

Phytochemical Analysis, Anti-Oxidant and Anti-Obesity Inhibitory Activity of Pancreatic Lipase from the Ethanol Extract of *Camellia sinensis*

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ABSTRACT: The anti-obesity inhibitory activity of pancreatic lipase is analyzed with the extract of *Camellia sinensis*. The aim of this study is to determine and estimate the amount of antioxidants, phenolic content and flavonoids with the help of various assays such as FRAP, DPPH etc. Initially the amount of phytochemicals such as saponins, tannins, proteins, steroids etc., is analyzed. Finally the in vitro inhibitory activity of pancreatic lipase is determined with the help of sample whose readings are compared with that of Orlistat (tetra hydro lipstatin) and the substrate used for enzyme preparation is PNPB (p- Nitro phenyl butyrate).

I. INTRODUCTION

HERBALISM:

Herbalism (also herbal medicine) is the study of botany and use of plants intended for medicinal purposes. Plants have been the basis for medical treatments through much of human history, and such traditional medicine is still widely practiced today. Modern medicine makes use of many plant-derived compounds as the basis for evidence-based pharmaceutical drugs.

PHYTOTHERAPY

Phytotherapy is a science-based medical practice and thus is distinguished from other, more traditional approaches, such as medical herbalism, which relies on an empirical appreciation of medicinal herbs and which is often linked to traditional knowledge

PHYTOCHEMICALS

Phytochemicals literally mean “plant chemicals.” Scientists have identified thousands of different phytochemicals, found in vegetables, fruits, beans, whole grains, nuts and seeds. Eating lots of plant foods rich in phytochemicals may help to prevent at least one in every five cases of cancer, as well as other serious ailments.

GREEN TEA

Green tea is a type of tea that is made from *Camellia sinensis* leaves that have not undergone the same

withering and oxidation process used to make oolong teas and black teas. Green tea originated in China, but its production and manufacture has spread to many other countries in Asia.

ANTIOXIDANTS

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms.

OBESITY

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. A crude population measure of obesity is the body mass index (BMI), a person's weight (in kilograms) divided by the square of his or her height (in metres)

LIPASE

Lipase is an enzyme that breaks down dietary fats into smaller molecules called fatty acids and glycerol.

INHIBITION OF FAT ABSORPTION

The digestion and absorption of nutrients should be decreased in order to reduce energy intake.

PANCREATIC LIPASE INHIBITORS FROM NATURAL PRODUCTS

Pancreatic lipase inhibitory properties have been extensively examined for the determination of the potential effect of natural products as anti-obesity agents.

II. REVIEW OF LITERATURE

This study aimed to compare in vitro antioxidant power of different types of tea (*Camellia sinensis*) the ferric reducing/antioxidant power (FRAP) assay was used to measure the total antioxidant power of freshly prepared infusions of 25 types of teas. Results showed that different teas had widely different in vitro antioxidant power and that the antioxidant capacity was strongly correlated ($r = 0.956$) with the total phenolics content of the tea.

III. MATERIALS AND METHODS

PHYTOCHEMICAL ANALYSIS:

TEST FOR TANNINS

1ml of sample was taken, to that few drops of 0.1 % ferric chloride was added and observed for brownish green or blue-black coloration.

TEST FOR SAPONINS:

1 ml of sample was taken, to that 2 ml of water was added. The suspension was shaken in a graduated cylinder for 15 minutes. A layer of foam indicates the presence of saponins.

TEST FOR FLAVONOIDS:

1 ml of sample was taken, to that add NaOH absorb yellow color and hydrochloric acid concentrated was added and observed for White color.

TEST FOR ALKALOIDS:

1 ml of sample was taken, to that few drops of drangandoff reagent was added. A prominent yellow precipitate indicates the test as positive.

TEST FOR PROTEIN:

1 ml of sample was taken, to that few drops of Bradford reagent was added. A blue color indicates the presence of Protein.

TEST FOR STEROIDS:

1 ml of sample was taken, to that two drops of 10% concentrated sulphuric acid was added and observed for brown color.

TEST FOF ANTHRAQUINONES:

1 ml of sample was taken, to that aqueous ammonia was added and observed for change in color. Pink, red, or violet color in aqueous layer indicates the presence of anthraquinones.

TEST FOR PHENOL:

1 ml of sample was taken, to that 3 ml of 10% lead acetate solution is added a bulky white precipitate indicates the presence of phenolic compounds.

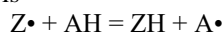
ANTIOXIDANT ASSAY

DPPH ASSAY:

DPPH (1,1-diphenyl-2-picrylhydrazyl) is characterized as a stable free radical by virtue of the delocalization

of the spare electron over the molecule as a whole, so that the molecules do not dim rise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 520 nm. When a solution of DPPH

is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Blois,1958) with the loss of this violet color (although there would be expected to be a residual pale yellow color from the picryl group still present). Representing the DPPH radical by $Z\cdot$ and the donor molecule by AH, the primary reaction is



where ZH is the reduced form and $A\cdot$ is free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorized) by one molecule of the reductant.

IV. PROCEDURE

Aliquot 3.7 ml of absolute methanol in all test tubes and 3.8ml of absolute methanol was added to blank. Add 100 μ l of BHT to tube marked as standard and 100 μ l of respective samples to all other tubes marked as tests. 200 μ l of DPPH reagent was added to all the test tubes including blank. Incubate all test tubes at room temperature in dark condition for 30 minutes. The absorbance of all samples was read at 517nm. Ferric Ion Reduction Potential (FRAP) :(Benzie and Strain, 1996). FRAP assay is a simple novel method for assessing "Antioxidant reduction at low pH causes a coloured ferrous-tripyridyltriazine complex to form. FRAP values are obtained by comparing the absorbance change at 593 nm test reaction mixtures with those containing ferrous ion in known concentrations.

V. PREPARATION OF FRAP REAGENTS

- Reagent A-Acetate Buffer (300mM, pH 3.6)
16 ml of glacial acetic acid was added to 3.1g of sodium acetate trihydrate; the solution was then made up to 1L using distilled water. The pH of the solution was checked using pH meter.
- Reagent B-TPTZ (2,4,6 tri[2 pyridyl] s-triazine)Solution
0.031g of TPTZ was added to 10ml of 40mM HCl .
- Reagent C-Ferric Chloride Solution
0.054g of ferric chloride was dissolved in 10ml of distilled water.
Reagents B and C were freshly prepared every time when the assay was performed.
2.5ml of Reagent B and 2.5ml of Reagent C were added to 25ml of Reagent A to make 30ml of the FRAP reagent. This was placed in a 37°C water bath for a minimum of 10 minutes. Standard-Ascorbic acid: 1.76 mg of Ascorbic acid was dissolved in 100 ml of distilled water.

STANDARD CALIBRATION CURVE

A standard solution of 1mM ferrous sulphate was prepared by dissolving 0.139g of $FeSO_4 \cdot 7H_2O$ in 580ml of distilled water. Serial dilutions were made and the absorbance at 593nm measured by performing the assay as described above with ferrous sulphate in place of test samples.

CALCULATION

FRAP value of sample (μ M)= (Change in absorbance of sample from 0 to 4 minute/change in absorbance of standard from 0 to 4 minutes)x FRAP value of standard (1000 μ M).

ESTIMATION OF TOTAL PHENOLIC CONTENT.

The total phenolic content (TPC) of the Sample was determined by spectrophotometric method 1 ml of sample (1 mg ml⁻¹) was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture, followed by the addition of 13 ml of deionized distilled water and mixed thoroughly. The mixture was kept in dark for 90 min at 23°C, after which absorbance was read at 750 nm. The TPC was determined from extrapolation of calibration curve which was made by preparing gallic acid solution. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per gm of sample.

TOTAL FLAVONOIDS ESTIMATION

PROCEDURE: (Aluminum Chloride Colorimetric Assay)

One ml of sample and 4 ml of distilled water were taken into a 10ml volumetric flask. To this flask, 0.3 ml of 5 % sodium nitrite was added and 0.3ml of 10 % aluminum chloride was mixed, after 5min. Thereafter, 2 ml of 1M sodium hydroxide was treated after 5min. and the content was diluted to 10 ml with distilled water. A standard curve was prepared with quercetin solution (100,200,300,400 and 500µg/ml) as the procedure described earlier. The absorbance readings were recorded for test and standard solutions against blank at 510 nm in UV/Visible spectrophotometer. The total flavonoid content was expressed as µg of QE/g of extract.

PANCREATIC LIPASE INHIBITION ACTIVITY

REAGENTS PREPARATION

BUFFER:

100mM PBS with 150mM Sodium Chloride and 0.5 % (v/v) Triton-X-100, pH 7.2.

ENZYME PREPARATION (CONCENTRATION OF ENZYME)

Porcine pancreatic lipase enzyme solution was prepared by dissolving 6mg of the enzyme in 10 ml of buffer solution by gentle vortexing. It was prepared immediately before use.

PNPB:

Substrate used was p-Nitrophenyl butyrate (PNPB).

PNPB working solution was prepared with 8.403 µl of PNPB stock solution in a vial and was made up to 10ml by acetonitrile.

Orlistat

The positive control Orlistat was prepared with varying concentrations of viz: 10,20,30,40 and 50 µg/ml with

DMSO:

Test samples were also prepared with different concentrations with that of positive control.

INVITRO PANCREATIC LIPASE INHIBITION ASSAY

Test solution and positive control were taken separately (25µleach). Both the tubes were added with 50µl of enzyme solution, 100µl of buffer and 25µl of PNPB solution .The mixtures were shaken well and incubated for 30 minutes at 37°C. Lipase activity was determined by measuring the hydrolysis of PNPB to P-nitro phenol. After incubation OD values were taken at 400 nm using spectrophotometer. The percentage inhibitory Activity was calculated using the following formula:

$$\% \text{Inhibition} = \frac{\text{Absorbance of Blank} - \text{Absorbance of test}}{\text{Absorbance of Blank}} \times 100$$

VI. RESULTS and DISCUSSION

Phytochemical analysis

The phytochemical screenings of green tea extract were analyzed and the results were shown in the table 1 as flavonoids, alkaloids, proteins, phenol were present whereas tannins, steroids, saponins and anthroquinones were absent.



Table 1: The Phytochemical studies of the sample Green tea

TEST	Sample
	Green tea
TANNINS	-
SAPONINS	-
FLAVONOIDS	+
ALKALOIDS	+
PROTEINS	+
STEROIDS	-
ANTHROQUINONES	-
PHENOL	+

(+) = Positive
 (-) = Negative

ANTIOXIDANT ASSAY

I. DPPH assay

The DPPH assay were done at different concentrations such as 200, 400, 600, 800, 1000 respectively and The DPPH activity % were observed in the increasing order were shown in the table 2.

Table:2

SI No.	Concentration (µg/ml)	O.D	DPPH Activity %
1	200	0.078	25
2	400	0.073	29.80
3	600	0.065	37.50
4	800	0.060	42.30
5	1000	0.050	51.92

Control: 0.104

II. FRAP assay

The FRAP assay for the given sample of ethanol extract of green tea were observed at different concentrations such as 200,400,600,800 and 1000 respectively and the O.D value at 0 min and at 4 min were noted and by substituting the O.D values in the formula the FRAP values can be estimated and shown in table 3.

Table 3 .

SI No.	Concentration ($\mu\text{g/ml}$)	O.D Value for Sample at 0 min	O.D Value for Sample at 4 min	FRAP(μM)
1	200	1.022	0.192	415
2	400	0.506	0.422	420
3	600	0.456	0.195	1305
4	800	0.514	0.107	2035
5	1000	1.038	0.376	3310

O.D Value for Ascorbic acid at 0 to 4 min = 0.2

Pancreatic Lipase Inhibition Activity

The pancreatic lipase inhibition assay was determined by using the test solution and positive control whose OD and the % of inhibition were determined separately by using spectrophotometer at 400nm. The percentage inhibitory activity of test sample were in the increasing order whereas the percentage inhibition activity of orlistat, the positive control will also be in the increasing order in which the both were estimated by using different OD as shown in the table 6.

Table 6:

S.No	Concentration ($\mu\text{g/ml}$)	Test sample		Orlistat	
		OD	% inhibition	OD	% inhibition
1	10	0.616	33.76	0.323	58.95
2	20	0.561	39.67	0.264	66.45
3	30	0.496	46.66	0.178	77.38
4	40	0.403	56.66	0.141	82.08
5	50	0.341	63.33	0.101	87.16

Control: 0.930

Among extracts studied, *C. sinensis* presented a strong in vitro inhibitory effect on lipase pancreatic. Other plant extracts, such as *Baccharistrimera*, *C. sinensis* (white tea), *Cymbopogoncitratus* and *Fragariaanansa* also had significant, but weaker inhibitory effect on the pancreatic lipase activity, whereas *Solanummelongen* were ineffective.

VII. SUMMARY AND CONCLUSION

Some studies show that green tea leads to increased weight loss. It may be particularly effective at reducing the dangerous abdominal fat.

As our lifestyle is now getting techno-savvy, we are moving away from nature. While we cannot escape from nature because we are part of nature. As herbs are natural products they are free from side effects, they are comparatively safe, eco- friendly and locally available. Traditionally there are lot of herbs used for the ailments related to different seasons. There is a need to promote them to save the humanlives.

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