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**ORIGINAL ARTICLE** **OPEN ACCESS**

# Screening of phyto-chemicals and determination of total phenolic content, antioxidant and anti-diabetic activity of water extract of *Camellia assamica* leaves

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**ABSTRACT**
*The study involves cold extraction of the powdered dried leaves of Camellia assamica, which helps in ensuring that all the plant components are extracted to the maximum for better outcome. Phytochemical analysis was carried out using standard procedure. Total phenolic content was determined using standard Folin-Ciocalteau's (FC) method. In vitro antioxidant property was analyzed using both DPPH method and $H_2O_2$ scavenging activity method. The alpha-amylase inhibition activity was used for evaluating the in-vitro anti-diabetic activity. The plant extract was used at different concentrations to ensure which concentration causes the highest inhibition. The phytochemical screening of the green tea extract showed positive results for steroids, Saponin, Tannins, Flavonoid, Phenols etc.The total phenolic content of aqueous extract for green tea is 630µg/ml of GAE or 0.63mg of phenol compound and the antioxidant analysis shows maximum inhibition at 250µg/ml concentration of water extract with a inhibition percentage of 76.5433 ±0.157% in case of $H_2O_2$ method whereas it was 73.9633 ±0.0693% of inhibition at 250µg/ml concentration of water extract with the DPPH method. The anti-diabetic property was maximum at 200µg/ml concentration of water extract with an inhibition percentage of 83.3533 ±0.0693%.*
**Keywords:** *Camellia assamica,* aqueous extraction, phytochemical, phenolic content, Antioxidant assay, Anti-diabetic Activity.

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## INTRODUCTION

Green tea is considered one of the oldest and most popular therapeutic beverages consumed all over the world. It is prepared from the plant *"Camellia assamica"* and can be consumed as a drink or the extract can be used as medicine. It contains numerous bioactive agents and is very rich in polyphenols which are known to be key players in prevention and treatment of different types of diseases. Many type of tea are produced from this plant species but due to the difference in the oxidation level and processing, they have slightly different chemical composition. Green tea is non-fermented tea and contains higher level of catechin, which is a strong antioxidant, than the fermented black tea.[1]

Many researchers have found that the polyphenols extracted from green tea has a number of health benefits such as anti-cancer,[2] nervous system protection, anti-oxidation, blood sugar control effects, etc.[3] Patients having hypertension, coronary heart disease, hyperlipidemia, diabetes and arteriosclerosis have been using green tea as a form of remedy due to its high content of polyphenols like catechins and phenolic acids.[4] Wu et al. analyzed the effect of tea polyphenols in insulin activity in Sprague-Dawley rats and found that green tea polyphenols increases the basal and insulin stimulated glucose uptake by adipocytes.[5]

There is also a small negative side effect to consumption of green tea but these effects are minute and do not appear if consumed in moderation. Hsu et al, in 2011 evaluated the toxicity of green tea extracts in mice and concluded that adverse effect is observed if green tea extracts is consumed in a number more than 2500mg/kg body weight per day.[6] Again in 2018, Hu et al, performed a systemic review of different published researches on toxicology and human intervention report and concluded that 338mg ECGC per day for adults is the safe consumption level as solid bolus dose and 704mg ECGC per day is the

safe level for consumption of green tea as beverage.[7] Taking all these in consideration, pregnant women, children and elderly persons should monitor their intake to not cross the safety limit.
This study focused on screening of phytochemicals and determination of its total phenolic content along with its in-vitro anti-oxidant and anti-diabetic properties of *Camellia assamica* leaves.

## MATERIAL AND METHODS

### Study area:

The study was conducted at Laboratory of Biotechnology program, Assam down town University, Assam.

### Sample collection:

The sealed packed plant sample of *Camellia assamica* (green tea) leaves were collected from a local market in Guwahati, Assam in the month of January, 2022. The collected sample was then measured and grinned into fine powdered form using a grinder machine and later was stored in an airtight container.

### Preparation of plant extract:

10g of Green tea powdered extract was weighted out using the electronic weighing balance. It was then added in 100ml of distilled water in a sterile plastic container and kept in a deep freezer (-20$^0$C) for 72 hours. Later, the extract was defrosted at room temperature and was filtered using filter paper. The filtrate was centrifuged at 5500 rpm for 5 minutes.

### Test for phytochemicals:

Plants produced different bio-active compounds through primary or secondary metabolism which play an important role in plant growth and its defense against any pathogen. They are also known to have different pharmacological effect on human. Screening of the different phytochemical of the extract was carried out using the previously described standard protocols.[8,9]

### Estimation of total phenolic content:

Phenolic compounds are important plant constituents with redox properties responsible for anti-oxidant activity. The current procedure was carried out using Folin - Ciocalteu (FC) method with slight modifications.[10]To determine the phenolic contents in the sample, the same protocol was used where the extract were added instead of the gallic acid (which is used as a standard) and was measured using spectrophotometer at 725nm

### Anti-oxidant Activity Assay:

The antioxidant assay of bioactive components was normally estimated using two different spectrophotometry methods, namely DPPH and H2O2 scavenging activity.[11]

### DPPH Method:

The antioxidant activity of the aqueous extract of the plant material was examined according to the method developed by Blois (1958) with slight modifications. The test extract was prepared with the concentration of 1000 µg/ml and the DPPH activity was carried out in a UV-Vis spectrophotometer by measuring the absorbance at 517 nm against a blank solution taking ascorbic acid as standard.[12] The percentage of inhibition was observed using

$$\% \text{ of inhibition} = \frac{Positive\ control - Absorbance\ of\ sample}{Positive\ control} \times 100$$

### Hydrogen Peroxide ($H_2O_2$) Scavenging Activity:

The ability of the plant extracts of *Camellia assamica to* scavenge the hydrogen peroxide was estimated according to the method by Ruch *et at* (1989) with slight modification.[13] Absorbance of the test sample at 230nm was determined 10 mins later against a blank solution taking ascorbic acid as standard. The percentage was calculated using

$$\% \text{ of inhibition} = \frac{Positive\ control - Absorbance\ of\ sample}{Positive\ control} \times 100$$

### Anti-diabetic Activity Test:

A starch solution (0.1% w/v) was obtained by stirring 0.1g of starch in 100ml of 16mM of Sodium Acetate buffer. The enzyme solution was prepared by mixing 27.5mg of Alpha Amylase in 100ml of distilled water. DNS solution was prepared by mixing 1gm DNS with 200mg crystalline phenol and 50mg sodium sulphite in 100ml of 1% NaOH. Both control and plant extract were added with starch solution and left to react with amylase solution under alkaline conditions at 25ºC. The reaction was measured over 10 minutes. The generation of maltose was quantified by the reduction of 3, 5 dinitro salicylic acid to 3-amino5- nitro salicylic acid· [9]Absorbance was measured at 540 nm. Percentage of inhibition was calculated using

$$\% \text{ of inhibition} = \frac{Positive\ control - Absorbance\ of\ sample}{Positive\ control} \times 100$$

## RESULTS AND DISCUSSION

### Phytochemical Analysis:

Medicinal properties of a plant are determined by the different bioactive compounds present on it which may have diverse pharmacological effect on human. The current study, indicate the presence of different phytochemical like steroids, saponin, tannins, phenol and flavanoids which are already reported by numerous previous studies like Narmada et al, in 2020.[14] Jain et al., have also reported similar result regarding the presence of phytochemicals in green tea.[15]Presence of difference phytochemicals in water extract of *Camellia assamica* leaves is presented in Table 1.

Table 1: Result of phytochemical screening of the leaf extract

| Sl. No | Phytochemicals | Results |
|---|---|---|
| 1 | Steroids | +ve |
| 2 | Terpenoids | -ve |
| 3 | Saponin | +ve |
| 4 | Glycoside | -ve |
| 5 | Tannins | +ve |
| 6 | Alkaloids | -ve |
| 7 | Carbohydrates | -ve |
| 8 | Phenols | +ve |
| 9 | Flavonoid | +ve |

### Determination of total phenolic content:

Table 2: Table showing phenol content in water extract

| Sl. No | Concentration of Gallic acid | O.D. at 725nm |
|---|---|---|
| 1 | 200 µg/ml | 0.23 |
| 2 | 400 µg /ml | 0.41 |
| 3 | 600 µg/ml | 0.60 |
| 4 | 800 µg/ml | 0.83 |
| 5 | 1000 µg/ml | 1.19 |
| Sample | - | 0.65 |

The total phenolic content of aqueous extract of *Camellia assamica* determined by FC method was found to be 630µg/ml of GAE or 0.63mg of phenolic compound. This result can be further backed up by a study done by Chen et al in 2007 where they found the total phenolic content of green tea to be 0.75mg of phenolic compound which does not have a significant difference with the result of the current study.[16] The presence of this slight difference can be due to the difference in the type of tea leaves used. Oliveira et al in 2022 have demonstrated a similar TPC determination where similar result of 0.56mg have been found and the green tea leaves are extracted using infusion method at 85 degrees.[17] Past studies have demonstrated that the presence of these phenolic compounds directly supported the antioxidant potential of green tea extracts and is also the main cause of reduction in oxidative stress.[18]

### Antioxidant assay:

The plant showed a significant antioxidant activity with both the $H_2O_2$ scavenging activity and DPPH methods.

### Hydrogen Peroxide Method ($H_2O_2$ Method):

Table 3: Result for anti-oxidant analysis of green tea using $H_2O_2$ method

| Sl. No | Concentration of sample | O.D. at 230nm | % of inhibition |
|---|---|---|---|
| 1 | 50 µg/ml | 0.110 | 49.77% |
| 2 | 100 µg/ml | 0.089 | 59.36% |
| 3 | 150 µg/ml | 0.081 | 63.01% |
| 4 | 200 µg/ml | 0.072 | 67.12% |
| 5 | 250 µg/ml | 0.051 | 76.71% |

Positive control = 0.219

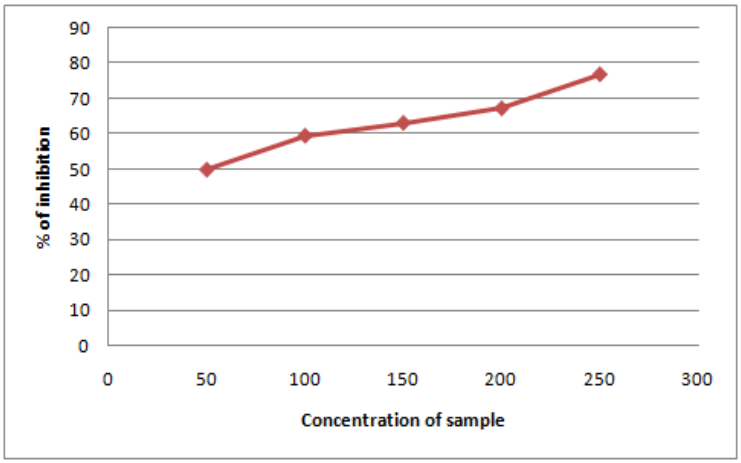


Figure1: Graph representing % of inhibition

The maximum percentage of inhibition for green tea is 76.5433 ±0.157% at 250 µg/ml using hydrogen peroxide method.

**DPPH METHOD:**

Table 4: Result for anti-oxidant analysis of green tea using DPPH method

| Sl. No | Concentration of sample | O.D. at 517nm | % of inhibition |
|---|---|---|---|
| 1 | 50 µg/ml | 0.414 | 49.32% |
| 2 | 100 µg/ml | 0.359 | 56.05% |
| 3 | 150 µg/ml | 0.302 | 63.03% |
| 4 | 200 µg/ml | 0.265 | 67.57% |
| 5 | 250 µg/ml | 0.213 | 73.92% |

Positive control = 0.817

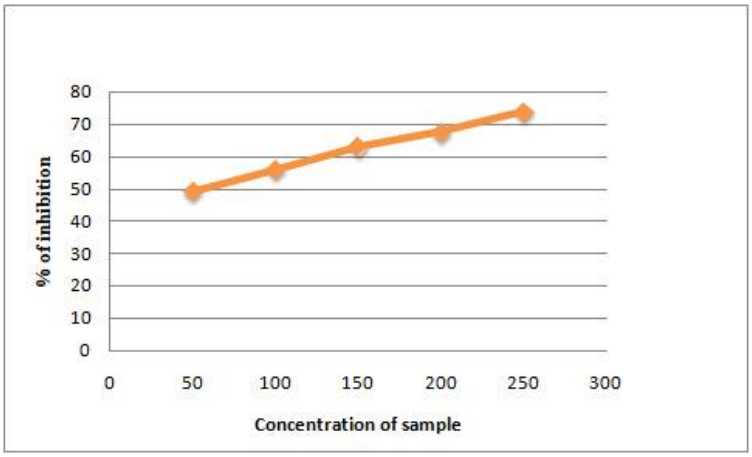


Figure 2: Graph representing % of inhibition

The maximum percentage of inhibition for green tea was observed to be 73.9633 ±0.0693% at a concentration of 250µg/ml using DPPH method with an IC50 value of 51.157. Whereas a maximum inhibition of 76.5433 ±0.157% was observed at a concentration of 250µg/ml using $H_2O_2$ scavenging activity method with an IC50 value of 44.677. Jain et al have also determined the percentage of inhibition by green tea extract to be 81.13% using the same extraction method followed in this paper which does not show a very large variation. This proves to show that green tea extract have a very high antioxidant capacity and is also a good candidate for further study regarding synergistic effects it can have.[14] Another study has found the $IC_{50}$ value of antioxidant activity of green tea to be 36.71µg/ml which have a close similarity to the $IC_{50}$ value of the current study. [15]This shows that green tea exhibit a very high

antioxidant activity but it is also important to note that these values differ due to various differences such as variety, mode of production and origin of the tea leaves, etc. The antioxidant activity is also greatly influenced by the temperature, as such the activity of green tea infusion increases as temperature rises.[19,20]

**IN VITRO α-AMYLASE INHIBITION ASSAY**

The study has also shown good α-amylase inhibition activity of the studied plant extracts. Different concentration of plant sample showed different inhibition of α-amylase for both the extract where the positive control was 0.761.

Table 5: Result of in-vitro anti-diabetic activity of green tea

| Sl. No | Concentration of sample | O.D. at 540nm | % of inhibition |
|---|---|---|---|
| 1 | 50 µg/ml | 0.213 | 72.01% |
| 2 | 100 µg/ml | 0.162 | 78.71% |
| 3 | 150 µg/ml | 0.138 | 81.86% |
| 4 | 200 µg/ml | 0.127 | 83.31% |

The aqueous extracts of *Camellia assamica* showed significant inhibitory effect on alpha-amylase activity. A maximum inhibition of 83.3533 ±0.0693% for green tea was observed. This result can be supported by a study conducted by (Miao et al., 2015) where they found the inhibition to be 63.5% which is a lesser but not by a huge number.[21]Miao et al performed the extraction of green tea using enzyme extraction method and α-amylase inhibition assay using the Nelson-Somogyi procedure and these difference in the procedure employed seems to be the cause behind the difference in the inhibition percentage albeit not much. It is also important to note that the extraction method employed have some effects in the overall inhibition percentage as is demonstrated by previous study by Zhu et al.[22]

**CONCLUSION:**

Green tea is a widely used beverage which is rich in phenolic compounds with a high antioxidant activity making it therapeutically potential against oxidative stress. It also possesses a strong alpha amylase inhibition in a dose dependent manner. Even though there are numerous studies, there is a need for studies done with human subject to know and use the full potential of green tea.

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**CONFLICTS OF INTEREST:** None

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