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RESEARCH ARTICLE

EXPLORING THE ANTIOBESITY AND ANTIOXIDANT POTENTIAL OF THE METHANOLIC EXTRACT OF *CAMELLIA SINENSIS*

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Abstract

The escalating problem of obesity has become a cause of great concern in the world today as it leads to adverse effects on human health, including cardiovascular diseases, cancer etc. The major causes of obesity may be attributed to sedentary lifestyle and bad food habits. Conventional modalities to tackle obesity are not free from side-effects. Urgency of a novel, nontoxic means needs to be developed to control obesity. In this study we aim to screen the phytochemical compounds of *Camellia Sinensis* and evaluate its antiobesity and antioxidant effects. The methanolic extract of *Camellia Sinensis* was analyzed for its phytochemical screening and assayed for its *in-vitro* activity against pancreatic lipase, its antioxidant potential and quantitative estimation of flavonoids and phenolics were done. The methanolic extract of *Camellia Sinensis* strongly inhibited pancreatic lipase by 63% and it also possesses a strong antioxidant effect and there was a significant positive correlation between phenolics, flavonoids and with alkaloid contents. From these results, it could be concluded that methanolic extracts of *Camellia Sinensis* possesses antipancreatic lipase compounds. It also possesses antioxidant effect. It is suggested that the phytochemical compounds from there plants may be applied for the prevention and treatment of obesity or hyperlipidemia.

Keywords: Obesity, *Camellia Sinensis*, Pancreatic lipase, Antioxidant, Phenolic

Introduction

Obesity is becoming a worldwide epidemic, resulting in a major risk factor for coronary heart diseases including diabetes mellitus, metabolic syndrome, stroke, and some cancers (Yun, 2010). Therefore, prevention and treatment of obesity

become an important factor for a healthy condition. The reduction of nutrient digestion and absorption by developing of enzyme inhibitors without altering major mechanism in gastrointestinal system became the most important strategies in the treatment of obesity (Martin *et al*., 2015; Loli *et al*., 2015). The major source of unwanted calories is dietary lipids, therefore, lipid metabolism play a major role in maintaining energy homeostasis (Sukhdev and Singh, 2013). The identification and characterization of several enzymes involved in lipid metabolism have yielded a rich pool of potential targets for drugs to treat obesity and other metabolic disorders. The flavonoid could inhibit α-amylase and α-glucosidase activity to decrease the absorption of carbohydrates from food (Haripriya *et al*., 2017). Pancreatic lipase is the key enzyme for dietary fat digestion and absorption. Therefore, inhibition of this enzyme would be in effect to reduce lipid absorption from intestine and lead to a consequence suppress of weight gain. Orlistat, a specific drug for inhibiting pancreatic lipase that reduces dietary fat absorption by 30%, has been approved for clinical use (Hill *et al*., 1999; Birari and Bhutani, 2007; Weigle, 2003). However, Orlistat can result in adverse side effects, such as fecal incontinence, flatulence, and steatorrhea (Hasani-Ranjbar *et al*., 2013; Ado *et al*., 2013). Therefore, the investigation to find new safety medication for anti-obesity is still needed. The significant progress of the development of anti-obesity from medicinal plants has provided potential therapeutic targets for obesity.

Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. India has about 45,000 plant species and among them, several thousands have been claimed to possess medicinal properties. Traditional system of medicine is found to have utilities as many accounts. Due to population rise adequate supply of drug and high cost of treatment in side effect along with drug resistance has been encountered in synthetic drugs, which has lead to an elevated emphasis for the use of plants to treat human diseases. The affordability of herbals has also drawn the attraction towards their use. India is one of the oldest civilizations which is known for rich repository of medicinal plants. *Camellia sinensis* is the species of plant whose leaves and leaf buds are used to produce Chinese tea. It is of the genus *Camellia*, a genus of flowering plants in the family Theaceae (Parmar, 2012).

Materials and method

Preparation of plant extracts

Extraction of the dried leaves The *C. Sinensis*leaves were dried in an oven to remove any moisture content and were coarsely powdered. The powdered material was defatted with petroleum ether (40- 60˚C). Further extraction were made by using Soxhlet apparatus in different solvents like ethanol, methanol.

Phytochemical Qualitative Analysis.

The plant extracts and methanolic and ethanolic aqueous solutions were assessed for the existence of the phytochemical analysis by using the following standard methods.

Test for Anthraquinones

10 ml of benzene was added in 6 g of the Ephedra powder sample in a conical flask and soaked for 10 minutes and then filtered. Further 10 ml of 10% ammonia solution was added to the filtrate and shaken vigorously for 30 seconds and pink, violet, or red color indicated the presence of anthraquinones in the ammonia phase.

Test for Tannins

10 ml of bromine water was added to the 0.5 g aqueous extract. Discoloration of bromine water showed the presence of tannins.

Test for Saponins

5.0 ml of distilled water was mixed with aqueous crude plant extract in a test tube and it was mixed vigorously. The frothing was mixed with few

drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins.

Tests for Flavonoids

Pieces of magnesium ribbon and Hcl concentrated were mixed with aqueous crude plant extract after few minutes and pink color showed the presence of flavonoid.

Test for Steroids.

2 ml of chloroform and concentrated H2SO4 were added with the 5 ml aqueous plant crude extract. In the lower chloroform layer red color appeared that indicated the presence of steroids.

Detection of Phenols

Ferric chloride test:

About 2ml plant extract was taken to water and warmed at 45-50°C. Then 2 ml of 0.3% FeCl3 was added. Formation of green or blue color indicates the presence of phenols.

Test for Alkaloids. Test for flavonoid used is as reported (Hikino *et al*., 1984). Extraction of component from 2 grams of each wood powder sample was carried out using 5% tetraoxosulphate (VI) acid (H2SO4) (20 cm3) in 50% ethanol by boiling for 2 minutes and filtered through Whatman filter paper number 42 (125 mm). The filtrate was made alkaline using 5 cm3 of 28% ammonia solution (NH3) in a separating funnel. Equal volume of chloroform (5.0 cm3) was used in further solution extraction in which chloroform solution was extracted with two 5 cm3 portions of 1.0 M dilute tetraoxosulphate (VI) acid. This final acid extract was then used to carry out the following test: 0.5 cm3 of Dragendorff ’s reagent (Bismuth potassium iodide solution) was mixed with 2 cm3 of acid extract and precipitated orange colour infers the presence of alkaloid.

Test for Proteins: Biuret test:

To the methanolic extracts, 4% sodium hydroxide and 1% copper sulfate solution were added and formation of violet or pink color indicated the presence of proteins.

Pancreatic lipase inhibition assay:

Lipase activity was measured using p-NPB as a substrate. The method was modified from the previously described (Kim *et al*., 2010). Briefly, an enzyme buffer was prepared by the addition 30 mL of solution of porcine pancreatic lipase (2.5 mg/mL in 10 mmol/L morpholine propane sulphonic acid and 1 mmol/L ethylene diaminetetraacetic acid, pH 6.8) to 850 mL of Tris buffer (100 mmol/L Tris–HCl and 5 mmol/L $CaCl_2$, pH 7.0). Then, either 100 mL of the plant extracts (100 mg/mL) or Orlistat was added and incubated for 15 min at 37˚C. Ten microliter of substrate (10 mmol/L p-NPB in dimethyl formamide) was then added and incubated for 30 min at 37˚C. Lipase activity was determined by measuring the hydrolysis of p-NPB to p-nitrophenol at 405 nm using an ELISA reader (Biochrome, England). The inhibitory activity (I) was calculated according to the following formula:
% Inhibitory activity was calculated using the following formula:
% Inhibition= Absorbance of blank- Absorbance of test/ Absorbance of blank × 100

Determination of total phenolic content

According to a previously described protocol (Kaur and Kapoor, 2002). Folin– Ciocalteu reagent was used to determine the total phenolic content of extracts. Absorbance was measured at 725 nm. All Tests were performed 6 times. The phenolic content was calculated based on a gallic acid standard curve.

Determination of total flavonoid content

Total flavonoid content was determined according to a previously discussed method (Chang, 2002) using quercetin as a standard. The absorbance

was measured at 510 nm. The flavonoid content was calculated based on a quercetin standard curve.

DPPH Radical Scavenging assay

Total free radical scavenging capacity of the extracts from different plant samples were estimated according to the previously reported method (Villano *et al*., 2007) with slight modification using the stable DPPH radical, which has an absorption maximum at 515 nm. A solution of the radical is prepared by dissolving 2.4 mg DPPH in 100 ml methanol. 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. All the determinations were performed in triplicate. The capability to scavenge the DPPH radical was calculated using the following equation. DPPH Scavenged (%)= ((AB–AA)/AB)×100…..(1), where, AB is absorbance of blank at t= 0 min; AA is absorbance of the antioxidant at t= 30 min. A calibration curve was plotted with % DPPH scavenged versus concentration of standard antioxidant (Trolox).

Ferric reducing antioxidant power

The antioxidant capacity of the medicinal plants was estimated spectrophotometrically following the procedure of Benzie and Strain (Benzie and Strain, 1996). The method is based on the reduction of Fe3+ TPTZ complex (colorless complex) to Fe^{2+}-tripyridyltriazine (blue colored complex) formed by the action of electron donating antioxidants at low pH. This reaction is monitored by measuring the change in absorbance at 593 nm.

Statistical analysis

Statistical analysis of the data was performed using the SPSS 16.0 program. The comparison between Orlistat control and extract group was conducted using the Mann–Whitney U test, and the correlations between parameters were determined using the Spearman's rank test.

Result

Phytochemical analysis

Table: 1 The Phytochemical studies of the sample *Camellia Sinensis*

Phytochemicals	*Camellia Sinensis*
Tannins	-
Saponins	-
Flavonoids	+
Alkaloids	+
Proteins	+
Steroids	-
Anthroquinones	-
Phenol	+

(+) = Positive (-) = Negative

The phytochemical screenings of *Camellia Sinensis* were analyzed and the results observed were shown in table 1. The results show the presence of flavonoids, alkaloids, proteins and phenols. Whereas, tannins, steroids, saponins and anthroquinones were absent.

Free radical scavenging activity:

The methanolic extract of Camellia Sinensis was tested for its antioxidant activity. The results of DPPH and FRAP assay were shown in table 2 & 3. The results show that it possesses a strong antioxidant activity with increasing concentration.

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 : DPPH assay

S. No.	Concentration (µg/ml)	DPPH Activity %
1	200	25
2	400	29.80
3	600	37.50
4	800	42.30
5	1000	51.92

Control: 0.104

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.

Total phenolics content

Total phenol contents of the samples expressed in terms of Gallic acid equivalent (mg of GAE/gm of extract). The methanolic extract of Camellia Sinensis was estimated for its total phenol and flavonoids content. The results were shown in table 4 & 5. The results shown that it's a good source of phenol and flavonoids compound with increasing concentration.

The total phenol content of the sample was determined in the decreasing order of the concentration range and the OD was estimated at 750nm which shows the observation of concentration in the increasing order shown in the table 4.

Total flavonoid content

The total flavonoids estimation was determined by using aluminum chloride colorimetric assay where standard curve is plotted with quercetin solution in the range of 100 to 500. The absorbance readings were taken against the blank at 510nm which was observed in decreasing order as shown in the table 5.

1.1.Pancreatic Lipase Inhibition Activity

The methanolic extract of *Camellia Sinensis* was examined for its inhibitory effect of pancreatic lipase. The results shown in figure 1. Camellia Sinensis showed a strong inhibitory effect on pancreatic lipase in vitro which is near to the value of the standard drug Orlistat.

The pancreatic lipase inhibition assay was determined by using the test solution and positive control that's 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 both the samples were estimated by using different OD as shown in the figure 1.

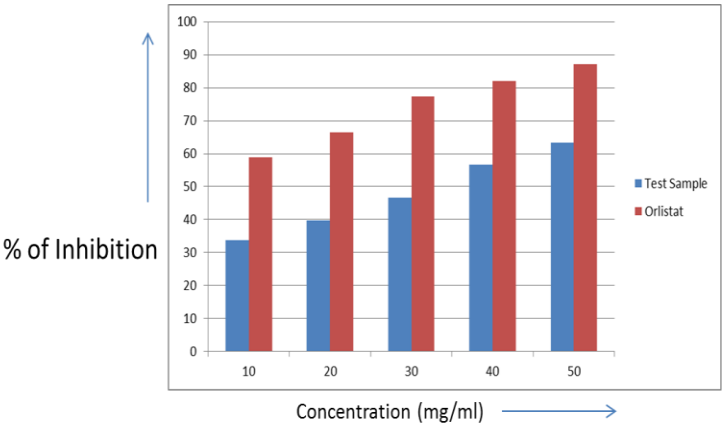

Figure 1

Table 3: FRAP assay

S. No.	Concentration (µg/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

Table 4: Total phenolics content

Name of the Sample /Extract	Sample Concentration (µg)	mg of GAE/gm of extract
Camellia Sinensis	500	37
	250	28
	125	15
	62.5	11

Table 5: Total flavonoid content

Name of the Sample /Extract	Sample Concentration	Concentration (µg/ml)
Camellia Sinensis	500	380
	250	250
	125	180
	62.5	80

Discussion

Obesity is a common disorder usually caused by the interaction of genetic, nutritional, and environmental factors. It has now become one of the most important health issues of the modern society around the world (Mohamed *et al*., 2014). It is often associated with other diseases such as arteriosclerosis, hypertension, cancer, diabetes, and osteoarthritis (Must *et al*., 1999; Poulos *et al*., 2010). The incidence of obesity is increasing exponentially, and it has been revealed that about 500 million adults are obese worldwide (Sweeting *et al*., 2015). Pancreatic lipase (PL), secreted by the pancreas, is a key enzyme, responsible for the digestion of 50–70% of fat into monogylceride and free fatty acids for absorption by the entherocytes. Inhibition in the digestion and absorption of fat usually reduces its accumulation in the adipose tissue (Hanefeld and Sachse, 2002). Therefore, one of the key targets for anti-obesity agent is inhibition of PL (Chakrabarati, 2009). On the other hand, lipoprotein lipase (LPL) is a rate-limiting enzyme that hydrolyzes the triglycerides (TG)-rich lipoproteins, chylomicrons, and very low-density lipoproteins (VLDL), resulting into the release of non-esterified fatty acids (NEFA) and monoacylglycerol. These fatty acids and monoacylglycerol are either used by the muscles for metabolic energy or re-esterified into TG and stored as neutral lipids in adipose tissue. Any imbalance in LPL activity affects the distribution of TG between muscle and adipose tissue and thus influences obesity (Kusunoki 2013). Consequently, compounds, which can inhibit activity of these lipases, are supposed to function as anti-obesity agents (Gargouri 1997). Pancreatic lipase is a key enzyme for lipid absorption by hydrolysis of total dietary fats. Therefore, inhibition of pancreatic lipase is suggested to be an effective therapy in the regulation of obesity. Although Orlistat has anti-obesity effects by inhibiting pancreatic lipase activity. However, it can cause adverse side effects such as fecal incontinence, flatulence and steatorrhea. Therefore, the investigation of new agent for pancreatic lipase inhibitor is still needed.

Our results show that methanolic extract of *Camellia Sinensis* exhibited strong anti-lipase activity. This suggests that *Camellia Sinensis*seems to be the potential candidates as the inhibitor of pancreatic lipase. However, further in vivo studies on animal model must be conducted in order to confirm this hypothesis. Our results showing a significant positive correlation between phenolic, flavonoid, alkaloid contents and inhibition activity, which provide strong support that these phytochemical compounds are key agents for pancreatic lipase inhibition. Published research also reported that flavonoids and alkaloid be able to inhibit pancreatic lipase [26]. The study in vivo model indicated that polyphenols and flavonoid glycoside derived from *Salix matsudana*leaf showed decreased in body weight gain in wistar rats (Han *et al*., 2003).

Our results suggest natural resources that possess strong antioxidant and pancreatic lipase inhibitory activities with potential applications in the treatment and prevention of 18 Evidence-Based Complementary and Alternative Medicine obesity and overweight problem. Tea extracts of Camellia sinensis, Ceratoniasiliqua, Curcuma longa, Sarcopoteriumspinosum, and Menthaspicata have shown possessing strong antioxidants and PPLI potentials. However, future studies are needed for screening in-depth phytochemical, clinical, and possible studies on molecular mechanism of action and identification of the constituents responsible for the antioxidant and PPLI activities. At the same time, efforts should be made to normalize the plant extracts with potent antioxidants and PPLI activities and formulate best alternative herbal products in order to substitute man made drugs which are presently in use.

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