

Lipid Lowering Activity and Free Radical Scavenging Effect of *Cinnamomum tamala* (Fam: Lauraceae)

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ABSTRACT

Lipid profile is a blood test that measures the amount of lipids, or fats, in your blood. The lipids measured are usually total cholesterol, HDL-C, LDL-C and triglycerides. When levels of these lipids are abnormal, there is an increased risk of heart attack and stroke. In our study, we use methanol crude extract for lipid lowering activity on rabbit and found that lipid profile was reduced by 14.0, 1.0, 4.0 and 15.0 mg/dl for total cholesterol, HDL-C, LDL-C and triglyceride respectively after using the plant extract (dose 500mg/rabbit for 10 days); where atorvastatin (0.005mg/rabbit) was used as standard lipid lowering agent. We also focused on the antioxidant property of crude methanol extract. Here we also carried out free radical-scavenging activity study and found the IC₅₀ value for *C. tamala* is 6.00 µg/ml where the standard antioxidant (ascorbic acid) gave the value of 3.21 µg/ml.

Key words: *Cinnamomum tamala*, lipid profile test, free radical scavenging effect, ascorbic acid

INTRODUCTION

The *Lauraceae* or Laurel family comprises a group of flowering plants included in the order Laurales. The family contains about 55 genera and over 2000 species world-wide, mostly from warm or tropical regions, especially Southeast Asia and Brazil. Most are aromatic evergreen trees or shrubs, but *Sassafras* and one or two other genera are deciduous, and *Cassytha* is a genus of parasitic vines. *Cinnamomum* is a genus of evergreen trees and shrubs belonging to the Laurel family, *Lauraceae*. The species of *Cinnamomum* have aromatic oils in their leaves and bark. The genus contains over 300 species, distributed in tropical and subtropical regions of North America, Central America, South America, Asia, Oceania and Australasia.

Notable *Cinnamomum* species include *C. tamala* (*Malabathrum*, also known as *C. tejpata*; *tejpat* or *tejpat* in Hindi; or, inaccurately, "Indian bay leaf"). *Cinnamomum tamala* is a moderate sized evergreen tree attaining a height of 8 m, and a girth of 150 cm. Its bark produces mucilage. Leaves lanceolate, glabrous; alternately placed, opposite and short stalked. 3- nerved from the base. The genus *Cinnamomum* has about 250 tropical tree and shrub species. The etymology is derived from the Greek word 'kinnamomon' (meaning spice). The Greeks borrowed the word from the Phoenicians, indicating that they traded with the East from early times. The specific epithet 'tamala' is after a local name of the plant in India [1,2,3]. Previous phytochemical study has revealed the presence of eugenol [4,5], β-

caryophyllene & linalool, trans-sabinene hydrate, (Z)-β-ocimene, myrcene, α-pinene and β-sabinene, germacrene A and α-gurjunene [6]. *Cinnamomum* leaf contains three flavonoid compounds namely quercetin, kaempferol and quercetrin which are responsible for its antioxidant activity [7,8]. The present work was an endeavor to screen the methanolic extract (ME) of *Cinnamomum tamala* for lipid lowering activity and antioxidant activities.

MATERIALS AND METHODS

Leaves of *Cinnamomum tamala* (Fam: *Lauraceae*) was collected from Gazipur and Joydevpur area in March 2009 and were taxonomically identified by a Scientific Officer, Bangladesh National Herbarium (BNH) and one voucher specimen has been deposited there.

The intact plant/plant part(s) is collected as a whole and sun-dried. In fresh condition, it is then oven-dried at reduced temperature (not more than 50°C) to make suitable for grinding purpose. The coarse powder is then stored in airtight container with marking for identification and kept in cool, dark and dry place for use.

Extraction of dried and powdered plant (250.0mg) of *C. tamala* was done by cold extraction process by using methanol as a solvent [9]. And after that the fractions were evaporated by roto-dryer at low temperature (40 – 50°C) to dryness. Crude methanol extract was subjected to lipid profile test and antioxidant activity study.

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Table 1. Lipid profile of the tested rabbit against standard atorvastatin (R-5) and blank (R-4).

Lipid profile (mg/dl)	Before taking plant extract			After taking plant extract		
	Tested Rabbit (R-1, R-2, R-3)	Standard Rabbit (R-5)	Blank Rabbit (R-4)	Tested Rabbit (R-1, R-2, R-3)	Standard Rabbit (R-5)	Blank Rabbit (R-4)
TC	44±5.13	40	35	30±5.00	28	45
HDL-C	17±1.53	20	24	16±1.54	19	21
LDL-C	15±2.89	22	19	11±3.21	20	19
TG	81±5.13	80	70	66±6.03	55	68

Lipid tests are routinely performed on plasma, which is the liquid part of blood without the blood cells. Lipids themselves are a group of organic compounds that are greasy and cannot be dissolved in water, although they can be dissolved in alcohol.

Lipid tests include measurements of total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol. The test is done to determine the risk of coronary heart disease. The test is good indicator of heart attack or stroke leaded by atherosclerosis. In our study, the typical lipid test was done that includes the measurement of total cholesterol (TC), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C) and triglycerides (TG).

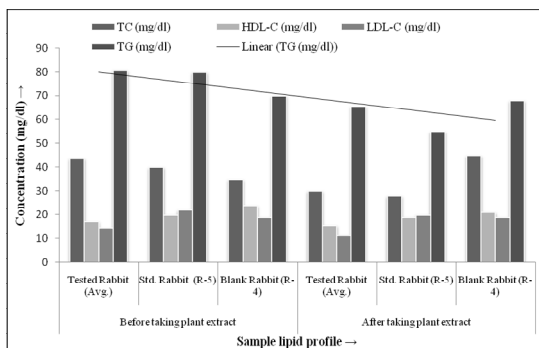


Figure 1. Comparison among the lipid profile (in mg/dl) of all tested rabbit in two states.

Five rabbits (of both sex, age 09-10 weeks) were collected from Dhaka and used as experimental model (*in vivo* analysis). They were marked as R-1, R-2, R-3, R-4 (blank) and R-5 (standard). Each rabbit was kept in same place with same treatment in terms of food and environment. Then methanol extract was given to three of them (R-1, R-2, R-3) with a dose of 500mg/rabbit for 10 days while the atorvastatin (0.005mg) was used to R-5 (standard rabbit) as positive control. Two blood samples were withdrawn (before and after treatment with plant extract) from all rabbits and tested for lipid profile by diagnostic method [10,11,12].

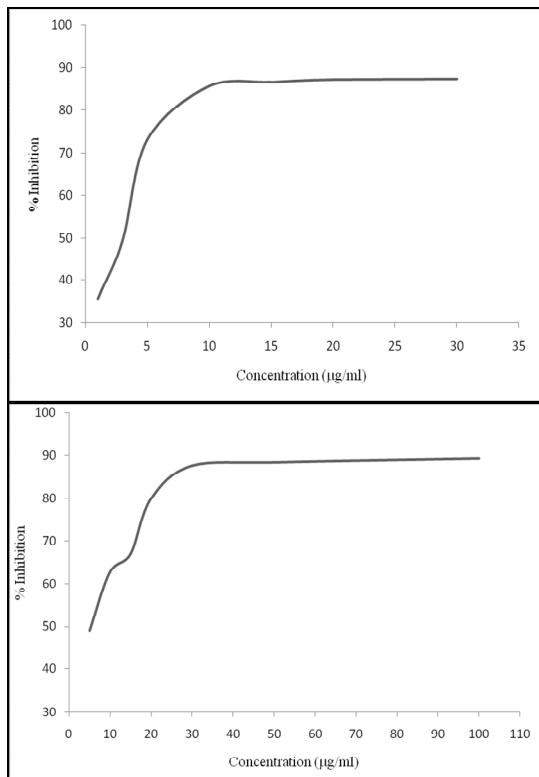


Figure 2. % Inhibition for standard ascorbic acid (a) and test sample, extract of *C. tamala* (b)

Antioxidant activity of the plant extracts and the standards was assessed on the basis of the radical scavenging effect of the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical, using Brand-Williams method [13]. In the experiment, 2.0 mg of extract was dissolved in methanol. Solution of varying concentrations such as 500, 250, 125, 62.50, 31.25, 15.62, 7.8125, 3.91, 1.95 and 0.98 µg mL⁻¹ were obtained by serial dilution technique. 2.0ml of a methanol solution of the extract of each concentration was mixed with 3.0ml of a DPPH-methanol solution (20µg/ml) and was allowed to stand for 20 min for the reaction to occur. Then the absorbance was determined at 517 nm and from these values the corresponding percentage of inhibitions were calculated by using the following equation.

$$\text{Inhibition (\%)} = 1 - \frac{\text{ABS}_{\text{sample}}}{\text{ABS}_{\text{control}}} \times 100$$

Then % inhibitions were plotted against respective concentrations used and from the graph IC₅₀ was calculated by using ascorbic acid, a potential antioxidant, was used as positive control.

Table 2. Comparison of IC₅₀ values between the extract of *C. tamala* and a standard antioxidant, ascorbic acid.

Sl	Abs. of blank (at 517nm)	Scavenging activity of Ascorbic acid				Scavenging activity of <i>C. tamala</i>			
		Conc. (µg/ml)	Abs. after reaction	% Inhibition	IC ₅₀ (µg/ml)	Conc. (µg/ml)	Abs. after reaction	% Inhibition	IC ₅₀ (µg/ml)
1		1	0.251	35.476		5	0.198	49.1	
2		3	0.197	49.357		10	0.145	62.725	
3		5	0.105	73.004		15	0.128	67.095	
4	0.389	10	0.056	85.604	3.21	20	0.078	79.949	6.00
5		15	0.052	86.632		30	0.048	87.661	
6		20	0.05	87.147		50	0.045	88.432	
7		30	0.049	87.404		100	0.042	89.203	

Three replicates of each sample were used for statistical analysis and the values are reported as mean \pm SD (n=3). Probability (P) value of 0.05 or less ($P < 0.05$) was considered significant.

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RESULTS AND DISCUSSION

In this study, TC, HDL-C, LDL-C and TG were measured from the blood sample of test animals treated with methanolic extracts of *Cinnamomum tamala*. The results were shown in Table 1 and Figure 1. In our study, lipid profiles on rabbit were reduced by 14.0, 1.0, 4.0 and 15.0 mg/dl for TC, HDL-C, LDL-C and TG, respectively after using the plant extract (dose 500mg/rabbit for 10 days) where atorvastatin (0.005mg/rabbit) was used as standard lipid lowering agent. If we compare with the lipid lowering drug, atorvastatin, our test sample showed significant reduction of lipid profiles.

In the DPPH assay, the ability of the investigated extracts to act as donors of hydrogen atoms or electrons in transformation of DPPH radical into its reduced form DPPH^{*}-H was investigated. All the assessed extracts were able to reduce the stable, purple-colored radical, DPPH^{*}, into the yellow-colored DPPH^{*}-H reaching 50% of reduction^[14]. In the present study we have evaluated the free radical scavenger activity of methanolic extract of *Cinnamomum tamala*.

In antioxidant activity study, ascorbic acid was used as standard whose IC₅₀ value was 3.21µg/ml where the plant extract give the IC₅₀ value of 6.0µg/ml (Table 2 and Figure 2). Thus the study showed a potent antioxidant property of *C. tamala*.

Therefore, it can be concluded that, the methanolic extract of *Cinnamomum tamala* reduced lipid profiles like TC, HDL-C, LDL-C and TG significantly compare to that of atorvastatin, a lipid lowering drug. The test sample also revealed significant antioxidant activity.

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