In Vitro Lipase, Cholesterol Esterase and Cholesterol Micellization Inhibitory Activities of Ceylon Cinnamon (*Cinnamomum Zeylanicum* Blume)

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Abstract— *Hyperlipidaemia is a group of metabolic disorders characterized by the elevated levels of serum triglycerides and cholesterol. It contributes significantly in pathogenesis of diabetes, obesity, hypertension and cardiovascular diseases. Hence, there is an imperative need for development of antilipidemic agents preferably from natural sources. Cinnamomum zeylanicum Blume is indigenous to Sri Lanka and used as a spice in several countries. According to some Sri Lankan traditional physicians the bark of this plant is claimed to possess antilipidemic effects by inhibition of lipid digestion and/or absorption. This study was initiated to investigate antilipidemic potential of Ceylon cinnamon in vitro.*

Ethanolic (95%) and 1:1 dichloromethane: methanol (DCM:M) brak extracts of Ceylon cinnamon were used in this study. Different concentrations of ethanolic and DCM:M bark extracts (anti-lipase: 3.75 - 600 µg/ml, n = 3; cholesterol esterase: 3.125 - 100 µg/ml, n = 3; cholesterol micellization: 0.25 - 1 mg/ml, n = 6) were used in testing of antilipidemic effects.

The results revealed that both extracts possess moderate lipase inhibitory activity (ethanol bark IC_{50} 301.09 ± 5.73 µg/ml and DCM:M bark IC_{50} 297.57 ± 11.78 µg/ml), high cholesterol esterase inhibitory activity (ethanol bark IC_{50} 30.62 ± 1.67 µg/ml and DCM:M bark IC_{50} 34.39 ± 0.91 µg/ml) and moderate to high cholesterol micellization inhibitory activities (mean percentage inhibition of cholesterol solubility in micelles in ethanolic bark and DCM:M bark 98.09 ± 1.25 and 73.94 ± 1.95, at 1 mg/ml; 69.48 ± 1.99 and 62.15 ± 2.37 at 0.5 mg/ml and 49.48 ± 1.90 and 19.36 ± 4.57 at 0.25 mg/ml respectively). Further, anti-lipase and anti-cholesterol esterase activities were dose dependent.

It is concluded that Ceylon cinnamon bark possess anti-lipase, cholesterol esterase and cholesterol micellization inhibitory activities. This is a novel finding having therapeutic potentials and indicates the potential of using bark as a functional food for hyperlipidaemia.

Keywords— Ceylon cinnamon, bark extracts, lipid lowering, *Cinnamomum zeylanicum*

I. INTRODUCTION

Hyperlipidaemia is a group of metabolic disorders characterized by the elevated levels of serum triglycerides and cholesterol (Jacobson *et al*., 2007). It contributes significantly in pathogenesis of diabetes, obesity, hypertension and cardiovascular diseases (Fauci *et al.,* 2008). Several factors, such as diet high in saturated fats and cholesterol, lack of physical activity, stress and life style factors play a significant role in development of hyperlipidaemia and related chronic diseases.

Currently, available antilipidaemic drugs to treat hyperlipidaemia include HMG-CoA reductase inhibitors, cholesterol absorption inhibitors, bile acid sequestrants and omega -3- fatty acids (Fauci *et al.,* 2008). These drugs are very effective in management of hyperlipidaemia and related chronic diseases. However, most of these drugs are expensive and beyond the reach of many persons in developing countries and some of these drugs are associated with undesirable side effects such as myalgias, arthralgias, elevated liver enzymes, elevated blood glucose, dyspepsia and constipation (Fauci *et al.,* 2008). Research conducted in recent years has shown that many of the natural products having antilipidaemic activity via multiple mechanisms (Adisakwattana *et al.,* 2012; Kumar et al., 2011; Uahiyama *et al.,* 2011; Ikeda *et al.,* 2010).

These natural products are proven to be safe and less expensive compared to the available antilipidaemic drugs.

Cinnamomum zeylanicum Blume is indigenous to Sri Lanka and used as a spice in several countries. According to some Sri Lankan traditional physicians the bark of this plant is claimed to possess antilipidemic effects by inhibition of lipid digestion and/or absorption. Scientifically antilipidaemic properties of cinnamon have shown in various *in vitro* (Sheng *et al.,* 2008) and *in vivo* models (Ranasinghe *et al.,* 2012; Lee *et al.,* 2003; Sambaiah & Srinivasan, 1991). However, antilipidaemic properties of Ceylon cinnamon via anti-lipase activity, cholesterol esterase and cholesterol micellization inhibitory activities were not previously reported. In this connection this study was initiated to investigate antilipidemic potential of Ceylon cinnamon via *in vitro* anti-lipase activity, cholesterol esterase and cholesterol micellization inhibitory activities.

II. MATERIALS AND METHODS

A. Materials

Alba grade cinnamon bark samples were collected from L.B spices (Pvt) Ltd, Aluthwala, Galle, Sri Lanka and G. P. De Silva and Sons spices (Pvt) Ltd, Ambalangoda, Sri Lanka.

B. Chemicals and equipments

Porcine pancreatic lipase (PPL, type II), p- nitro phenyl butyrate (p-NPB), pancreatic cholesterol esterase, oleic acid, phosphatidylcholine, cholesterol, epigallocatechin gallate (EGCG) and taurocholic acid were purchased from Sigma-Aldrich, USA. Cholesterol test kits were purchased from Fortress diagnostics, UK. All the other chemicals and reagents were of analytical grade.

C. Preparation of bark extracts of Ceylon cinnamon

Preparation of ethanolic bark extracts: Powdered bark (20 g) was extracted into 200 ml of 95% ethanol for 4-5 h and 4-6 cycles in a soxhlet extractor till the solvent in the Siphon tube and extractor becomes colourless. The extract was then filtered and evaporated under reduced pressure and freeze dried. The freeze dried extracts were stored at -20 ^{o}C and used for the following assays.

Preparation of dichloromethane:methanol (DCM:M) bark extracts: Powdered bark (20 g) was extracted into 200 ml of dichloromethane:methanol in a ratio of (1:1 v/v) at room temperature for 7 days with occasional shaking . The extract was filtered and evaporated under reduced pressure and freeze dried. The freeze dried extracts were stored at -20 ^{o}C and used for the following assays.

D. Anti-lipase activity of Ceylon cinnamon

Pancreatic lipase inhibitory activity of bark extract of Ceylon cinnamon was carried out according to the method describe by Kim *et al.,* (2010) with some modifications. Porcine pancreatic lipase (PPL, type II) stock solution (2.5 mg/ml) was prepared in 0.1 M Tris HCl buffer with 5 mM $CaCl_2$ (pH 7.0). Reaction volume of 200 µl, containing 30 µl of 2.5 mg/ml enzyme and 120 µl of different concentrations of bark extracts (37.5, 75, 150, 300, 600 µg/ml; n = 3) were pre-incubated at 37 ^{0}C for 15 min. Reaction was then started by addition of 5 µl of 10 mM p-NPB in dimethylformamide and was allowed to proceed at 37 ^{0}C for 30 min. Lipase inhibitory activity of bark extracts were determined by measuring the hydrolysis of p-NPB to p-nitrophenol at 405 nm using SpectraMax384micro plate reader. Inhibition of lipase activity was expressed as the percentage decrease in optical density when pancreatic lipase was incubated with bark extracts. Lipase inhibition (%) was calculated according to the following formula;

$$\text{Inhibition (\%)} = \frac{(A-a) - (B-b)}{(A-a)}$$

Where, A is the activity without inhibitor, a- the negative control without inhibitor, B- the activity with inhibitor and b is the negative control with inhibitor.

E. Cholesterol esterase inhibitory activity of Ceylon cinnamon

Pancreatic cholesterol esterase inhibitory activity of bark extracts of Ceylon cinnamon were performed according to the method described by Pietch & Gutschow, (2005) with some modifications. Reaction volume of 200 µl, containing different concentrations of bark extracts (3.12, 6.25, 12.5, 25, 50, 100µg/ml; n=3) were pre-incubated with 50 µl of 24 mM taurocholic acid and 5 µl of 8 mM p-NPB in acetonitrile in 0.1M sodium phosphate buffer containing 0.1M NaCl (pH 7.0) at 25^{0}C for 10 min. Reaction was then initiated by addition of 42.5 µl of (1.25 µg/ml) cholesterol esterase enzyme and it was

monitored at 25^{0}C for 6 min at 405 nm using SpectraMax384 micro plate reader. The IC_{50} values were calculated from the linear steady state turnover of the substrate.

F. Cholesterol micellization inhibitory activity of Ceylon cinnamon

Artificial micelles were used as a model system for *in vitro* cholesterol solubilization, which contains predominantly uniform particles based on sodium taurocholate, egg lecithins, cholesterol and oleic acid to reflect the natural mixed micelle. Further, they were prepared according to the method described by Kirana *et al.,* (2005) with some modifications. Briefly, the solution containing 2 mM cholesterol, 1 mM oleic acid and 2.4 mM phosphatidylcholine were dissolved in methanol. Then these samples were dried under nitrogen before adding 15 mM phosphate-buffered saline (PBS) containing 6.6 mM taurocholate salt, pH 7.4. The suspension was sonicated twice for 30 min using a sonicator (Bandelin SANOREX electronic, RK 510) and incubated overnight at 37 °C. Different concentration of ethanol and DCM:M bark extracts (0.25, 0.5 and 1.0 mg/ml; n=6) and were added to the mixed micelle solution and were incubated at 37 °C for further 2 h. The solution was centrifuged at 16,000 rpm for 20 min. The supernatant was collected and cholesterol concentration was determined using total cholesterol test kit (BXC0261, Fortress diagnostics, UK). PBS was used as the control and EGCG as the positive control in the assay.

G.Statistical analysis

Data represented as mean ± SD/SE. Data of each experiment were statistically analysed using SAS version 6.12. One way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT) were used to determine the differences among treatment means. P < 0.05 was regarded as significant.

III. RESULTS

A. Anti-lipase activity of Ceylon cinnamon

Both ethanol and DCM:M bark extracts of Ceylon cinnamon showed moderate anti-lipase activity and anti-lipase activity among ethanol and DCM:M bark extracts were statistically insignificant (p < 0.05). The IC_{50} values of ethanol bark and DCM:M bark extracts were 301.09 ± 5.73 and 297.57 ± 11.78 µg/ml respectively. The dose response relationship of ethanol bark and DCM:M bark extracts is given in Table 1.

Table 1. Anti-lipase activity of bark extracts of Ceylon cinnamon

Concentration (µg/ml)	% Inhibition	
	Ethanol bark	DCM:M bark
37.5	55.27 ± 3.59	55.66 ± 3.07
75	49.54 ± 0.29	52.07 ± 1.96
150	27.35 ± 4.43	24.14 ± 3.11
300	5.30 ± 1.28	17.95 ± 5.72
600	5.74 ± 0.80	12.50 ± 1.32
IC_{50}	**301.09 ± 5.73[a]**	**297.57 ± 11.78[a]**

Data represented as mean ± SD (n=3). IC_{50} values in the columns superscripted by different letters were significantly different at p < 0.05.

B. Cholesterol esterase inhibitory activity of bark extracts of Ceylon cinnamon

Cholesterol esterase inhibitory activity of bark extracts of Ceylon cinnamon is given in Fig 1. Ceylon cinnamon possesses significant (p < 0.05) *in vitro* cholesterol esterase inhibitory activity in a dose dependent manner. However, ethanol bark had significantly high activity compared to DCM:M bark extract (p < 0.05). The IC_{50} values of ethanol bark and DCM:M bark extracts were 30.62 ± 1.67 and 34.39 ± 0.91 µg/ml respectively.

C. Cholesterol micellization inhibitory activity of bark extracts of Ceylon cinnamon

Both ethanol and DCM:M bark extracts of Ceylon cinnamon showed Cholesterol micellization inhibitory activity in a dose dependent manner. However, ethanol bark extract had significantly high (p < 0.05) activity compared to DCM bark extract. The IC_{50} values of ethanol bark and DCM:M bark extracts were 0.23 ± 0.02 and 0.48 ± 0.02 mg/ml respectively. The dose response relationship of ethanol bark and DCM:M bark extracts for Cholesterol micellization inhibitory activity is given in Table 2.

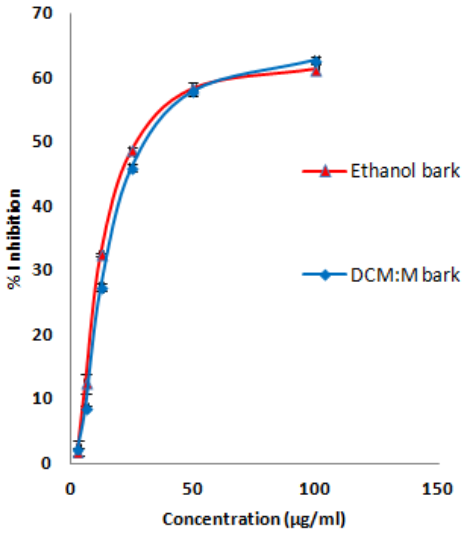

Figure 1:

Fig 1. Cholesterol esterase inhibitory activity of ethanol and DCM:M bark extracts of Ceylon cinnamon. IC_{50} values: Ethanol bark 30.62 ± 1.67[a]µg/ml; DCM:M bark: 34.39 ± 0.91[b]µg/ml. IC_{50} values superscripted by different letters are significantly different at $p < 0.05$.

Table 2. Cholesterol micellization inhibitory activity of bark extracts of Ceylon cinnamon

Concentration (µg/ml)	% Inhibition of cholesterol solubility in micelles		
	Ethanol bark	DCM:M bark	EGCG
0.25	98.09 ± 1.25	73.94 ± 1.95	96.75 ± 2.40
0.5	69.48 ± 1.99	62.15 ± 2.37	69.78 ± 2.58
1	49.48 ± 1.90	19.36 ± 4.57	55.16 ± 1.29
IC_{50}	**0.23 ± 0.02[b]**	**0.48± 0.02[a]**	**0.15 ± 0.01[c]**

Data represented as mean ± SD (n=6). IC_{50} values in columns superscripted by different letters are significant different at $p < 0.05$.

IV. DISCUSSION

Hyperlipidaemia is a group of metabolic disorders characterized by elevated levels of serum triglycerides and cholesterol (Jacobson et al., 2007). It plays an important role in pathogenesis of obesity, diabetes, hypertension and cardiovascular diseases (Fauci et al., 2008). The prevalence and incidence of hyperlipidaemia is increasing rapidly throughout the world due to variety of factors. These factors include sedentary life styles, lack of physical exercise, stress and consumption of high fat diets (Fauci *et al.,* 2008). Currently, available antilipidaemic drugs are expensive and induce undesirable side effects (Fauci *et al.,* 2008). In this regard many natural products have been evaluated for antilipidaemic properties and can be used in prevention and management of hyperlipidaemia (Adisakwattana *et al.,* 2012; Kumar *et al.,* 2011; Uahiyama *et al.,* 2011; Ikeda *et al.,* 2010).

In this study we evaluated the antilipidaemic properties of bark extracts of Ceylon cinnamon via anti-lipase activity, cholesterol esterase and cholesterol micellization inhibitory activities. Ceylon cinnamon bark possesses all the antilipidaemic properties tested in this study. The cholesterol esterase and cholesterol micellization inhibitory activities showed potent activities whereas lipase inhibitory activity was moderate (Orlistat IC_{50} = 0.35 µl/ml, Bustanji *et al*; 2010). Some studies have shown that cinnamon has antilipidaemic properties via HMG-CoA reductase inhibitory activity (Lee *et al.,* 2003) and through activation of peroxisome proliferator-activated receptors mechanisms (Sheng *et al.,* 2008). Recently antilipidaemic properties of Ceylon cinnamon have shown in a rat study by Ranasinghe et *al.,* (2012). An inhibition of fat absorption and suppression of lipid absorption can be mediated by three main mechanisms: inhibition of pancreatic cholesterol esterase activity (Kumar *et al.,* 2011); impairment of cholesterol micellization (Vermeer *et al.,* 2013): and inhibition of bile acid binding (Adisakwattana *et al.,* 2012). However, inhibition of fat digestion and absorption and thereby suppression of lipid digestion and absorption via anti-lipase activity, cholesterol esterase and cholesterol micellization inhibitory activities of Ceylon cinnamon were not previously reported. Therefore, these novel antilipidaemic properties add values to Ceylon cinnamon as a natural good source with antilipidaemic activity via multiple mechanisms. As Ceylon cinnamon is called as true cinnamon worldwide and currently Sri Lanka produces > 90 % of the world's production these novel findings with therapeutic value may be useful in value addition to Ceylon cinnamon in the international trade. Further, consumption of bark of Ceylon cinnamon in daily life may be important for prevention and management of hyperlipidaemia and related chronic diseases. Moreover, there is a possibility to isolate active compounds from bark of

Ceylon cinnamon with anti-cholesterol esterase activity and cholesterol micellization inhibitory activities for development of functional foods, nutraceuticals, cosmaceuticals and pharmaceuticals for prevention and management of hyperlipidaemia and related chronic diseases.

We have previously reported anti-oxidant properties of bark extracts of Ceylon cinnamon (Abeysekera *et al.,* 2013). Bark extracts of Ceylon cinnamon had potent anti-oxidant activities via multiple mechanisms (Abeysekera *et al.,* 2013). Oxidative stress is now known to be involved in hyperlipidaemia; it is indeed an early event in the evolution of hyperlipidaemia (Jin *et al.,* 2013). As free radicals are involved in lipid peroxidation and related hyperlipidaemic activities anti-oxidants can play a vital role in antilipidaemic activities. It has been reported that phenolic compounds show the ability to inhibit the formation of cholesterol micelles (Vermeer *et al.,* 2013). Therefore, observed antilipidaemic activities of bark extracts of Ceylon cinnamon may be due to the presence of anti-oxidative compounds. Further, experiments are in progress to isolate active compounds and efficacy *in vivo* studies.

V. CONCLUSIONS

It is concluded that bark extracts of Ceylon cinnamon possess lipase, cholesterol esterase and cholesterol micellization inhibitory activities. This is the first study to report anti-lipase, cholesterol esterase and cholesterol micellization inhibitory activities of Ceylon cinnamon worldwide.

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