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Unveiling the Multifaceted Health Benefits of Kangra Green Tea Leaves: Antioxidant, Antidiabetic and Antibacterial Properties

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ABSTRACT

This research focused on the *in vitro* evaluation of the hypoglycemic and antioxidant effects of leaf extracts of *Camellia sinensis* using ethanol, methanol and water. The assessment techniques employed included α-amylase, α-glucosidase inhibitory assays, glucose adsorption, glucose diffusion methods, glucose uptake potential in yeast cells, as well as DPPH, hydrogen peroxide activity and ferric reducing power radical scavenging activity. The green tea extracts demonstrated significant antioxidant activity by eliminating free radicals: 65-90.21% (DPPH), 60-80% (HO_2O_2) and 55-76% (FRAP). Although the *in vitro* glucose diffusion retardation, glucose uptake capacity and glucose adsorption suggested potential therapeutic value for green tea, further research is necessary to identify the relevant bioactive compounds and to validate their pharmacological properties *in vivo.*

Key words: Oxidative stress, diabetes, epigallocatechin-3-gallate, antioxidant, oxidative damage

INTRODUCTION

Green tea has garnered significant attention for its extensive range of health benefits, largely attributed to its rich composition of bioactive compounds. Among the various types of green tea, Kangra green tea, cultivated in the pristine environment of the Kangra district, Himachal Pradesh, in India, has emerged as a notable exemplar of both superior quality and diverse therapeutic properties. This unique variety of green tea is not only for its distinctive flavour and aromatic profile but also for its substantial health-promoting attributes (Basu *et al.,* 2021). The health benefits of Kangra green tea are underpinned by its high concentration of antioxidants, particularly catechins and polyphenols, which play a pivotal role in mitigating oxidative stress and reducing the risk of chronic diseases. These antioxidants are crucial in neutralizing free radicals, thereby preventing cellular damage and promoting overall health (Dong *et al.,* 2022). Additionally, green tea demonstrates significant anti-diabetic properties by regulating blood glucose levels and enhancing insulin sensitivity, making it an asset in the management of diabetes and related metabolic disorders. The antioxidant, anti-inflammatory and antidiabetic potential are due to being rich in catechins, particularly epigallocatechin gallate (EGCG), epicatechin gallate (ECG) and epicatechin (EC). These compounds effectively neutralize free radicals and reduce oxidative damage, which is critical for preventing cellular aging and disease. The different assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl), H_2O_2 (Hydrogen Peroxide), FRAP (Ferric Reducing Antioxidant Assay) and TBARS (Thiobarbituric Acid Reactive Substance) are demonstrating that Kangra green tea possesses strong radical scavenging activity. Collectively, these findings affirm Kangra green tea's significant antioxidant potential, contributing to its health-promoting properties and making it a valuable component of a health-conscious diet (Basitere *et al.,* 2022). This research paper aims at providing comprehensive research on the multifaceted health benefits of Kangra green tea leaves, with a focus on their antioxidant, antidiabetic and antibacterial properties (Ahmed *et al.,* 2021). By examining the underlying mechanisms and presenting recent scientific findings, this study seeks to elucidate the potential of Kangra green tea as a functional beverage with significant therapeutic value.

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MATERIALS AND METHODS

The dried leaves of Kangra green tea were gathered from district of Kangra, Himachal Pradesh, India, in the summer of 2023, which were recently harvested and processed. Subsequently, the dried components were finely ground into a powder to facilitate storage. Meteorological details are given in Table 1.

The extracts were prepared using three different solvents: methanol, ethanol and petroleum ether along with water as standard or aqueous by maceration method. Ten g of desired plant powder was poured into the required solvent and kept overnight at room temperature. After 24 h, the extract was filtered using a vacuum filter to obtain the particle free extract. The extract was concentrated by using a hot air oven maintained at 37°C. Finally, the dried and concentrated extract was kept at 4°C for further use to estimate the efficacy. The computation of the gallic acid equivalent was performed utilizing the formula y= 0.003x + 0.002. In this equation, x represented the quantity of the test component, c denoted the extinction coefficient, m signified the slope value and y represented the amount of flavonoid. The determination of the total flavonoid content was carried out using the $AlCl_3$ colorimetric method (Farman *et al.,* 2017; Ahmed *et al.,* 2021).

The scavenging efficacy of aqueous, methanolic and ethanolic extracts of Kangra green tea was estimated by using the DPPH method (Apetrei and Munteanu, 2021). Hydrogen peroxide (H_2O_2) solution was prepared in 300 ml of 1M phosphate buffer to maintain pH at 7.4, and then absorbance was measured at 230 nm in the blank containing phosphate buffer. Fernado and Munbeanu Approach was used to assess the ability to decrease superoxide anions (Superoxide Dismutase assay, SOD). The TBARS assay was carried out according to the instructions. To 1 ml of sample solution, 2 ml of 20% trichloroacetic acid and 2 ml of 0.67% thiobarbituric acid were added. For 10 min, the mixture was placed in a boiling water bath. After chilling for 20 min at 3000 rpm, it was centrifuged. At 552 nm, the absorbance of the supernatant was measured. Percentage inhibition was used to define antioxidant activity.

For alpha amylase inhibition assay, 0.125 g potato starch in 20 mM phosphate buffer was dissolved with 6.7 mM NaCl (pH 6.9), boiled for 15 min, and mixed with 1 UU/ml alpha-amylase. 1000 µl starch solution and 1000 µl enzyme inhibitor were added to 1000 µl DNS solution, heated for 15 min, diluted with distilled water, and absorbance was measured at 540 nm for IC50 for percentage inhibition. 100 µl of IU/glucosidase enzyme was blended with 100 µl phosphate buffers and 100 µl enzyme inhibitors, then mixed with maltose solution and incubated at 37°C for 60 min. It was boiled for 2 min to stop the reaction and cooled. Glucose reagent was added, and absorbance was measured at 540 nm.

For yeast preparation, baker's yeast in water was dissolved, incubated overnight at 25°C and centrifuged to prepare a 10% v/v yeast suspension. Plant extracts in DMSO were combined with glucose solution and incubated with yeast suspension at 37°C for 60 min to measure glucose concentration using a UV spectrophotometer at 520 nm. Glucose diffusion inhibition was performed using 200 µg/ml leaf extracts on dialysis membrane strips at 37°C with orbital shaking for 3 h (Aziz *et al.* 2021)

RESULTS AND DISCUSSION

The phytochemical screening of different extracts: water, ethanolic, methanolic and petroleum ether revealed the presence of several key phyto-constituents. Flavonoids, steroids, glycosides, phenols, carbohydrates, tannins and terpenoids were consistently present in all extracts, indicating their broad solubility across these solvents. Saponins were

Table 1. Ecological details when collecting *Camellia sinensis* (Kangra green tea)

S. No.	Sample	Area	Part used	Soil type	Average temperature (°C)	Average rainfall	Altitude	Collecting time (2022-23)
1.	*Camellia sinensis,* green tea	Himachal Pradesh	Leaves	Well drained, loamy	27	1200 to 2000 mm	1500 m	September

detected in water, methanolic, and petroleum ether extracts, but not in the ethanolic extract. This suggests that ethanol may not be the ideal solvent for extracting saponins. Overall, the study demonstrates that a wide range of bioactive compounds can be effectively extracted using these solvents, with slight variations in the solubility of certain constituents like saponins (Table 2). The total phenolic content (TPC) and total flavonoid content (TFC) were measured across different extracts of Kangra green tea extract (KGTE) using methanolic, ethanolic, aqueous, and petroleum ether solvents. The methanolic extract showed the highest TPC (0.601±0.1 mg/g) and TFC (0.85±0.1 mg/g), indicating that methanol is the most efficient solvent for extracting phenolics and flavonoids from KGTE. The ethanolic extract had slightly lower values with a TPC of 0.521±0.3 mg/g and a TFC of 0.701±0.6 mg/g. The aqueous extract followed with TPC and TFC values of 0.421±0.9 and 0.601±0.21 mg/g, respectively. The petroleum ether extract exhibited the lowest TPC (0.265±0.7 mg/g) and TFC (0.3±0.32 mg/g), suggesting it is less effective for extracting these compounds (Table 3).

To estimate the antioxidant potential of *Camilla sinensis,* five concentrations (50, 100, 150, 200 and 250 µg/ml) were used with different solvent systems i.e. aqueous, ethanol, petroleum ether, and methanol. A DPPH radical scavenging assay was performed and the maximum antioxidant activity (% scavenging) for *C. sinensis* was found (70.11±0.01 to 92.11±0.1) with methanol, ethanol, petroleum ether, aqueous and

Table 3. Quantitively estimation of total phenolic and flavonoid content in crude extract of Kangra green tea

Samples	Total phenolic content (TPC) (mg/g)	Total flavonoid content (TFC) (mg/g)
Methanolic KGTE	0.601±0.1	0.85±0.1
Ethanolic KGTE	0.521±0.3	0.701±0.6
Aqueous KGTE	0.421±0.9	0.601±0.21
Petroleum ether	0.265±0.7	0.3±0.32

standard (Fig. 1a). In addition, the scavenging activity of SOD and TBARS of *C. sinensis* was found in the range of 17.89±0.01 to 33.43 and 11.21±0.1 to 26.11±0.01, respectively (Fig. 1c and 1d). Free radical scavenging activity for hydrogen peroxide assay with *C. sinensis* was found to be (66.34±0.01 to 85.43±0.3) (Fig. 1b). Kangra green tea (KGTE) demonstrated strong antioxidant activity in four assays, surpassing the standard in all. KGTE's DPPH scavenging (265.42 µg/ml) was notably higher than the standard (103.03 µg/ml), as was its hydrogen peroxide scavenging (270.33 vs. 88.30 µg/ml). In the SOD scavenging assay, KGTE (229.65 µg/ml) outperformed the standard (77.47 µg/ml), and its TBARS scavenging activity (551.86 µg/ml) far exceeded the standard (70.22 µg/ml), indicating superior antioxidant potential (Table 4). For further antidiabetic analysis, only three different extracts were used including methanolic, ethanolic and aqueous based on higher radical percentage activity. The inhibitory effect of the KGTE (Kangra green tea extract) and acarbose on α-amylase activity was assessed in a dose-dependent manner, with concentrations ranging from 50 to 100 µg/ml. As the concentration increased, the

Table 2. Qualitative phytochemical analysis of different phytoconstituents

S. No.	Phytoconstituents	Solvents used			
		Water extract	Ethanolic extract	Methanolic extract	Petroleum ether
1.	Flavonoids	+	+	+	+
2.	Saponins	+	-	+	+
3.	Steroids	+	+	+	+
4.	Glycosides	+	+	+	+
5.	Phenol	+	+	+	+
6.	Carbohydrates	+	+	+	+
7.	Tannins	+	+	+	+
8.	Terpenoids	+	+	+	+

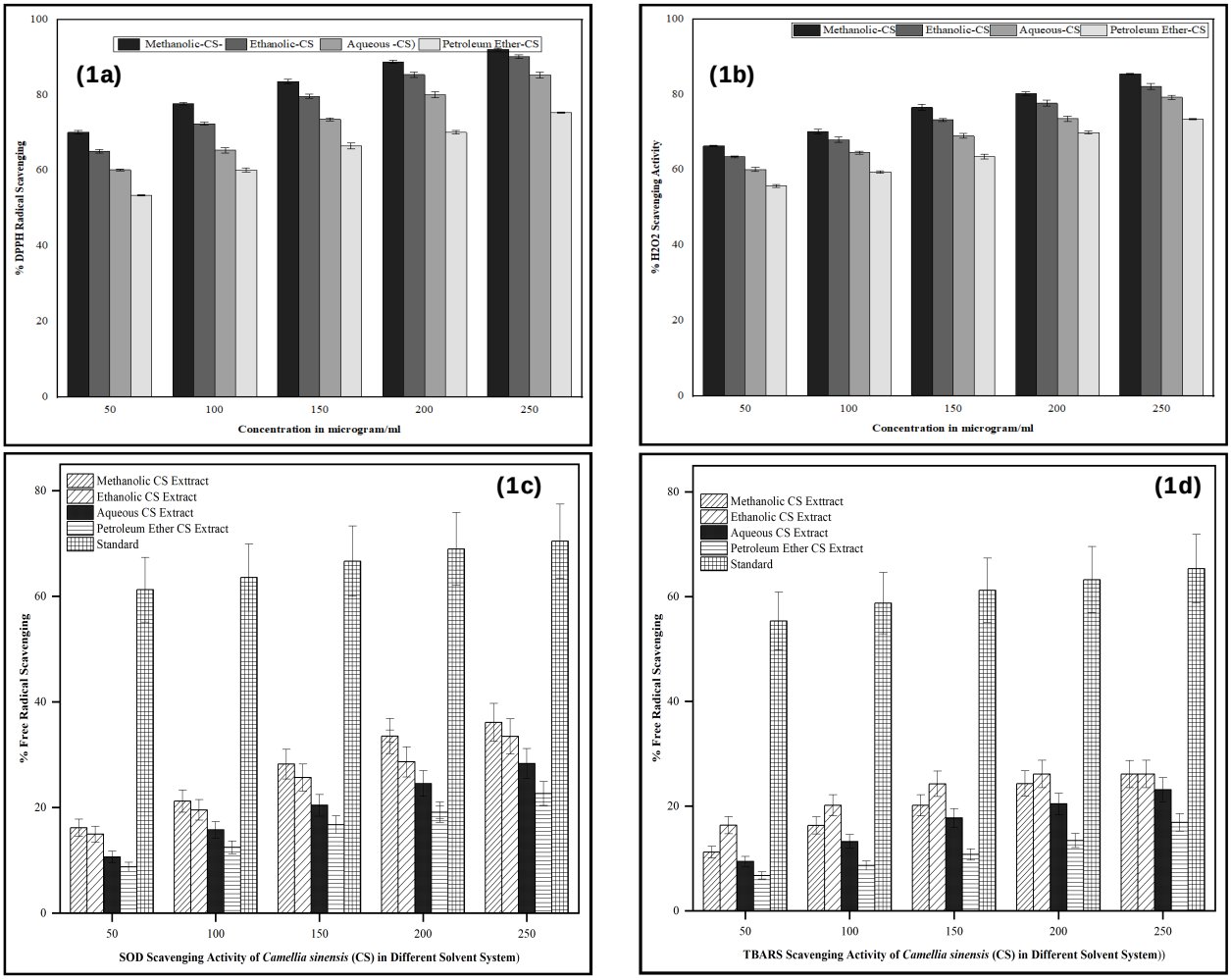

Fig. 1. Optimization of a solvent system for a DPPH (1a), H_2O_2 (1b), SOD (1c) and TBARS assay (1d).

Table 4. Comparative assessment of distinctive methanolic extracts conducted using various assays including DPPH, H_2O_2, SOD and TBARS to determine their IC_{50} values

S. No.	Sample (µg/ml)	DPPH scavenging	Hydrogen peroxide scavenging	SOD scavenging	TBARS scavenging
1.	Standard	103.026	88.301	77.47	70.22
2.	Kangra green tea	265.42	270.33	229.65	551.86

inhibition action also increased. The inhibitory percentages of methanolic, ethanolic, aqueous extracts and acarbose were found to be in the range of 55, 53.43, 50.21, 55.43 and 60%, respectively (Fig. 2a). In all the extracts from the same plant, a reduction of α-amylase activity was observed in all solvents, which was dependent on the dose. In this study, the selected herbal plants green tea extracts showed comparable results to acarbose. Similarly, in the alpha-glucosidase inhibitory assay, the KGTE leaves showed comparable inhibitory activity as compared to acarbose at the same concentration which was 36.78±0.5 to 48.7±0.01, 35.76±0.5 to 48.70±0.4, 30.11±0.3 to 45.77 and 38.78±0.5 to 55.43±0.01 in the methanolic, ethanolic, aqueous and standard shown in Fig. 2b. The extracted phytochemical responsible for α-amylase inhibition may be linked to the differentials in solvent selectivity (Rupasinghe and Sekhoon-Loodu, 2019). Further, dietary polyphenols are responsible for the anti-hyperglycemic effect of binding with membrane glucose transporters to generate a competitive inhibition response against digestive enzymes

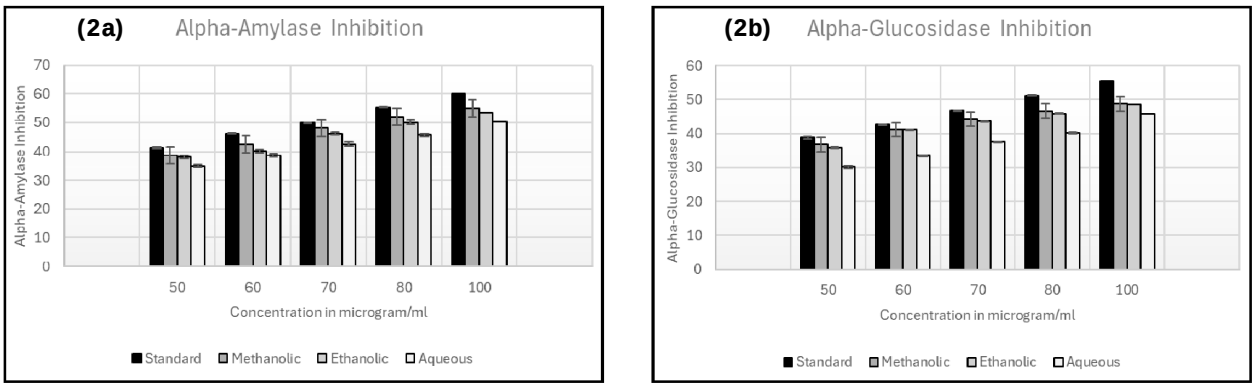

Fig. 2. Inhibition of alpha-amylase (2a) and alpha-glucosidase (2b) by Kangra green tea extract (KGTE) at various herbal concentrations.

(Alqahtani *et al*., 2019). Fig. 3 illustrates the rate at which glucose is taken up by the yeast cells across the membrane. The KGTE's glucose absorption was approximately equivalent to the well-known antibiotic metronidazole at starting concentrations of 5 and 10 mM. In the aqueous extract, the % glucose uptake capacity was approximately lower than the standard, which was in the range of 34.55 to 47.89% at 5 mM glucose concentration (Fig. 3a), and 41.22 to 53.21% at 10 mM glucose concentration (Fig. 3b). However, as compared to KGTE, metronidazole had a somewhat larger effect on glucose uptake by yeast cells at 10 mM glucose concentration, which was 50.11% of KGTE (Fig. 3b). It is well documented in various studies that glucose transportation across the membrane of yeast cells occurs by facilitated diffusion (Bhutkar *et al.,* 2018).

Table 5 illustrates how KGTE extracts (GDRI) can retard the diffusion of glucose. It was found that regardless of the plant extract or the chosen concentration levels, the attenuation proportion improved with time. At the end of 180 min, the methanolic leaf extract (200 g/ml) showed the maximum (29.43±0.01) and least (23.02±0.05) inhibition, respectively. Similar ethanolic KGTE at the 200 µg/ml concentration showed a minimum (20.76±0.1) and maximum (25.87±0.6). In comparison to other extracts, the aqueous extract of KGTE demonstrates a significant increase in the inhibition of glucose transport. To illustrate,

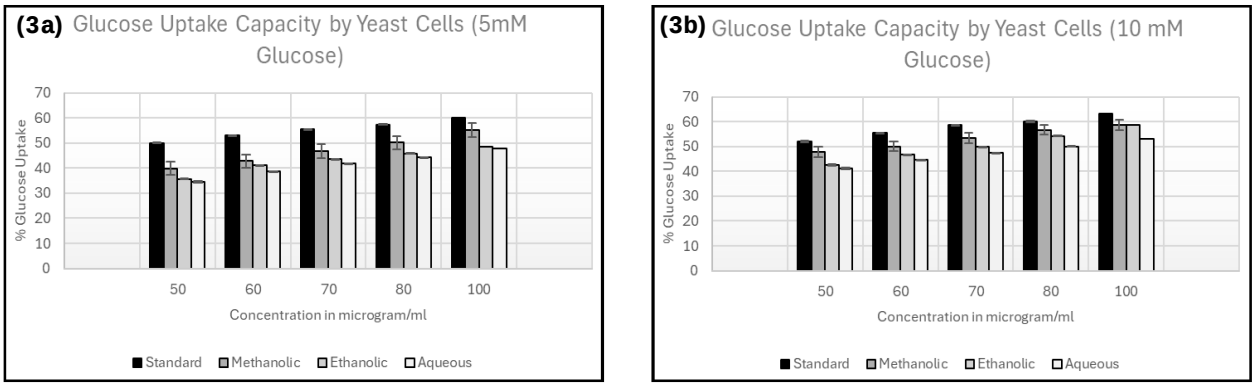

Fig. 3. Changes in glucose uptake capacity in yeast cells in the presence of Kangra green tea extract (KGTE) at glucose concentrations of 5 mM (3a) and 10 mM (3b).

Table 5. Glucose diffusion assay *in vitro* estimation

Test sample (200 mg/ml)	Glucose concentration in external solution at 30 min	Glucose concentration at 60 min	% GDRI at 30 min	% GDRI at 60 min
Control	0.921±0.002	1.42+0.3	0	0
Methanolic extract (*C. sinensis*)	0.812	1.221±0.6	23.02±0.05	29.43±0.01
Ethanolic leaves extract (*C. sinensis*)	0.621+0.0	1.021±0.09	20.76±0.1	25.87±0.6
Aqueous extract (*C. sinensis*)	0.521±0.7	0.9±0.03	18.92±0.5	22.54±0.05

when compared to the control group, a much higher dosage (50 mg/ml) of agrimony and avocado extracts was required to achieve a 60 and 71% inhibition, respectively, in glucose diffusion. In one of the studies, it was discovered that the flow of glucose across the dialysis membrane could be reduced from 309.15 to 200.15 mg/dl (equivalent to a 35% inhibition) by using a 50 g/l aqueous extract of *Psidium guajava* leaves (Barooah *et al.,* 2021).

CONCLUSION

Kangra green tea (KGT) extract exhibits strong antioxidant and antidiabetic properties, as demonstrated by its ability to neutralize free radicals and inhibit key enzymes like alpha-amylase and alpha-glucosidase. Its effectiveness in enhancing glucose uptake and reducing oxidative stress underscores its potential as a natural remedy for health and wellness applications.

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