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Comparative Evaluation Of Antioxidant, Anti-Inflammatory And Anti-Diabetic Activities In Different Varieties Of Green Tea Extracts

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ARTICLE DETAILS

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ABSTRACT

One of the best sources of bioactive ingredients is green tea (*Camellia sinensis*), which is high in polyphenols. In the current investigation, samples of four different types of green tea were collected and using established procedures, the phytochemicals, ash and moisture content, antioxidant potential, anti-inflammatory activity, and anti-diabetic activity were all examined. All of the samples contain total phenols, amino acids, flavonoids, alkaloids, tannins, and carbs, according to the phytochemical analysis. *Camellia sinensis* has higher ash content than moisture, and sample C has higher ash content than the other samples. The antioxidant capacity of various *Camellia sinensis* samples was also assessed in this study. Based on these criteria, *Camellia sinensis* was shown to have a high potential for antioxidants due to its various phenolic compounds, which help to prevent oxidative stress, which is the process by which free radicals cause damage. Green tea contains a high concentration of polyphenolic compounds, tannins, and flavonoids, which are important components responsible for antioxidant properties. Green tea was found to have 75% to 95% anti-inflammatory efficacy when tested in vitro against compounds derived from egg albumin and bovine serum albumin. This finding demonstrated the involvement of *Camellia sinensis* in the denaturation of proteins, which triggers the inflammatory response. This investigation also ascertained *Camellia sinensis's* in vitro antidiabetic efficacy. The anti-diabetic effect of the green teas ranges from 66% to 91%. This study suggested that people with diabetes may experience less hyperglycemia if they drank green tea. The strong anti-inflammatory and antioxidant properties of *Camellia sinensis* have been revealed by this research.

1. Introduction

One of the ingredients in tea brew, a popular and restorative beverage all over the world, is green tea (*Camellia sinensis*). It is grown in regions with acidic soils and high humidity [5, 4]. Caffeine, a well-known stimulant, is the main active component. Approximately 4000 bioactive components are found in green tea, including vitamins, β-carotene, fiber, chlorophyll, minerals, amino acids, tannin, alkaloids, flavonoids, and polyphenols. Of these, polyphenols make up 33% and catechins are the main component. It also raises metabolism and speeds up fat burning [6]. As the least processed tea, green tea is created from unoxidized leaves, which means it has a higher concentration of antioxidants. These antioxidants may aid in weight loss, increase metabolic rate, fight against cancer, reduce risk of heart disease, and enhance brain function. Catechins found in

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Camellia sinensis are known as epigallocatechin-3-gallate (EGCG). They are very beneficial to health and help to prevent damage to cells [2, 3]. Green tea's anti-inflammatory qualities and ability to reduce oxidative stress are attributed to its polyphenols, which are also responsible for the beverage's associated lower cancer risk, according to numerous studies [7, 8].

The body's initial reaction to an infection or damage is inflammation, which is essential for both innate immunity and adaptive immunity [1]. Inflammation plays a role in aging, cancer, arthritis, and cardiovascular disorders. It may be beneficial to human health to look for natural substances and phytoconstituents that can tamper with the systems underlying inflammation [8]. Understanding the mechanism underlying the anti-inflammatory effect of herbal components is aided by the in vitro investigations [1].

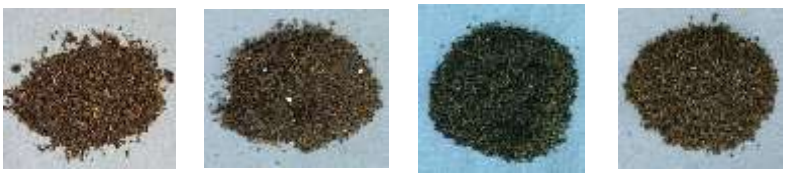
There is evidence that green tea's epigallocatechin-3-gallate (EGCG) has anti-inflammatory properties in a number of research studies. A possible explanation for the anti-inflammatory benefits could be the upregulation of the anti-inflammatory cytokine interleukin-10. As a result, drinking green tea beverages high in bioactive substances inhibits the production of inflammatory cytokines in the form of proteins, which controls the process of inflammation [1, 8]. The two main chemical components of green tea that affect the liquor's color and brightness are called theaflavin (TF) and thearubigin (TR). These are the distinctive by-products that arise from catechins when tea is oxidized enzymatically during production. Theaflavin gives tea its orange orangish red color, which enhances the mouthfeel and prolongs the creation of cream [4].

From the review studied, very limited data is available about the nutraceutical properties and medicinal effects of different green tea samples. Therefore, this research work is aimed at the qualitative and quantitative analysis of phytochemicals and biochemical constituents found in the watery extracts of *Camellia sinensis*. After preliminary analysis, the samples were subjected to the evaluation of medicinal properties of green tea, such as antioxidant, anti-inflammatory, and antidiabetic activities.

2. Materials and methods

2.1. Collection of Samples

The four ideal brands of green tea (*Camellia sinensis*) was collected from the retail department stores, Erode and they are named as Sample A, Sample B, Sample C and Sample D.



A **B** **C** **D**

Figure 1: *Camellia sinensis* Sample A, B, C and D

2.2. Preparation of aqueous extracts

The collected four different green tea samples (*Camellia sinensis*) was boiled separately with distilled water at 90^0C for 5 minutes. After boiling the mixture was filtered through Whatman No.1 filter paper. The filtrates were stored at refrigerator until further analysis.

2.3. Preliminary Phytochemical Screening

Phytochemical analysis of the aqueous extract of the green tea was performed to investigate the presence or absence of the different phytochemical constituents such as Carbohydrates, Amino acids, Proteins, Alkaloids, Terpenoids, Flavonoids, Phenols, Tannins and Anthraquinones, Glycosides using standard protocol.

2.3.1. Test for Carbohydrates

(a) Molish's test: To a few drops of individual extracts, 2 ml of molish's reagent was added. The mixture was shaken well and 2.0 ml of Concentrated H_2SO_4 was added slowly along the sides of the test tube and allowed to stand. A reddish ring formed at the junction of two solutions indicates the presence of carbohydrates.

(b) Fehling's test: To a few drops of individual extracts, 2ml of fehling's reagent was added. The mixture was shaken well and kept in a boiling water bath for five minutes. A formation of brick red precipitate indicates the presence of sugar.

(c) Benedict's test: To a few drops of individual extracts, 2ml of benedict's reagent was added. The mixture was shaken well and kept in a boiling water bath for five minutes. A formation of brick red precipitate indicates the presence of sugar.

2.3.2. Test for Alkaloids

(a) Dragendorff's test: To a few drops of individual extracts, two drops of draggendorff's reagent was added by the side of the test tube. An orange red coloured precipitate confirms the test as positive.

(b)Wagner's test: To a few drops of individual extracts, two drops of Wagner's reagent were added by the side of the test tube. A reddish-brown coloured precipitate confirms the test as positive.

2.3.3. Test for Tannins

(a) Lead Acetate Test: To a few drops of individual extracts, add few drops of 1% lead acetate. The mixture was shaken well. A yellowish precipitate indicates the presence tannins.

2.3.4. Test for Flavonoids

(a) Shinode test: To a few drops of individual extracts, few fragments of magnesium metal were added and then followed by few drops of concentrated HCL. A pink or orange or red to purple colouration indicates the presence of flavonoids.

2.3.5. Test for Terpenoids

(a) Acetic anhydride test: To a few drops of individual extracts, 2 ml of acetic anhydride and Concentrated H_2SO_4 was added. Formation of blue, green rings indicate the presence of terpenoids.

2.3.6. Test for Amino Acids

(a) Ninhydrin test: To a few drops of individual extracts, few drop of Ninhydrin solution was added in a test tube. A characteristic blue colour indicates the presence of amino acids.

2.3.7. Test for Proteins

(a) Biuret Test: Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink colour.

2.3.8. Test for Glycosides

(a) Libermann's test: To a few drops of individual extracts, 2ml of chloroform and 2 ml of acetic anhydride was added. Formation of violet to blue to green reddish-brown ring indicates the presence of glycosides.

2.3.9. Test for Total Phenols

To a few drops of individual extracts, 3% of $FeCl_2$ was added. Formation of deep blue colour indicates the presence of total phenol.

2.3.10. Test for Anthraquinones

To a few drops of individual extracts, 2ml of $FeCl_2$ was added and few drops of concentrated H_2SO_4 was added along the sides of the test tube and cooled and add 2ml of diethyl ether. Formation of red colour indicates the presence of anthraquinones.

2.4. Analysis of Ash and Moisture Content

The ash and moisture content were performed according to American Association of Cereal Chemists (AACC) method. They were performed in triplicate. The green tea samples were measured into ash dishes in amounts of 3 g. Then samples were placed in a muffle furnace at 550°C. They were incinerated until light gray ash or constant weight was obtained. After cooling, the samples were weighed, and the ash contents were calculated. The green tea samples (3 g) were measured into glass weighing bottles and placed in a laboratory dryer for 3 h. The samples were dried at 105 °C to constant weight. After cooling, the samples were weighed, and the moisture contents were calculated

2.5. Analysis of Theaflavin (TF), Thearubigin (TR), Colour and Brightness

Tea infusion was prepared by boiling green tea with distilled water continuously for 10 minutes. Then, filtering the tea infusion through a cotton cloth and allowed cool at to room temperature. Equal volume of green tea infusion and aqueous solution of Na_2HPO_4 were mixed and the mixture solution was extracted with 10 mL of ethyl acetate by quickly repeatedly inversion for 1 minute. After draining out, the separated bottom layer, the TF fraction (ethyl acetate layer) was diluted with 5 mL ethyl acetate. The absorbance of the extracts (E1, E2 and E3) was obtained at 380nm and 460nm on a UV-Visible spectrophotometer. E1 is the TF factor (10 mL) diluted to 25 mL with methanol; E2 is the infusion (1 mL) diluted to 10 mL with water and made up to 25 mL with methanol; E3 is the infusion (1 mL) mixed with aqueous oxalic acid (10% w/v,1 mL) and water (8 mL) and made up to 25 mL with methanol. Percent TF and percent TR were calculated at 380nm while at 460nm the total colour and percent brightness were calculated.

2.6. Analysis of Secondary Metabolites

The secondary metabolites such as total phenols, flavonoids and tannins were estimated using standard protocol of Folin-Ciocalteau reagent method, Aluminium chloride method and Folin-denis method respectively.

2.7. Determination of Antioxidant Activity

2.7.1. DPPH Assay

A test solution (5 µl) was added to 3.995 ml of methanolic DPPH. The mixture was shaken vigorously and kept at room temperature for 30 min in the dark. Absorbance of the reaction mixture was measured at 515 nm spectrophotometrically. Absorbance of the DPPH radical without antioxidant, i.e. blank was also measured. The test was performed in triplicate. The capability to scavenge the DPPH radical was calculated.

2.7.2. Hydrogen peroxide radical scavenging capacity

2.4 ml of 40mM hydrogen peroxide was mixed with 0.6 ml of green tea extracts and incubated for 10 minutes. The absorbance of the reaction mixture was measured at 230 nm spectrophotometrically against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentages of hydrogen peroxide scavenging of Green tea extracts were calculated. The test was performed in triplicate.

2.7.3. β Carotene Bleaching Assay

2 ml of β carotene in chloroform, 0.2 ml of linoleic acid and 0.2 ml of Tween 20 were transferred into a 100 mL round bottom flask. A 0.2 ml of green tea extract was added to the mixture. After evaporation to the dryness under vacuum at room temperature, 50 mL of distilled water was added into the flask. The mixture was agitated vigorously to form an emulsion was transferred to another test tube and immediately placed in water bath at 50^0C. The absorbance of the sample was measured every 20 minutes for 2 hours at 470nm using a UV-Visible spectrophotometer. The β carotene bleaching activity (CBI) was calculated. The test was performed in triplicate.

2.8. In-Vitro Anti-Inflammatory Activity

The anti-inflammatory activity was studied by using inhibition of protein denaturation technique. For this work, we choose the bovine serum albumin and egg white. To the various concentrations of green tea extracts, 450µl of bovine serum albumin and 1400µl of phosphate buffer saline was added to the test tubes. Distilled water instead of extracts with above mixture is used as negative control. Afterward, the reaction mixtures were incubated at 37^0C for 15 minutes and then heated

at 70^{0}C for 5 minutes. After cooling, their absorbance's are measured at 660nm.The percentage of protein denaturation was calculated and the test was performed in triplicate. The same procedure was followed for the egg white protein denaturation process.

2.9. In-Vitro Anti-Diabetic Activity (α-amylase inhibition method)

The different concentrations of green tea extract and 1.0 ml of α-amylase enzyme solution were mixed together and incubated at 25^{0}C for 10 minutes. After incubation,1.0ml of starch solution was added to the mixture and further incubated at 25^{0}C for 10 minutes. The reaction was then stopped by adding 2.0 ml of DNS colour reagent and heat the mixture in a boiling water bath for 5 minutes. After cooling the absorbance was measured UV-visible spectrophotometer at 565nm. The inhibition percentage was calculated and the test was performed in triplicate.

3. Results and discussion

In the present study, evaluated the phytochemical analysis, Antioxidant activity and quantification of secondary metabolites of extracts of different green tea samples and also analysed the anti-diabetic and anti-inflammatory potential of green tea. The obtained results are as follows

3.1. Phytochemical Screening

This study revealed the presence of medicinally active constituents and are summarized in following Table

Table 1: Phytochemical screening of extracts of *Camellia sinensis* varieties

S. No	Phytochemicals Tested	Sample A	Sample B	Sample C	Sample D
1	Carbohydrates	+	+	+	+
2	Alkaloids	+	+	+	+
3	Tannins	+	+	+	+
4	Flavonoids	+	+	+	+
5	Terpenoids	+	+	+	+
6	Amino acids	-	-	-	-
7	Proteins	-	-	-	-
8	Glycosides	-	-	-	-
9	Total phenols	+	+	+	+
10	Anthraquinones	-	-	-	-

The results of phytochemical screening of green tea samples (A, B, C, and D) extracts showed the presence of carbohydrates, alkaloids, tannins, flavonoids, terpenoids, amino acids, and total phenols, whereas amino acids, proteins, glycosides, and anthraquinones were absent in all the samples. The phytochemicals present in the leaves of *Camellia sinensis* indicate their potential as sources of bioactive compounds that may supply novel medicines. These findings have shown that the extract of green tea is extensively rich in secondary metabolites. The plant leaves have a high potential for a vast number of bioactive compounds, which justified their use for various ailments by traditional practitioners.

3.2. Determination of Ash and Moisture Content

The results of the ash and moisture content analysis are presented below Table. Ash and Moisture, contents are expressed in percentages.

Table 2: Showed the results of ash and moisture content

Green Tea Samples	Ash (%)	Moisture (%)
A	79.0 ± 2.2	20.4 ± 0.9
B	75.0 ± 1.9	24.7 ± 1.0
C	80.0 ± 2.4	25.9 ± 1.2
D	74.0 ± 2.9	24.2 ± 1.2

A **B** **C** **D**

Figure 2: The obtained ash content of Green Tea samples

The current study reported that the ash and moisture content of green tea samples A, B, C & D were found to be 79 % and 20.4 %, 75 % and 24.7 %, 80 % and 25.9 % & 74 % and 24.2 % respectively. From the above results, the ash content was found to be higher than the moisture content in *Camellia sinensis,* and Sample C shows a high ash content when compared to other samples. The presence of ash content in any plant sample is an indication of the presence of mineral content in an optimum amount.

3.3. Analysis of Theaflavin, Thearubigin, Color and Brightness

Theaflavin, thearubigin, color, and brightness of *Camellia sinensis* were identified, and the obtained results are as follows:

Table 3: Shows the results of TF, TR, Color & Brightness of *Camellia sinensis* varieties

Green Tea Samples	TF %	TR %	Colour %	Brightness %
A	0.33	9.10	**22.0**	4.0
B	0.22	7.76	1.50	0.41
C	0.27	5.90	6.50	11.0
D	**0.36**	**12.0**	4.75	21.0

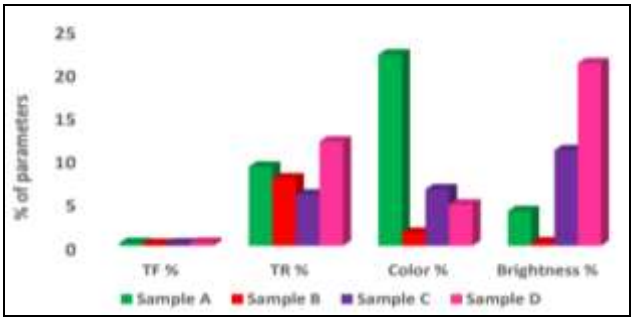


Chart 1: Levels of TF, TR, Color & Brightness of *Camellia sinensis* varieties

From the above results, the TF, TR, color, and brightness of samples A, B, C, and D were calculated. Comparatively, the percentage of TF, TR, and brightness of samples A, B, C, and D were higher in sample D than others, but the percentage of color was higher in sample A than others. Finally, there was a significant difference between all four varieties of samples.

3.4. Estimation of Secondary Metabolites of Green Tea

Green tea contains varying amount of secondary metabolites, estimated by the standard protocol and the obtained results were as follows

Table 4: Showed the results of total phenol, flavonoids and tannins

Green Tea Samples	Concentration in mg/g		
	Total Phenols	Flavonoids	Tannins
A	8.2 ± 0.2	14.0 ± 0.7	2.4 ± 0.1
B	9.6 ± 0.4	12.0 ± 0.6	6.4 ± 0.2
C	8.9 ± 0.5	13.4 ± 0.5	6.0 ± 0.2
D	9.9 ± 0.5	7.2 ± 0.3	13.2 ± 0.5

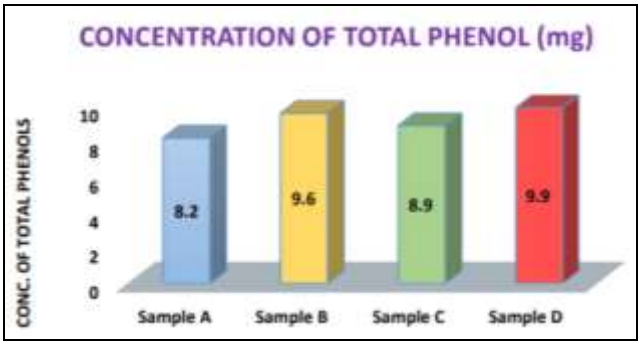


Chart 2: Different concentrations of total phenol in *Camellia sinensis* varieties

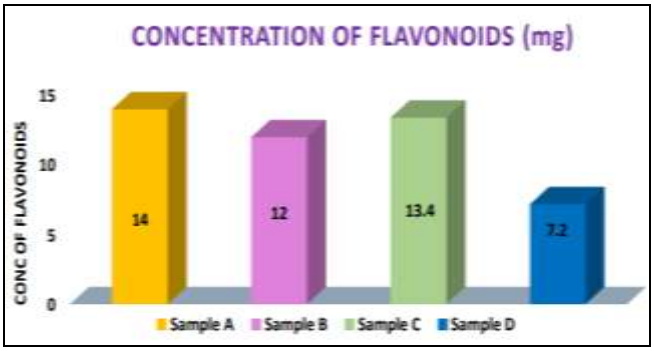


Chart 3: Different concentrations of flavonoids in *Camellia sinensis* varieties

Chart 4: Different concentrations of tannins in *Camellia sinensis* varieties

Total Phenols **Flavonoids** **Tannins**

Figure 3, 4 & 5: Showed the pictures of total phenol, flavonoids & tannins respectively

The amount of total phenol in the samples of Green tea is found to be 8.2, 9.6, 8.9 and 9.9 mg respectively. Therefore, the green tea sample B and D contain rich amount of total phenol than Sample A and C. The amount of flavonoids in the samples of Green tea is found to be 14, 12, 13.4 and 7.2 mg respectively. Therefore, the green tea sample A and C contain rich amount of flavonoids than Sample B and D. The amount of tannins in the samples of Green tea is found to be 2.4, 6.4, 6.0 and 13.2 mg respectively. Therefore, the green tea sample C and D contain rich amount of tannins than Sample A and B. Hence, the average amount of total phenol, flavonoids and tannins in the four different green tea samples was 9.15 mg/g, 11.65 mg/g and

7.0 mg/g respectively. The antioxidant activity of green tea samples lies in the presence of total phenol, flavonoids and tannins that reduces damage caused by free radicals. Therefore, the solvent extraction method plays a significant role in the extraction of antioxidants from food material in which the selection of extracting solvent is important for the recovery of antioxidant compounds

3.5. Antioxidant Activities of Green Tea

Green tea samples contains the different percentage of DPPH and H_2O_2 scavenging Activities and the obtained results were as follows

3.5.1. DPPH Assay

Table 5: Shows the results of DPPH assay

Green Tea Samples	Free radical scavanging activity in %
A	7.0 ± 0.3
B	19.0 ± 0.9
C	11.0 ± 0.5
D	9.0 ± 0.4

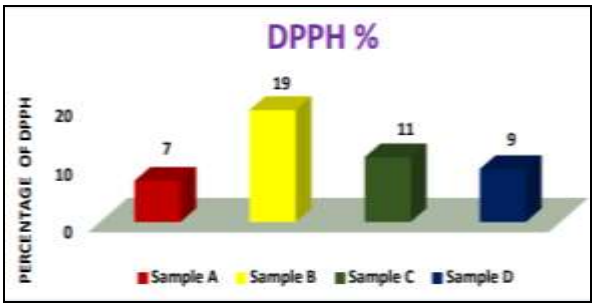


Chart 5: Represented the DPPH activity of *Camellia sinensis* varieties

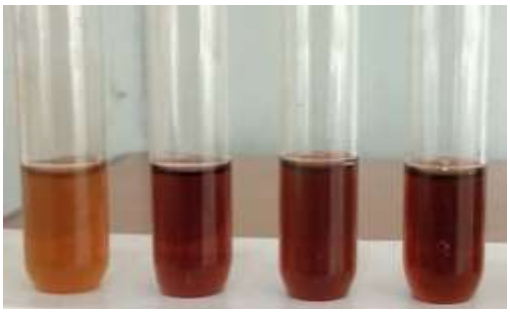


Figure 6: Results of DPPH assay of *Camellia sinensis*

The percentage of DPPH activity in the Green tea samples is found to be 7, 19, 11 and 9 % respectively. Therefore, the green tea sample B possess high percentage of DPPH free radical scavenging activity than other samples.

3.5.2. Hydrogen peroxide scavenging activity

Table 6: Shows the results of H_2O_2 Assay

Green Tea Samples	Free radical scavanging activity in %
A	12.5 ± 0.6
B	15.6 ± 0.7
C	14.0 ± 0.7
D	19.3 ± 0.9

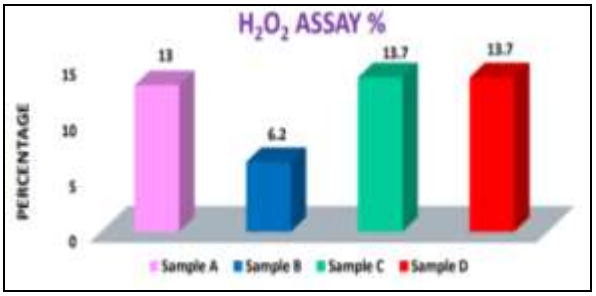


Chart 6: Represented the Hydrogen peroxide scavenging capacity of *Camellia sinensis* varieties

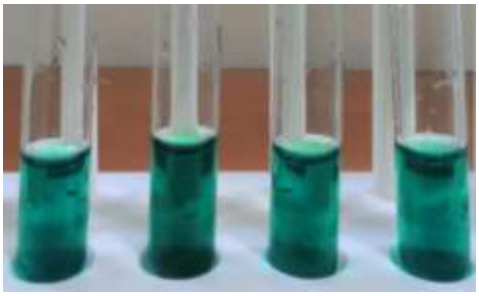


Figure 7: Results of H_2O_2 Assay of *Camellia sinensis* varieties

The percentage of H_2O_2 scavenging activity in the Green tea samples is found to be 12.5, 15.6, 14 and 19.3 % respectively. Therefore, the green tea samples D possess higher percentage of FRAP scavenging activity than the other samples.

The overall, the average % of DPPH and H_2O_2 activity in the four different green tea samples was 11.5 % and 15.35 % respectively. Comparatively, the green tea samples have high percentage of hydrogen peroxide activity than DPPH. It was observed that sample D is significantly more effective in scavenging these three free radical activities followed by B, C and D.

3.5.3. β Carotene Bleaching Assay

An assay on beta carotene activity of different samples of *Camellia sinensis* was done.The results are shown below.

Table 7: Showed the results of β Carotene Bleaching Assay

Green Tea Samples	Beta Carotene scavanging activity in %					
	20 minutes	**40 minutes**	**60 minutes**	**80 minutes**	**120 minutes**	**140 minutes**
A	21	25	30	35	**42**	29
B	58	**63**	58	54	45	42
C	20	31	34	41	**47**	43
D	43	60	**83**	79	70	66

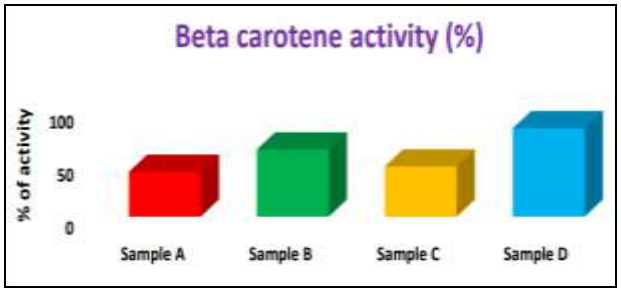


Chart 7: Represented the Beta Carotene Bleaching assay

Figure 8: Showed the picture of β Carotene Bleaching Assay

The beta carotene activity of the green tea samples are found to have high percentage in their optimal time period only, that is sample A, B, C and D contains 42% in 120 minutes, 62.5 % in 40 minutes, 47 % in 120 minutes and 83 % in 60 minutes respectively. Comparatively, the abobe result repoted that the sample D have highest potential of beta carotene activity.

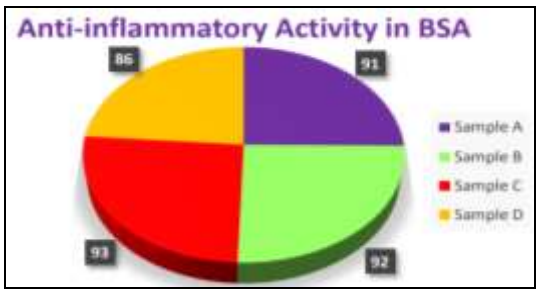
3.6. In-Vitro Anti-Inflammatory Activity

The inhibition of egg and bovine albumin denaturation technique was studied by anti-inflammatory activity and the results are as follows

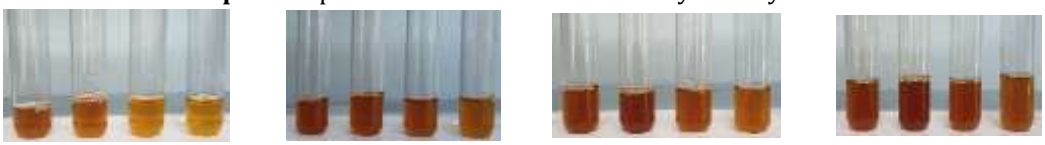
3.6.1. Using Bovine Serum Albumin:

Table 8: Shows the anti-inflammatory activity of different samples of *Camellia sinensis* in bovine serum albumin

Green Tea Samples	Anti-inflammatory activity in %			
	0.5 ml	**1.0 ml**	**1.5 ml**	**2.0 ml**
A	83	86	**91**	90
B	81	84	91	**92**
C	74	80	**93**	90
D	63	81	83	**86**



Graph 8: Represented the anti-inflammatory activity for BSA



0.5 ml **1.0 ml** **1.5 ml** **2.0 ml**

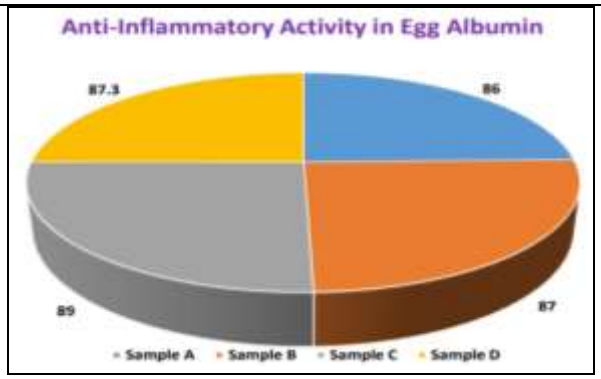
Figure 9: Result shows the anti-inflammatory activity of *Camellia sinensis* in denaturation of bovine albumin

In this work, checked the anti - inflammatory activity of 4 green tea samples by using 4 different concentrations in BSA. From the above results, 1.5 ml of sample A & C produced higher anti-inflammatory activity while 2.0 ml of sample B & D possess higher anti-inflammatory activity. Therefore, the green tea samples were good in anti-inflammatory activity with BSA

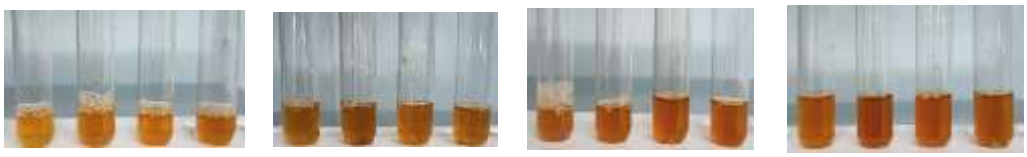
3.6.2. Using Egg Albumin

Table 9: Shows the anti-inflammatory activity of *Camellia sinensis* in egg albumin

Green Tea Samples	Anti-inflammatory activity in %			
	0.5 ml	1.0 ml	1.5 ml	2.0 ml
A	40	75	**86**	82
B	68	78	**87**	87
C	66	80	**89**	85
D	75	85	**87.3**	87



Graph 9: Represented the anti-inflammatory activity for egg albumin



0.5 ml **1.0 ml** **1.5 ml** **2.0 ml**

Figure 10: Result showed the anti-inflammatory activity of *Camellia sinensis* in Egg albumin

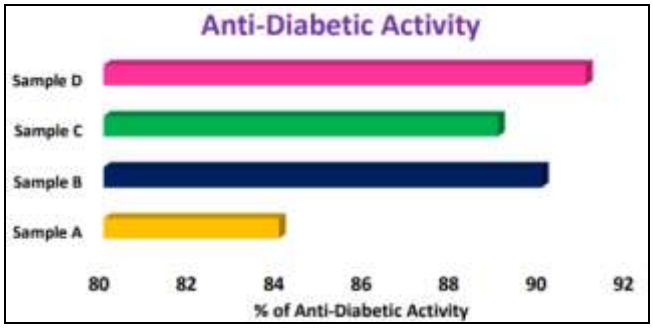
In this work, checked the anti - inflammatory activity of 4 green tea samples by using 4 different concentrations in Egg albumin. From the above results, 1.5 ml of sample A, B, C & D produced higher anti-inflammatory activity Therefore, the green tea samples were good in anti-inflammatory activity with Egg albumin.

3.7. In-Vitro Antidiabetic Activity

The anti-diabetic activity of green tea was determined by using inhibition of α amylase activity and their results are as follows

Table 10: Showed the results of Anti-diabetic activity

Green Tea Samples	Anti-diabetic activity in %			
	0.5 ml	1.0 ml	1.5 ml	2.0 ml
A	70	**84**	66	73
B	75	**90**	81	81.5
C	82	**89**	82	78
D	78	**91**	87	82



Graph 10: Result shows the anti-diabetic activity of *Camellia sinensis*

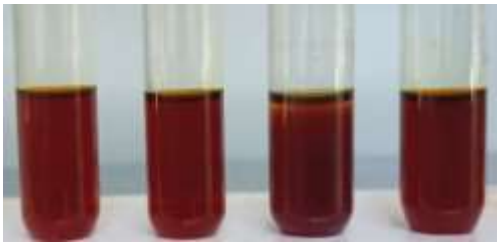


Figure 11: Showed the picture of β Carotene Bleaching Assay

Table 10 shows the anti-diabetic activity of different samples of *Camellia sinensis*. The results are exhibited in four different concentrations. From the above results, *Camellia sinensis* sample A, B, C & D have 84, 90, 89 & 91 % of anti-diabetic activity by inhibiting α amylase enzyme. When comparing the 4 concentrations of 4 samples, the 1.0 ml extract containing mixture have highest anti-diabetic activity. Therefore, the green tea samples have enormous anti-diabetic activities.

4. Summary and Conclusion

Green tea is becoming more and more popular around the world due to its various health benefits. The current study focused on the phytochemical screening of a *Camellia sinensis* aqueous extract. This indicates that total phenols, tannins, flavonoids, terpenoids, and carbohydrates are present in all of the samples. As a result, green tea has medicinal qualities that support a variety of nutritional benefits. The green tea samples have enough ash and moisture content; therefore, all have a high nutritive content. In this work, we estimated the theaflavin, thearubigin, color, and brightness of *Camellia sinensis*. This provides much better results; therefore, the green tea gives a bright orange-red color and contributes to a better mouth-feel sensation. The antioxidant capacity of *Camellia sinensis* samples is also assessed in this work through the use of DPPH, hydrogen peroxide scavenging, and beta-carotene bleaching assays. Based on the variety of phenolic compounds found in *Camellia sinensis* samples, it can be concluded that these samples have a high potential for antioxidants, which can prevent

oxidative stress, which is the process by which free radicals cause damage. Green tea is an excellent source of flavonoids and polyphenolic chemicals, which are significant compounds with a variety of nutritional effects. In summary, the antioxidant activity may offer a different approach to treating a range of illnesses.

There are certain problems associated with the use of animals in experimental pharmacological research, like ethical issues. Hence, in the present study, the protein denaturation bioassay was done by in vitro assessment of the anti-inflammatory properties of aqueous extracts of green tea using natural egg albumin and artificial bovine serum albumin. The green teas provide 75% to 95% anti-inflammatory activity. This result showed that *Camellia sinensis* has the ability to suppress carcinogenic progression and is involved in the denaturation of proteins, which are responsible for the inflammation reaction. Hence, it is used as an anti-inflammation agent.

This study also determined the antidiabetic activity of *Camellia sinensis* in in vitro conditions. The green teas provide 66% to 91% anti-diabetic activity. The experimental results showed that all four green tea samples significantly inhibited alpha-amylase enzyme activities. This study recommended that the consumption of green tea by diabetic patients may reduce the level of hyperglycemia and also regulate it.

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6. References

1. Aafrin S, Thasleema , Variq Razukas, " Bioactive components of Green tea and their Antioxidant Effects",Antioxidant, December 2004,Volume 4.
2. Baolu Zhao, "Antioxidant effects of Green tea polyphenols",Chinese Science Bulletin,Volume 48,2013
3. Cinthura C, R.V.Geetha, "Evaluation of anti-inflammatory activity of *Camellia sinensis* extract using protein denaturation assay", Drug invention today, Volume 12,2019,Special issue 1.
4. Hardoko,BintiNafi AH,Bambang B, Sasmito and YuniwatyHalim, "Antidiabetic activity of herbal green tea extract from white mangrove(*Avicennia marina*) leaves towards blood glucose level of diabetics",International journal of food studies, Volume 8,2019
5. J.Ananthi and R.Sagaya Giri "Screening of Bioactive compounds of Green Tea(*Camellia sinensis*)",World Journal of Pharmaceutical and Medical Research,Volume 4,Issue 9,2018.ISSN 2455-3301
6. JelamVazir, Priyal Inamdar, Sharav Desai, .Dhara Patel, Dhananjay Meshram, "Phytochemical Screening and of *Camellia sinensis",* Indo American Journal Of Pharmaceutical Research, 2014 , Volume 4, Issue 02 ISSN NO: 2231-6876.
7. Jigisha Anand ,Bhagwati Upadhyaya , Pramod Rawat Nishant Rai, " Biochemical characterization and pharmacognostic evaluation of purified catechins in green tea (Camellia sinensis) cultivars of India", 3 Biotech (2015) 5:285–294
8. Ki Won Lee ,Jacob Lee., " Antioxidant capacities of Green Tea and Bioactive components",Food Science and Research, Volume 2,2002 ISSN: 6521 6573
9. Lee Shan Chua,Norul Liza A. Rahaman,Nur Ardawati Adnan, "Antioxidant activity of green tea samples in relation with their biochemical components", Hindawi Publishing corporation, Journal of Analytical methods in chemistry,Volume 2013,Article ID 313798
10. Nor Qhairul Izzreen,M.N and Mohd Fadzelly,A.B "Phytochemicals and antioxidant properties of different parts of *Camellia sinensis* leaves from Sabah tea plantation in Sabah,Malaysia",International food research journal 20(1): 307-312(2013)
11. Pervin Monira,Tomokazu Ohishi,Shingo Goto,Mamoru Isemuru,Yoriyuki Nakamura, "Anti-inflammatory action of Green tea" ,Anti-inflammatory and anti-allergy agents in Medicinal chemistry, Volume 15,2016 No. 2.
12. Preethi Soyasa, Chamira Dilanka Fernando , "Extraction kinetics of phytochemicals and antioxidant activity during black tea(*Camellia sinensis*) brewing",Fernando and Soysa nutrition Journal,2015,14:74

13. Priyanka Chatterjee,Sangita Chandra,Sanjib Bhattacharya, "Evaluation of anti-inflammatory effects of green and black tea comparative in vitro study" ,Journal of advanced pharmaceutical technology and research, Volume 2,April-june 2012,Issue 2
14. Qiong Luo , Jia-Rong Zhang , Hua-Bin Li , Ding-Tao Wu , Fang Geng , Harold Corke , Xin-Lin Wei 1, and Ren-You Gan , "Green Extraction of Antioxidant Polyphenols from Green Tea (Camellia sinensis)" Antioxidants, 2020,Volume 9, 785; doi:10.3390
15. Ramesh B,Himani Vishnoi,Ravi Kant, "Green tea (*Camellia sinensis*)and its antioxidant property",International journal of pharmaceutical sciences and Research, Volme 5,November 12,ISSN: 2320 5148
16. Rani R,Nagpal D ,Gullaiya S, Madan S, Agrawal S.S, "Phytochemical,Pharamocological and Beneficial effects of Green tea", International journal of Pharmacognosy and Phytochemical Research Volume 6,2014,Issue 3,ISSN: 0975 4873.
17. Romsa Singh,Kalpana Singh "Phytochemical analysis and antioxidant potential of Green tea and Guduchi aqueous extract", Asian journal of Pharmaceutical Education and Research,Volume 6, Issue 3, 2017,ISSN 2278 7496.
18. Sarveswaran R., Jayasuriya W.J.A.B .N,Suresh T.S "In vitro assays to investigate the Anti-inflammatory activity of herbal extracts", World Journal of Pharmaceutical Research, Volume 6,Issue 17,2017.ISSN 2277-7105
19. Sergio Barreira ,Nazim Mohammad, "Phytochemical ,Antioxidant capacities of green tea", International Journals of Drug development and Research, Volume 2, April 2018,ISSN : 4563 9807.
20. Sowbagyalakshmi Prabha L, T.Venkatachalapathi., R.S.Venkatesan, "Phytochemical analysis of Green tea (Camellia sinensis) and assessment of its scavenging ability", International Journal of Science, Volume 3, Issue 8 – 2016, ISSN: 2348-5213.
21. Suhailah Hayaza ,Jibin Jacob.,(2020), " Phytochemical screening, Antioxidant capacity Functional groups and characterization analysis of EGCG of green tea", International Journal of food Science, Volume 4 , March 2020,ISSN : 6549 4324
22. Sumathi P, Subhashini R, U.S. Mahadeva Rao, and Gayathri Gunalan, "A comparative Phytochemical analysis and Antioxidant activity of green tea", Indian Journal of Science and Technology, Volume 3 No. 2 ,February 2010, ISSN: 0974- 6846.
23. Sumaya Farooq, Amit Sehgal, "Antioxidant activity of different forms of Green tea",Current research in Nutritional and Food Science, Volume 3, 2018, ISSN: 2322 0007
24. Tariq A, Reyaz.A.L, "Phytochemical analysis of *Camellia sinensis* leaves",International journal of durg development and research,Volume 4,December 2012 ISSN: 3110-3166
25. Thomas Efferth,Sergio Barreira,Carla Moutinho,Andre M.N.Silva,Jose Neves, Ean-Jeong Seo, Mohamed-Elamir F.Hegazy,,Ligia Rebelo Gomes "Phytochemical characterization and biological activities of green tea (*Camellia sinensis*) produced in Azores,Portugal", Phytomedicine plus 1,2021,100001
26. Vilma Armoskaite, Kristina Ramanauskiene, Audrius Maruska, Almantas Razukas, Audrone Dagilyte, Algirdas Baranauskas and Vitalis Briedis, "The analysis of quality and antioxidant activity of green tea extracts", Journal of Medicinal Plants Research Vol. 5,4 March, 2011, ISSN 1996-0875
27. Wen-Ying-Huang, Yu-Ru Lin,Ruei-Fen Ho,Ho-Yen Liu and Yung-Sheng Lin "Effect of water solutions on extracting Green tea leaves" ,The Scientific World Journal, Volume 2013,Article ID 368350
28. Yadav KC, Ashok Parajuli, Bishnu Bahadur Khatri and Lila Devi Shiwakoti "Phytochemicals and quality of Green and Black teas from different clones of tea plant", Journal of food quality, Volume 2020,Article ID 8874271
29. Yakuba A,Dallatu,Gana P.Jibrin,Musa Haliru, "Invitro evaluation of *Camellia sinensis* aqueous extracts effect on protein denaturation during acute inflammation", Biointerface research in applied chemistry,Volume 3,Issue 3,2013,ISSN: 2069 5837.
30. Yi-Fang Li,Bang-Tian Chen,Wei-Xi Li,Rong-Rong He and Hiroshi Kurihara , "Anti-inflammatory effects of a polyphenols rich extract form tea(*Camellia sinensis*) flowers",Hindawi Publishing corporation,Oxidative medicine and cellular longevity,Volume 2012,Article ID 537923