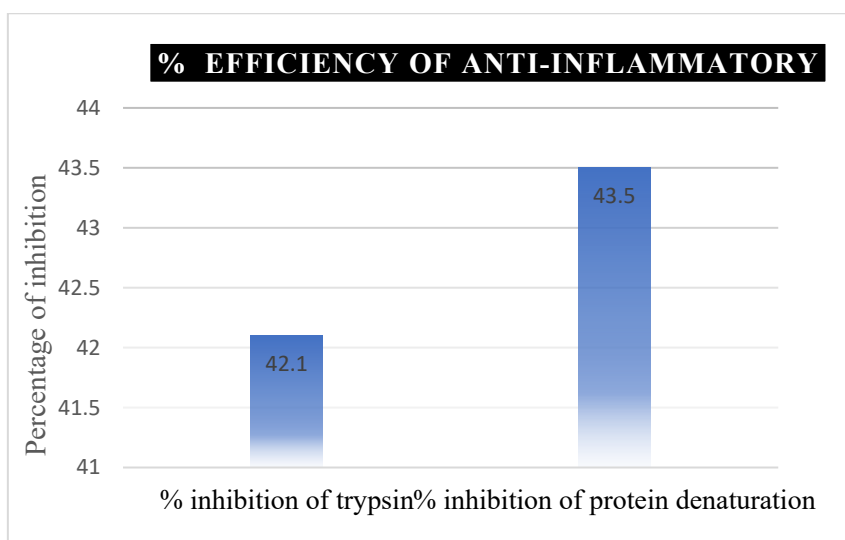
#### *In vitro* anti – inflammatory activity

The anti-inflammatory activity of *Camellia sinensis* bud extract was determined using protein denaturation and trypsin methods. The results were represented in table 3 and figure 4

**Table 3: Anti-inflammatory activity in *C. sinensis* bud extract**

TEST	PERCENTAGE (%) OF INHIBITION
Trypsin inhibition	42.1
Protein denaturation	43.5

**Figure 5: Anti-inflammatory activity in *C. sinensis* bud extract.**



The present investigation of anti-inflammatory activity of *Camellia sinensis* bud extract using protein denaturation and trypsin methods revealed that the extract has significant anti -inflammatory potential against protein denaturation when compared to inhibition of trypsin.

Singh *et al.*,2019 revealed that the investigation of protein denaturation analysis of *C.sinensis* dried green tea extract has higher percentage of inhibition and significant anti-inflammatory activity

compared to *Hibiscus rosa sinensis*, *Matricaria chamomilla*, *Rosa sp* and *Zingiber officinale* tea extract.[25]

### Summary and Conclusion

White tea (*Camellia sinensis*) was used as medicine for various diseases. It is shown to have many biological properties like Phytochemicals, Anti-oxidant, Anti-inflammatory, Anticancer, Antifungal activity, Antimicrobial activity and Other biological activities. The present study showed that *Camellia sinensis* bud extract has significant antioxidant and anti-inflammatory activity. This activities in the extract are may be due to the presence of phytochemicals such as alkaloids, flavonoids, phenols, tannins and saponins. The present study results indicate that white tea can be used as an effective therapeutic agent against various diseases. Further study can be done to isolate compound responsible for anti-oxidant and anti-inflammatory properties.

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