In-vitro Antidiabetic Activity of Ethanolic Extracts of *Camellia sinensis L.* (Green tea and Oolong tea) By Using α Amylase InhibitoryAssay

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Abstract:

Diabetes Mellitus (DM) is a long-term chronic disorder that leads to high blood glucose levels. Type 2 diabetes mellitus (T2DM) is the most common type of DM which includes defects like insulin resistance and lower insulin secretion. The general strategies that are incorporated by people to treat T2DM are regular exercise, diet control, and consumption of various oral antidiabetic drugs (OADs). Despite their therapeutic benefits, these drugs tend to produce undesirable side effects. With the advantage of having several anti-diabetic bioactive reagents, natural products (NPs) are being considered a better alternative for the treatment of T2DM. These products are considered to be less toxic and have fewer side effects than allopathic medicines. The increased dependency of the people on plant-based products shows their urge to attain a collective defence against various ailments and diseases. So, the prime aim of this study is to investigate anti-diabetic properties (at the in-vitro level) of ethanolic extracts *Camelliasinensis*L. (Green tea and Oolong tea) By Using α Amylase Inhibitory Assay abundantly available in Rajasthan and are being used by the traditional medicine system to cure several ailments. For this investigation, α-amylase DNSA have been performed. In this study, it has been observed that both the plants contain compounds like alkaloids, tannins, flavonoids, etc., and show several medicinal properties. However, the ethanolic extracts of *Camellia sinensis* L contains large amounts of phenolic and flavonoid compounds that exhibit the highest antioxidant and free radical scavenging potential. This plant even exhibited better in vitro antidiabetic potential as compared to the other plant extract in consideration.

Keywords: *Camellia sinensis,* Green tea, Oolong tea, α-amylase DNSA Method, Polyphenols, Type 2 diabetes mellitus,

INTRODUCTION

Diabetes mellitus is a leading metabolic disorder in worldwide and India is the capital for diabetes. Diabetes is a condition in which the body does not produce enough insulin or properly respond to insulin. Insulin is a hormone which is produced in β cells of pancrease. It stimulates the body cells to absorb glucose from blood. Diabetes mellitus is one of the most common endocrine metabolic disorders which causes the various micro vascular and macro vascular complications[1]. Microvascular complications are most common in type 1 diabetes mellitus which includes retinopathy, neuropathy and nephropathy and Macrovascular complications includes heart attack, stroke and peripheral vascular dis eases etc. Nearly 2.8% of the world's population affected by diabetes and it is expected to increases up to 5.4% in the year of 2025[2]. Various kinds of antidiabetic therapies such as sulphonylureas, biguanides, glinides etc, are available, but it causes various adverse effects. So, the researchers are investigating and try to find more effective and safer hypoglycaemic agent without causing any side effects[3.] Herbal medicines are derived from plant extract are being used to treat a wide variety of clinical diseases.[4]

Many of the pharmaceutical compounds which are present as a secondary metabolite in plants.Tea is the second most commonly consumed beverage in the world after coffee, beer, wine and carbonated soft drinks.[5,6]Green tea has number of pharmacological activities such as anticancer, lipid lowering, neuromuscular blocking action, immunomodulatory effect, antiviral, antibacterial[7], antispasmodic, antioxidant[8]. A large number of phytoconstituents like alkaloids (caffeine, theobromine), proteins, enzymes, carbohydrates, lipids, polyphenols, catechins (epicatechins, epigallocatechin and epicatechins 3-gallate, epigallocatechin-3-gallate), carbohydrates, tannins, vitamins and minerals have been reported to be present in this plant[9].

Free radicals can damage cells, and may play a role in heart disease, cancer and other diseases. Another study shows that green tea is also helpful in reducing stroke, myocardial infarction and coronary heart diseases[10].Oolong tea has been demonstrated to possess various pharmacological activities such as antioxidant activity by reducing oxidative stress, anti-cancer, anti-obesity, antidiabetic, preventive effect of atherosclerosis, heart disease, hypertension, anti-allergic effect, and antiseptic effects.[11-16]

Experimental Section:

Collection of plant materials

Green tea leaves were collected from Kurnool.

Oolong tea leaves were collected from Kurnool.

Preparation of extracts:

Maceration extraction was done for the tea leaves by taking 25gms of green tea leaves and oolong tea leaves in a separate beaker; ethanol was poured until the leaves covered. The container is then closed and kept for 3 days. The content is stirred periodically. At the end of extraction, the leaves are filtered the filtrate is placed on the heating mantle, the ethanol is evaporated until the required amount of extract is formed. This extract was screened for phytochemical screening, anti-diabetic activity.

Qualitative preliminary phytochemical screening of ethanolicextracts:

Ethanolic extract of green tea leaves, oolong tea leaves were screened for their chemical constituents. Phytochemical screening was done as explained in literature. A small amount of extract was used to determine the alkaloids, tannins, flavonoids, glycosides, steroids, terpenoids.

Determination of total flavonoid content:

Procedure:

Ethanolic extracts of green tea and oolong tea (camellia sinensis) were estimated for total phenolic content by Folin-Ciocalteau reagent method. Stock solution (1mg/ml) of the ethanolic extracts were prepared inrespective solvents. From the stock solutions 1ml of the extract was taken into a 25 mlvolumetric flask. To this 10 ml of water and 1.5 ml of Folin-Ciocalteaureagent were added. The mixture was kept aside for 5 min. & then 4 ml of 20% SodiumCarbonate solution was added and volume was made up to 25 ml with distilledwater. The mixture was kept aside for 30 min & absorbance of blue Colour developed was recorded at 765nm using UV-Visible spectrophotometer. Standard gallic acid was prepared in concentrations ranging between 50-150 µg/ml.Calibration curve for gallacidwas obtained by plotting absorbance onY-axis and their corresponding concentration on X-axis. The phenolic content of the test samples was computed from calibrationcurve of gallic acid and expressed as µg/ml.

Determination of flavonoid content:

Procedure:

This method was reported by chang et.at. was utilized for this estimation. Naringenin was utilized as a reference standard to construct calibration curve. 20mg of Naringenin was dissolved in methanol and then it diluted to give concentrations of 250, 500, 1000 and 2000µg/ml. one ml of each of the diluted standard solution was separately mixed with 2ml of 2,4-dinitrophenyl hydrazine reagent and 2 ml of methanol at 500C for 50 mins. After cooling to room temperature, the reaction mixture with 5 ml of 1%potassium hydroxide in 70% methanol and incubated at room temperature for 2mins.Then 1ml of the mixture was taken, mixed with 5 ml of methanol and centrifuged at 1000 rpm/min to remove the precipitate formed. The supernatant was collected and adjusted to 25 ml. The absorbance of the supernatant liquid was measured at 495 nm. Similarly, 5 ml of ethanol extract of green tea, Black tea, Oolong tea (5 mg /ml) were similarly treated with 2,4-dinito phenyl hydrazine reagent for detection of Flavonoid content as described above.For the blank the amount of 2,4-dinitri phenyl hydrazine reagent was substituted by methanol.

In-vitro **anti-Diabetic Activity**:

Alpha-amylase inhibitory assay:

Procedure:

The amylase inhibitory activity was determined by an assay modified from the Worthington Enzyme Manual. A total of 500 L of each sugar extract and 500 L of 0.02 M sodiumphosphate buffer (pH 6.9 with 0.006 MNaCl) containing -amylase solution (0.5 mg/mL) were incubated at 25°C for 10minutes. After preincubation, 500 L of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube at timed intervals. The reaction mixtures were then incubated at 25°C for 10 minutes. The reaction was stopped with 1.0 mL of dinitro salicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 minutes and cooledto room temperature. The reaction mixture was then diluted after adding 15 mLof distilled water, and absorbance was measured at 540 nm. Sample blanks (buffer instead of enzyme solution) and a control (buffer in place of sampleextract) were included as well. The -glucosidase inhibitory activitywascalculated according to the equation below:

$$\text{Inhibitory activity (\%)} = (Ac - As/Ac) \times 100$$

Where, As is the absorbance in the presence of test substance and Ac is the absorbance

RESULT AND DISCUSSION

The leaves extract of Camellia sinensis L. were subjected formaceration with ethanol. The yield was found to be 45 % w/w and 35 % w/wfor ethanolic extracts respectively. These extracts were subjected to variousqualitative phytochemical tests to identify the active constituents which showedpresence of Alkaloids, Tannins, Flavonoids, Glycosides, Steroids. Ethanolic root extract showed the presence of Alkaloids, Tannins, Steroids andterpenoids, Flavonoids, Glycosides.

Physical characteristics of extracts:

The Ethanolic extracts of Camellia sinensis L.(Green tea andOolong tea) was thick green in colour, sticky in nature and the percentage yield of the extracts was found to be,

Qualitative phytochemical screening was carried out usingseveral tests and results revealed that ethanolic extracts of *Camellia sinensis L.*leaves contains phenols, flavonoids, alkaloids, carbohydrates, and steroids andtriterpenoids.

Table 1: Summary of Phytochemical constituents.

S. no	Phytochemical Constituents	Ethanolic extract	
		Green tea	**Oolong tea**
1	Alkaloids	presences	presences
2	Carbohydrates	presences	presences
3	Amino acids and Proteins	presences	presences
4	Glycosides	presence	presence
5	Tannin	presence	presence
6	Flavonoids	presence	presence
7	Steroids and Triterpenoids	presence	presence

Table 2: Total Phenolic content in ethanolic extracts of *Camelliasinensis L.*

The quantity of total phenolic content was determined from gallic acid calibration curve using the regression equation y = 0.0043x + 0.0104, R^2 = 0.9968. The total phenolic content of the ethanolic extract was100.5±0.402& 89.5±0.358µggallic acid equivalents/gm of dry material.

S.no	Extracts	Total phenolic content
1	Ethanolic extract of Green tea	100.5±0.402
2	Ethanolic extract Oolong tea	89.5±0.358

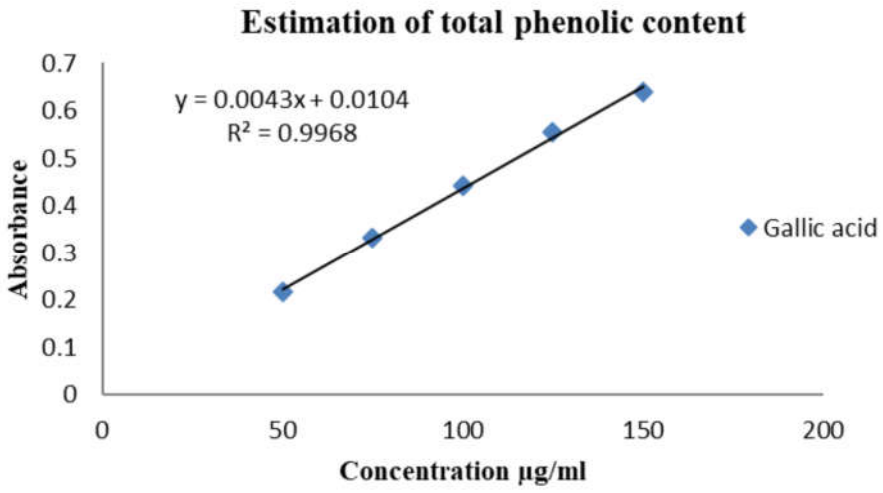

Fig 1: Standard calibration curve of gallic acid for estimation of total phenolic content

Table 3: Determination of flavonoid content of different tea leaves:

The quantity of total flavonoid content was determined from gallic acid calibration curve using the regression equation y = 0.017x + 0.036, R^2 = 0.993. The total flavonoid content of the ethanolic extract was43.5±0.174µgNaringin equivalents/gm of dry material.

S.no	Concentration(ug/ml)	Absorbance
1	10	0.204
2	20	0.394
3	30	0.56
4	40	0.778
5	50	0.895
6	Green Tea	0.254
7	Oolonga Tea	0.205

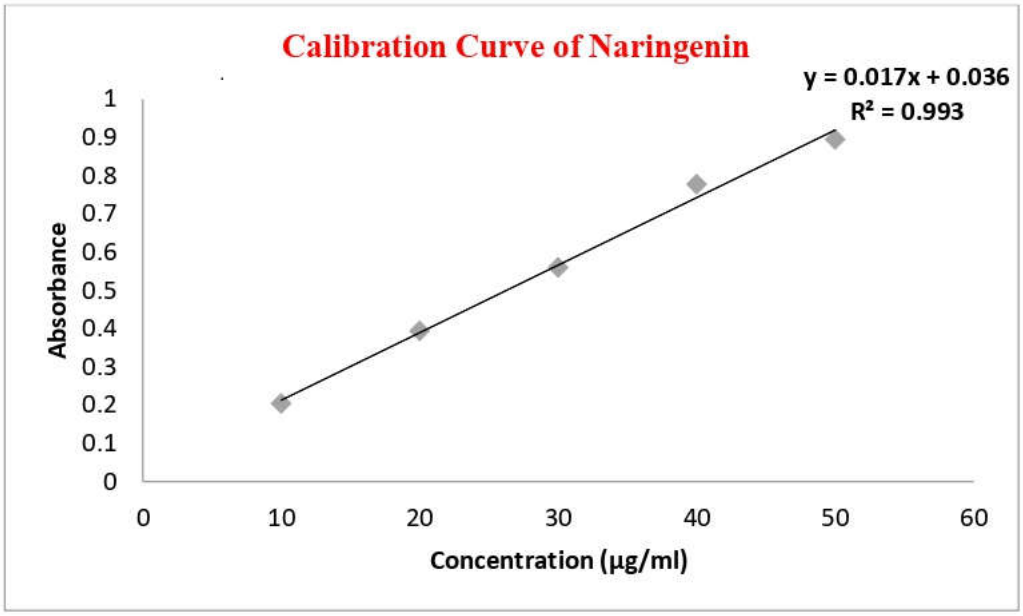

Fig 2: Standard calibration curve of Naringenin for estimation of total flavanoid content

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM.

INVITRO-ANTI-DIABETIC ACTIVITY:

Evaluation of *in vitro* α-amylase inhibitory activity using green tea &oolong tea extract:

The effect of various concentrations of Green tea & Oolong tea leavesextract on a-amylase activity is presented in table 16. Green tea& oolong teaextract potentially inhibited α-amylase activity in a dose-dependent manner.This high inhibitory activity was expressed in terms of IC50 value. Thecalculated IC50 showed that the inhibition potential of acarbose (203.52 µg/ml)and Green tea (206.66 µg/ml), Oolong tea (231.41 µg/ml) respectively. Theextract showed dose dependent percentage inhibition. The Green teaconcentration 500µg/ml of plant exhibited highest 65.49% inhibition of α-amylase activity and 68.42% inhibition was showed by standard drug acarboseat the 250 µg/ml concentration. The Oolong tea concentration 500µg/ml of plantexhibited highest 70.75% inhibition of α-amylase activity and 68.42%inhibition was showed by standard drug acarbose at the 250 µg/mlconcentration.

Table 4: α-amylase inhibition by *Camellia sinensis* L. ethanolic extract:

S.no	Standard	Concentration(ug/ml)	Absorbance	Percentage inhibition
1	Acarbose (203.52µg/ml)	7	0.0510±0.0005774	17.54%
2		15	0.0480±0.001155	29.20%
3		30	0.04933±0.0008819	29.07%
4		60	0.0420±0.0005774	44.85%
5		120	0.0220±0.001155	61.40%
6		250	0.0180±0.0005773	68.42%
7		500	0.02033±0.0008819	64.91%

Table 5: Anti-diabetic activity of *Camellia sinensis* L. (Green tea)

S.no	Sample	Concentration(ug/ml)	Absorbance(mean ± SEM)	Percentage inhibtion
1	Green Tea (206.66µg/ml)	7	0.0510±0.0005774	10.52%
2		15	0.0480±0.001155	15.78%
3		30	0.04933±0.0008819	13.45%
4		60	0.0420±0.0005774	26.31%
5		120	0.03233±0.001453	43.28%
6		250	0.02367±0.001453	58.47%
7		500	0.01967±0.001453	65.49%

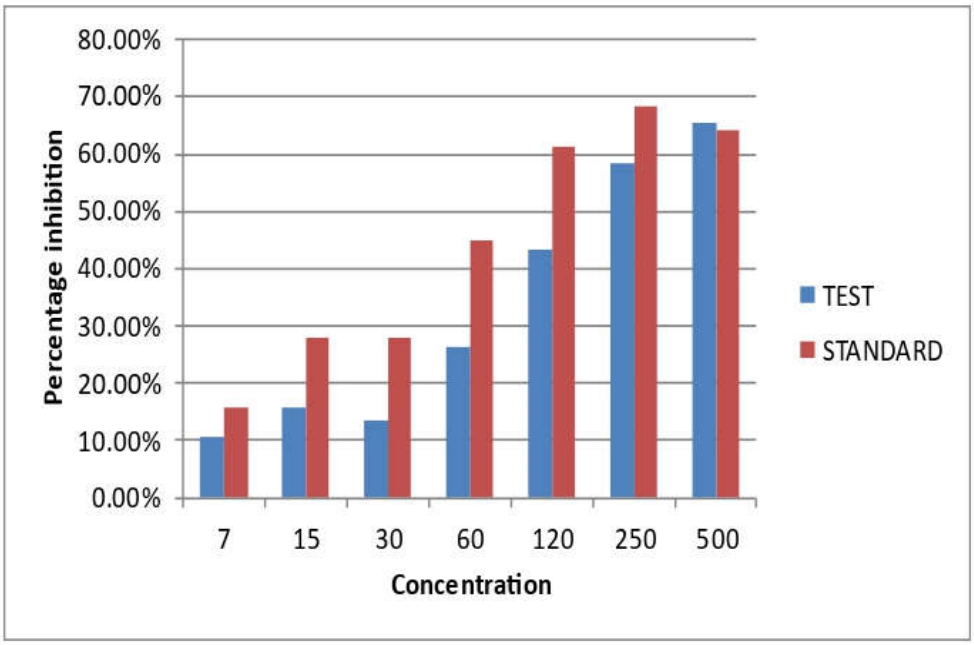

Fig 3: α-amylase inhibition assay of *Camellia sinensis L.* (Green tea)

Table 6: Anti-diabetic activity of *Camellia sinensis* L. (Oolong tea)

S.no	Sample	Concentration(ug/ml)	Absorbance(mean±SEM)	Percentage inhibition
1	Oolonga Tea (231.41µg/ml)	7	0.0540±0.001155	5.26%
2		15	0.0500±0.001155	12.28%
3		30	0.0470±0.0005774	17.54%
4		60	0.03967±0.002028	30.40%
5		120	0.0350±0.0005773	38.59%
6		250	0.0290±0.0005773	49.12%
7		500	0.01667±0.0008819	70.75%

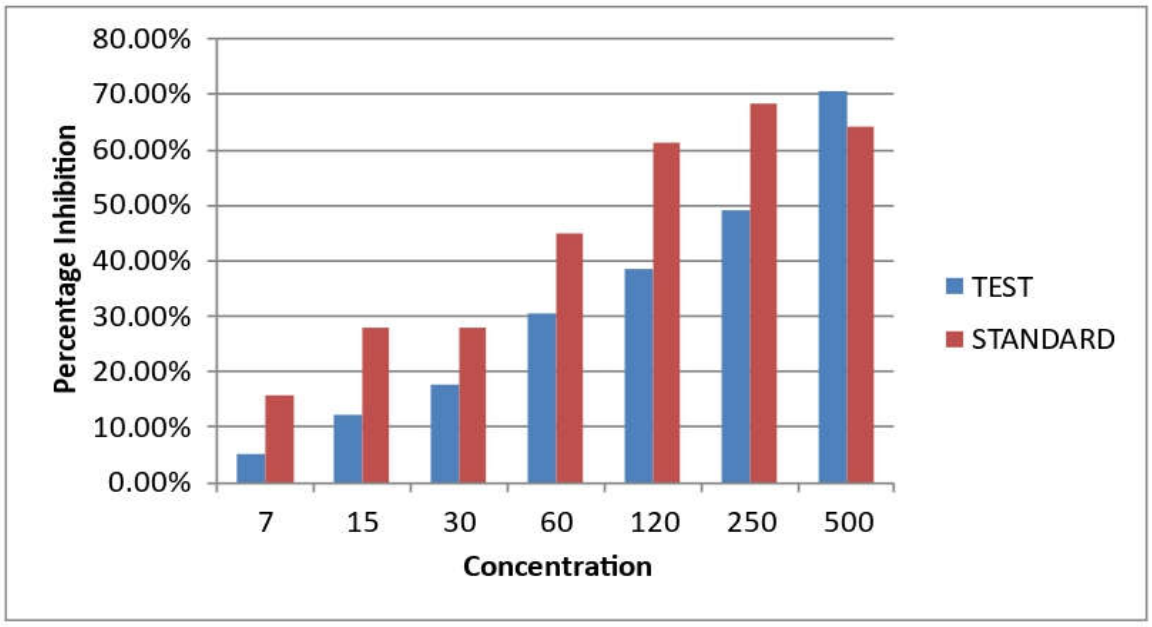

Fig 4: α-amylase inhibition assay of *Camellia sinensis*.L (Oolong

CONCLUSION

Diabetes has emerged as one of the most serious metabolic disorders in the world because the seriousness of related complications and its rapid onset worldwide. The crucial role natural products in drug discovery are evident as the use of medicinal plants in the prevention treatment of diabetes continues to grow because of their beneficial effects.

Natural products also being used as alternative sources for safer and cheaper diabetes treatment with fewer side effects. In this study, the antidiabetic activities of polyphenols isolated from green and oolong tea and their inhibitory potential against enzymes associated with diabetes were evaluated. Polyphenols are molecules naturally found in plants that are structurally characterized by the presence of one or more phenol groups. Generally, polyphenols are known possess antioxidant activities thereby protecting cells against oxidative stress in both plants and humans. In addition to this, polyphenols also possess antidiabetic activities, particularly as good glucosidase and α-amylase inhibitors.

The inhibitory activities of the compounds isolated against α -amylase were investigated in comparison with acarbose. The inhibitory activities observed in this study are consistent with recent reports on the inhibitory activities of polyphenolic compounds against α -amylase.
The preliminary investigations of active compounds in Green tea revealed presence of polyphenols such Catechin, Caffeine, Theanine, Theaflavin, Epigallocatechin gallate, Gallocatechin gallate, Gallocatechol, 2- amyifuran, 2- decenal, Gallic acid, Epicatechin gallate, y- aminobutyric acid and Oolong tea revealed presence of polyphenols such Catechins andTheaflvins, Gallocatechin, Epigallocatechin, Epicatechin, Catechin gallate, Epicatechin gallate, Epigallocatechin gallate, Allocatechin gallate, Gallic acid, Caffeine.

In summary, the findings of this study suggest that polyphenols isolated from Green tea & Oolong tea extract showed good hypoglycaemic activity that may be linked to the inhibition of crucial enzymes associated with diabetes. Further investigations are required to characterize these compounds and determine their potential antidiabetic pharmacological activity.

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