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CHOLESTEROL ESTERASE ENZYME INHIBITORY AND ANTIOXIDANT ACTIVITIES OF LEAVES OF *CAMELLIA SINENSIS* (L.) KUNTZE. USING *IN VITRO* MODELS

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ABSTRACT

The present study was to evaluate the *in vitro* cholesterol esterase enzyme inhibitory and *in vitro* antioxidant activity of the methanol extract of the leaves of *Camellia sinensis* (L.). Phytochemical screening of the extract shows the presence of flavonoids, phenolics and terpenoids. The extract shows ability to inhibit the enzyme with IC_{50} (82.46±0.74µg/ml) whereas that of standard, Orlistat (24.15±0.59µg/ml). Antioxidant and free radical scavenging activity were also assessed by using the methods, IC_{50} values for Nitric oxide radical scavenging activity (396.83±0.83µg/ml), whereas for standard curcumin (260.38±0.66µg/ml), hydroxyl radical scavenging activity (47.04±2.26µg/ml) and for quercetin (70.99±1.31µg/ml). Moreover, the extract was found to scavenge the superoxide with 50% inhibition at 308.17±23.25µg/ml and standard ascorbic acid at 225.08±2.44µg/ml, IC_{50} for ferrous chelating ability assay (44.12±4.63µg/ml) and of ascorbic acid (47.25±.89µg/ml). Total content of flavonoids present in 1mg of extract was 19.8±0.11 µg quercetin equivalents/mg. Results indicated that the extract shows potential bioactive compounds which might have a beneficial impact on diseases related to cholesterol synthesis and showed potential antioxidant and free radical scavenging activities.

INTRODUCTION: Dietary cholesterol comprised of free and esterified cholesterol. In diets rich in meats, significant percent of cholesterol is esterified. Hydrolysis of cholesteryl ester in the lumen is catalyzed by cholesterol esterase (CEase).

Cholesterol esterase (CEase) also is a lipolytic enzyme with wide substrate specificity capable of hydrolysing cholesteryl esters, triacylglycerol, phospholipids and lysophospholipids. It is synthesized primarily by the pancreas and is found as a component of pancreatic juice. This enzyme is also found in the milk of numerous species. Milk CEase is postulated to substitute for the pancreatic enzyme in the neonatal gastrointestinal tract before maturation of the pancreas [1]. Pancreatic cholesterol esterase is also known as bile salt-activated lipase, nonspecific lipase, phospholipase A_1 lysophospholipase, bile salt-dependent lipase, bile salt stimulated lipase, carboxyl ester lipase, and carboxyl ester hydrolase [2].

A number of studies have suggested a possible role for CEase in the absorption of free cholesterol at the brush border membrane of the small intestine [3]. Cholesterol and its esters are the major risk factors for the development of various cardiovascular diseases and are known to be one of the leading causes of death in developing and developed countries. Free radical is an atom with at least one unpaired electron in the outer most shell; this has the capability of independent

existence. It is easily formed when, breakage of covalent bond occurs between entities and remaining of one unpaired electron in each new atom. They are extremely reactive due to the presence of unpaired electrons. Free radicals are implicated in various diseases. Free radicals act directly on the cells and effect cholesterol levels by an increase in cholesterol biosynthesis and esterification, decrease in cell cholesterol ester hydrolysis, reduced cholesterol efflux from the cells [4].

Antioxidants counteract free radicals by donating one of their own electrons, ending the carbon-stealing reaction. They act as scavengers, helping to prevent cell and tissue damage that directs to cellular damage and disease.

Camellia sinensis belongs to the family Theaceae, and also known as tea. Tea is native to mainland China, Southern Asia, but it is today cultivated across the world in tropical and sub tropical regions. Leaves are 4-15 cm long and 2-5 cm broad [5]. Fresh leaves have 4% caffeine. Light green and young leaves are preferably produced for tea production. Leaves of *Camellia sinensis* reported antimicrobial properties, they contain flavonoids, their analysis and their functions [6], they inhibits the enzyme ribonuclease A [7], antioxidant activity [8], antibacterial activity [9], tea polyphenols used in the herbal infusions [10], protection against reactive oxygen species induced degradation of lipids, proteins and 2-deoxyribose by tea catechins [11], green tea polyphenols having antiatherogenic properties [12].

There is need to develop plant derived CEase inhibitory and antioxidant compounds as the synthetic drugs available are all found to have adverse effects. Various studies have been conducted to explore the potential CEase inhibitory and antioxidant compounds from plant extracts.

MATERIALS AND METHODS:

Materials: Leaves of *Camellia sinensis* used in the present study were procured from Tamil Nadu, India, during the month of July 2010. Plant was recognized and authenticated by Dr. G.V.S. Murthy, Joint Director, C-I/C, Botanical Survey of India and bearing no.- BSI/SRC/5/23/10-11/Tech.- 828. Bovine pancreatic cholesterol esterase (C3766) and pNPB (p-nitro phenyl butyrate) were purchased from the Sigma Aldrich, USA. Acetonitrile and taurocholate were purchased from Loba Chemicals, Mumbai. Orlistat drug was purchased from Annanya chemicals, Hyderabad. Quercetin and 2-deoxy-2-ribose, sodium nitroprusside and curcumin were purchased from SRL, Mumbai. Ascorbic acid, nitro blue tetrazolium (NBT) and EDTA were purchased from Hi Media labs. Pvt. Ltd., Mumbai. All the other drugs and chemicals used in this study were commercially obtained and were of analytical grade.

Plant collection and extraction procedure: Fresh green leaves were collected as shoot on fields in Ooty. 100g of fresh leaves were cut to 1-1.5mm size then immerse in solvent methanol (1:5 to 1:15 g/ml) for a certain time (0-90 minutes). Then it was transferred to flask and brewed, in to keep temperature not rise above 70^{o}C. After that, the infusion was let cool down to room temperature, filtered to separate solid. Final infusion was stored in refrigerator at 4°C [13].

Methods: Phytochemical screening of the methanolic leaves extract of *Camelia sinensis* was done for tannins and phenolics, flavonoids, terpenoids & steroids, glycosides and alkaloids [14].

Cholesterol esterase Enzyme Inhibitory Activity: Cholesterol esterase enzyme inhibitory activity was evaluated and performed in the presence of sodium taurocholate with p-nitrophenyl butyrate as chromogenic substrate [15]. Stock solution of CEase (19.5ng/ml) and taurocholate (12mM) were prepared by using 100mM sodium phosphate buffer of PH (7.O). Stock solution of pNPB (200µM) & inhibitors were prepared by using Acetonitrile (6%). A final volume of 1ml is taken into a cuvette containing 430µl of assay buffer, 500µl of TC solution, 40µl of acetonitrile, 10µl of pNPB solution and 10 µl of an inhibitor solution were added and thoroughly mixed. Incubation for 2 min at 25°C, the reaction was initiated by adding 10µl of the enzyme solution. Uninhibited enzyme activity was determined by adding acetonitrile instead of the inhibitor solution. Absorbance was measured at 405 nm. The percentage inhibition was calculated by using, [16].

$$\text{CEase inhibition \% I} = [1- a] \times 100$$

a= enzyme activity with inhibitor /enzyme activity without inhibitor

Anti-oxidant Models:

Hydroxyl Radical Scavenging Assay: Hydroxyl radical scavenging activity of the extract was assessed by using 2-Deoxy 2-Ribose method [17]. This assay was quantified by studying the competition between deoxyribose and extract for hydroxyl radical generated by $Fe3^{+}$-EDTA– Ascorbate-H_2O_2 system (Fenton reaction).The reaction mixture in a final volume of 1.0 ml contained 100µl of 2-deoxy2-ribose (28 mM in 20 mM KH_2PO_4 buffer, pH 7.4), 500µl of the extract at various concentrations (10-160µg/ml) in buffer, 200µl of 1.04 mM EDTA and 200 µM $FeCl_3$ (1:1v/v), 100µl of 1.0 mM hydrogen peroxide (H_2O_2) and 100µl of 1.0 mM ascorbic acid.

Test samples were kept at 37^{o}C for 1 h. The free radical damage imposed on the substrate, deoxyribose was measured using the thiobarbituric acid test. One ml of 1% thiobarbituric acid (TBA) and 1.0 ml 2.8% trichloroacetic acid (TCA) were added to the test tubes and were incubated at 100^{0}C for 20 min. After cooling, the absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Quercetin (10-160µg/ml) was used as the positive control. Percentage inhibition (%I) was calculated by comparing the results of the test with that of control.

Total Flavonoid Content: Samples were analyzed for total flavonoid content according to the aluminium nitrate method using quercetin as a standard [18]. Different concentrations of quercetin in the range of (10-320µg/ml) were made with 80% ethanol to construct a standard graph. Known concentration of extract (1000 µg) was added to 1ml of 80% ethanol. An aliquot of 0.5 ml is added to test tubes containing 0.1 ml of 10% aluminium nitrate, 0.1 ml of 1 M potassium acetate and 4.3 ml of 80% ethanol. The absorbance of the supernatant is measured at 415 nm after 40 min of incubation at room temperature.

Ferrous Chelating Ability: Fe^{2+} forms complexes with ferrozine quantitatively. But in the presence of ion chelating agents, the complex development is disturbed, following a reduction in the red color of the complex [19]. The reaction mixture containing different concentrations of the extract were added to 2mM ferrous chloride (0.1 ml) and 5mM ferrozine (0.2 ml) to initiate the reaction and the mixture is shaken vigorously and left to stand at room temperature for 10 min. The absorbance of the solution is measured at 562 nm. The percentage chelating effect of ferrozine-Fe^{2+} complex formation is calculated. The positive control used was ascorbic acid

Superoxide Anion Radical Scavenging Assay: Superoxide anions were generated in a non-enzymatic phenazine methosulfate- nicotinamide adenine dinucleotide (PMS- NADH) system through the reaction of PMS, NADH, and oxygen [19]. The scavenging activity towards superoxide anion radicals of the extract was measured. In this assay superoxide anions were generated in a final volume of 3 ml of 100 mM Tris-Hydrochloric acid buffer (pH 7.4) containing 0.75 ml of 300µM NBT solution, 0.75 ml of 936 µM NADH solution and 0.3 ml of various concentrations of the extract. The reaction was started by adding 0.75 ml of PMS (120 µM) to the solution. The mixture after incubation for 5 min at 25^{o}C, absorbance was measured at 560 nm in UV spectrophotometer. The positive control used was ascorbic acid [20] in different concentrations (50-800µg/ml). The super oxide anion scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = [(A_o - A_1) / A_o \times 100]$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Nitric Oxide Scavenging Assay: Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction. Sodium nitroprusside in water at physiological pH on impulse instinctively generates nitric oxide, which interacts with oxygen to generate nitrite ions that can be estimated by Greiss reagent [21]. Various concentrations of the extract and sodium nitroprusside (5mM) in phosphate buffer saline (0.025 M, pH 7.4) in a final volume of 3 ml are incubated at 25^{o} C for 150 min.

Control experiments without the test compounds but with equivalent amount of buffer are prepared in the same manner as done for the test. There after, 0.5 ml of incubation solution is removed and diluted with 0.5 ml Griess' reagent (1% sulphanilamide, 2% *O*-Phosphoric acid and 0.1% naphthyethylene diamine dihydrochloride) and allowed to react for 30 min. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with naphthyethylene diamine

dihydrochloride is read at 546 nm. The positive control used was curcumin and the percentage inhibition was calculated.

Statistical analysis: The data were analyzed by one way analysis of variance (ANOVA) to study the CEase inhibitory and antioxidant activity of the extract and between the concentrations. Probability level was fixed to $^{*}P < 0.001$.

RESULTS AND DISCUSSION: In present study, plant known for their therapeutic properties of the traditional medicine was selected for evaluating their potential as an anti-hyperlipidemic agent by testing the inhibitory effect against cholesterol esterase. Methanol extract of leaves of *Camellia sinensis* was prepared. Phytochemical screening of crude extract of the plant revealed the presence of flavonoids, phenolics, tannins and alkaloids. In addition steroids and terpenoids were found to be present in the leaves extract of *Camellia sinensis.* Flavonoids, tannins and phenolics were reported to possess antioxidant and other important pharmacological activities including anti-hyperlipidemic activity [22].

Cholesterol esterase Inhibitory Activity: CEase inhibitory activity can be attributed to the presence of flavonoids in the extract. Flavonoids have been reported to irreversibly bind with the cholesterol esterase enzyme in its active fatty acid pocket at Serine 194. It was also suggested that the flavonoids act as suicide substrates ahead of cholesterol esters [23]. The inhibitory effect of the extract against bovine pancreatic cholesterol esterase was performed, which showed good activity and the same was comparable with that of the standard used, Orlistat.

Extract exhibited good Cholesterol esterase activity ranging from 17.8 to 81.2% inhibition in comparison to corresponding values of standard Orlistat ranges from 15.89 to 93.9% (**Table 1 & 2**), activity increasing with higher concentrations. IC_{50} value of the extract was found to be 82.46µg/ml. These results indicate that the plant extract had a noticeable effect on the enzyme cholesterol esterase. The differences among the samples were found to be extremely significant as per analysis of variance *** ($P < 0.001$).

TABLE 1: CHOLESTEROL ESTERASE INHIBITION BY THE STANDARD, ORLISTAT

Conc. (µg/ml)	% inhibition			Mean ± SEM	IC_{50} (µg/ml)
	I	II	III		
5	16.1	14.8	16.8	15.89 ± 0.24	24.15 ± 0.59*
10	27.8	29.6	30.9	29.44 ± 0.35	
20	46.8	45.0	49.0	46.94 ± 0 .54	
40	70.4	69.1	71.4	70.3 ± 0 .19	
80	83.5	82.5	84.1	83.3 ± 0 .29	
160	94.8	93.9	92.1	93.6 ± 0.39	

Values are expressed as mean ± S.E.M. Mean values of six readings with three replications

TABLE 2: CHOLESTEROL ESTERASE INHIBITION BY METHANOL EXTRACT OF *CAMELLIA SINENSIS* LEAVES

Conc. (µg/ml)	% inhibition			Mean ± SEM	IC_{50} (µg/ml)
	I	II	III		
10	17.9	18.3	15.3	17.8 ± 0.23	82.46 ± 0.74*
20	34.6	31.9	30.5	32.3 ± 0.54	
40	43.8	41.8	40.0	41.8 ± 0.67	
80	53.2	50.6	53.9	52.5 ± 0.77	
160	62.2	67.9	69.2	66.4 ± 0.98	
320	79.3	83.2	81.2	81.2 ± 0. 54	

Values are expressed as mean ± S.E.M. Mean values of six readings with three replications

Anti-oxidant activity:

Superoxide Radical Scavenging Activity: Superoxide anions indirectly initiated lipid oxidation as a result of superoxide and hydrogen peroxide serving as precursors of singlet oxygen and hydroxyl radicals [19]. Various studies reported that the anti-oxidant properties of flavonoids are effective mainly via the scavenging of superoxide anion. In this assay, decline in absorbance at 560 nm indicates that anti oxidants shows the utilization of superoxide anion in the reaction [24]. Superoxide scavenging activity of extracts was increased markedly with an increase in concentrations having the percentage inhibition in the range from 14.2 to 74.8% and that of standard ascorbic acid starts from 13.2 to 85.2%. IC_{50} values of the extract and the standard was expressed in **Table 3**.

Hydroxyl Radical Scavenging Activity: Hydroxyl radicals are the major active oxygen species causing lipid oxidation and enormous biological damage. Ferric-EDTA was incubated with H_2O_2 and ascorbic acid at pH 7.4. Hydroxyl radicals were formed in free solution and were detected by their ability to degrade 2-deoxy-2-ribose into fragments that on heating with TBA at low pH form a pink chromogen [17, 18, 20]. In this assay, the extract, prevented the decomposition of

deoxyribose in a dose dependent manner. They exhibited concentration dependent scavenging activity against the hydroxyl radical generated in Fenton's reaction. Percentage inhibition of the extract increased distinctly with that of concentrations used in the range from 21.9 to 74.3% in comparison with that of standard quercetin ranges from 11.9 to 81.5% and the IC_{50} values were tabulated in the **Table 3**.

Ferrous Chelating Ability: In this assay, extract and standard compound interferes with the formation of ferrous complex with the reagent ferrozine, suggesting that it has chelating activity. The results of our study expressed that the extracts have ability to bind iron indicates its antioxidant potential [19, 20]. Moreover, the metal chelating capacity of the extracts established that they decrease the concentration of the catalyzing transition metal concerned in the peroxidation of lipids with the increase in the concentration of the extract in the solution and the percentage inhibition of the extracts ranges from 19.5 to 71.2% in similarity to that of the standard ascorbic acid having the inhibition values 13.7 to 79.1% and the IC_{50} values of both are mentioned in the **Table 3**.

Nitric Oxide Radical Scavenging Activity: It is well known that nitric oxide has an important role in various types of inflammatory processes in the animal body [17, 18]. In the present study, crude extract of the leaves of the plant reduced the quantity of nitrite formed from the breakdown of sodium nitroprusside. This might be due to the presence of antioxidant constituents in the extract which participate with oxygen to react with NO· thus inhibiting the production of nitrite. Different concentrations of the extract was checked for its activity and compared with that of the standard and percentage inhibition of the extract starts from 18.4% at 50 µg/ml concentration and ends up at 68.9% at 800 µg/ml concentration.IC_{50} values for both were expressed in **Table 3**.

TABLE 3: *IN VITRO* ANTIOXIDANT ACTIVITY OF THE METHANOL EXTRACT OF THE LEAVES OF *CAMELLIA SINENSIS*

Antioxidant Activity	IC_{50} µg/ml	
	Standard	CSLE
Superoxide Radical Scavenging Assay	225.08±2.44	308.17±23.25
Hydroxyl Radical Scavenging Assay	70.99 ±1.31	47.04±2.26
Ferrous Chelating Ability	47.25 ± 0.89	44.12±4.63
Nitric Oxide Radical Scavenging Assay	260.38±0.66	396.83±0.83

Mean values of five readings with three replications. Values are expressed as mean± S.E.M

Total Flavonoid Content: In aluminum nitrate colorimetric method, aluminum nitrate forms acid stable complex with the keto group and either the hydroxyl group in A or C ring of flavonoids [25] in addition it forms acid labile complexes with orthodihydroxyl groups in the A or B ring of flavonoids [18]. The aluminum nitrate complexes of flavonoid compounds show strong absorbance at 415 nm and flavonoids with more functional groups absorb stronger at 415 nm. We used quercetin as a standard compound because it is one of the widely spread flavonoid in most of the plant extracts. Total flavonoid content of the methanolic extract of the leaves of the *Camellia sinensis* was 19.8±1.26µg quercetin equivalents/mg.

CONCLUSION: From the results of our study, we can conclude that the plant extract was capable of inhibiting the enzyme cholesterol esterase, but at varied potencies and were found to be good sources of antioxidants which have beneficial effects in preventing oxidative damage to membranes. It showed direct relationship between the concentration of the tested plant extract and its activity which was evaluated. It might be useful in the clinical field and since being plant extracts can be expected to exhibit lower incidence of side effects. Isolation of phyto-constituents from the extract would help in determining the active principles responsible for the activities, even though it could be suggested that flavonoids might be the potential agent. The existence of poly phenols and flavonoids can be a reason for the prevalence of these activities. Further, *in vivo* studies are required to assess possible clinical potential and confirm its mechanisms of action.

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REFERENCES:

1. Li F and Hui DY: Synthesis and secretion of the pancreatic-type carboxyl ester lipase by human endothelial cells. Biochemistry Journal 1998; 329: 675-679.
2. Hui DY and Howells PN: Cholesterol Esterase. In: Mansbach II, CM. Tso P and Kuksis, A: (Eds.) *Intestinal Lipid Metabolism*2001; Kluwer Academic Publishers, New York, pp. 119-134.
3. Chen CHJ, Miercke LJW, Krucinski J, Starr JR, Gina S, Wang X, Spilburg CA, Lange LG, Jeff L, Ellsworth O and Stroud RM: .Structure of bovine pancreatic cholesterol esterase at 1.6 Å:

novel structural features involved in lipase activation. Biochemistry1998; 37: 5107-5117.
4. Gesqui`ere L, Loreau N, Minnich A, Davignon J and Blache D: Oxidative stress leads to cholesterol accumulation in vascular smooth muscle cells. Free Radical Biology & Medicine 1999; 27: 134–145.
5. Kiritikar K R and Basu BD: Indian Medicinal Plants Vol - II,. Dehradun, International Book Distributors; (2nd ed) 1987; pp. 1225, 2128 & 2327.
6. [6] Hamilton JMT: Antimicrobial properties of tea (*Camellia sinensis* L.). Antimicrobial Agents and Chemotherapy 1995; 39: 2375–2377.
7. Kalyan SG, Kanti TM and Dasgupta S: Green tea polyphenols as inhibitors of ribonuclease A. Biochemical and Biophysical Research Communications 2004; 325: 807-811.
8. Chan EWC, Lim Y and Chew Y L: Antioxidant activity of *Camellia sinensis* leaves and tea from a lowland plantation in Malaysia. Food Chemistry 2007; 102: 1214-1222.
9. Turkmen N, Sari F and Velioglu S: Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of black tea. Molecules 2007; 12:484-496.
10. Horžić D, Komes D, Belščak A, Ganić KK, Ivekovic D and Karlović D: The composition of polyphenols and methylxanthines in teas and herbal infusions. Food Chemistry 2009; 115: 441-448.
11. Raza H and John A: *In vitro* protection of reactive oxygen species-induced degradation of lipids, proteins and 2-deoxyribose by tea catechins. Food and Chemical Toxicology 2007; 10: 1814-1820.
12. Rosenblat M, Volkova N, Coleman R, Almago Y and Aviram M: Antiatherogenicity of extra virgin olive oil and its enrichment with green tea polyphenols in the atherosclerotic apolipoprotein-E-deficient mice enhanced macrophage cholesterol efflux. *The Journal of Nutritional Biochemistry* 2008; 19: 514-523.
13. Quan TP, Hang V T, Ha HN, Anh NT and Tuyen NT: Microwave-assisted extraction of polyphenols from fresh tea shoots. *Science and Technology Development* 2006; 9: 1-4.
14. Trease GE and Evans NC: Text book of Pharmacognosy, (15th ed.), London: Elsevier publishers 2005; pp. 137-149.
15. Pietch M and Gutshow M: Alternate substrate inhibition of cholesterol esterase by thieno[2,3-d][1,3]oxazin-4-ones. The Journal of Biological Chemistry 2002; 27: 24006-24013.
16. Ojeda D, Ferrer EJ, Zamilpa A, Arellanob AH, Tortoriello J and Alvarez L: Inhibition of angiotensin convertin enzyme (ACE) activity by the anthocyanins delphinidin and cyanidin-3-O-sambubiosides from *Hibiscus sabdariffa*. Journal of Ethnopharmacology 2010; 127: 7-10.
17. Kumar RS, Sivakumar T, Sundaram RS, Nethaji R, Gupta M and Mazumdar UK: Antimicrobial and antioxidant activities of *Careya arborea* Roxb. stem bark. Iranian Journal of Pharmacology and Therapeutic 2006; 5: 35-41.
18. Mohammedzadeb S, Sharriatpanahi M, Hamedi M, Amanzadeh Y, Esmaeil S and Ostad N Antioxidant power of Iranian propolis extract. Food Chemistry 2007; 103: 729–733.
19. Nagulendran KR, Velavan S, Mahesh R and Begum HV: *In Vitro* antioxidant activity and total polyphenolic content of *Cyperus rotundus* rhizomes. E-Journal of Chemistry 2004; 3: 440-449.
20. Subhadradevi V, Asokkumar K, Umamaheswari M, Sivashanmugam AT and Sankaranand R: *In vitro* antioxidant activity of *Vetiveria zizanioides* root extract. Tanzanian Journal of Health Research 2010; 12: 1-7.
21. Jagetia GC, Rao SK, Baliga MS and Babu KS:The evaluation of nitric oxide scavenging activity of certain herbal formulations *in vitro*: A Preliminary Study. Phytotherapy Research 2004; 18: 561–56.
22. Mukherjee PK. Plant products with hypocholesterolemic potentials. Advances in Food and Nutrition Research 2003; 47.
23. Heynekamp JJ, Hunsaker L, Vander Jagt TA, Royer R, Decka ML and Vander Jagt DL: Isocoumarin-based inhibitors of pancreatic cholesterol esterase. Bioorganic & Medicinal Chemistry 2008; 16: 5285–5294.
24. Salar RK and Dhall A: Antimicrobial and free radical scavenging activity of extracts of some Indian medicinal plants. Journal of Medicinal Plants Research 2010; 4: 2313-2320.
25. Hsu YC: Antioxidant activity of extract from *Polygonum aviculare* L. Biological Research 2006; 39: 281-288

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