Original Article

Antihyperlipidemic and antioxidant activities of *Bruguiera cylindrinca* (L)

Abstract

Background: Atheroclerosis is the major cause of morbidity and mortality world wide. The drugs used these days have some or other side effects. **Aim:** Therefore it is an urgent need to develop a safe and cheaper drugs for the society. We have planned this study to investigate lipid lowering and antioxidant activities of *Bruguiera cylindrinca* in triton and cholesterol fed rats. **Materials and Methods:** Different extract and fractions were collected from *B. cylindrica*. Two major compounds were isolated from there and have been tested for their lipid lowering activity along with increase in high density lipoprotein,cholesterol, compared with the cholesterol fed animals. **Results:** On chronic feeding of active fraction and pure compounds (at 100 mg / kg b.w.) for 30 days. The pure compounds isolated from the active fraction were found showing lipid lowering activity similar to the fraction. **Conclusion:** Structre modification of the two pure compounds isolated from the active fraction is required for further enhacement of biological activity.

Key words:
B. cylindrica, hypolipidemic activity, lipoprptein metabolism, triton model

Introduction

Atherosclerosis is the major cause of myocardial morbidity and mortality world wide. Lipoproteins are responsible for the initiation of atherosclerosis and associated disorders. Elevated level of plasma concentration of cholesterol, lipoproteins (LDL) and triglycerides are recognized as a major cause in the development of atherosclerosis and coronary heart disease[1,2] Oxidative stress starts in low density lipoproteins of the plasma by hydroxyl radicals generated by the metal ions prsent in the serum, due to alteration in their oxidation states. Currently several drugs are being used in the treatment of the dyslipidemia.[3] Statins are used to lower serum level, which are known or side effects, such as myositis, athralgias, gastrointestinal upset etc. Statins such as atarovastatin, atarovastatin, fluvastatin, simvastatuin and and provastatin act as a inhibitors of HMGCoA reductase enzyme.

Reductase is an enzyme involved in denovo synthesis of cholesterol and upgradation of LDL. Therefore it is required to develop drugs for the treatment of hyperlipidemia reducing their Side effects. The involvement of hydroxyl free radicals have been found to be a major causative factor for the peroxidative damage and progression of atherosclerosis in hypolipidemic subjects.[4]

The antioxidant compounds in a diet are mostly derived from the plant sources.[5] Little is known about the lipid lowering and anti oxidant potential of the mangroves. Oxygen via its transformation produces Reactive oxygen species (ROS) such as superoxide, hydroxy radicals and hydrogen peroxide.

Free radicals attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA.[6,7] The body possesses several defense systems comprising enzymesand radical scavengers.[8] Antioxidants act as a inhibitors in the oxidation process and are found to inhibit oxidative chain reactions at a small concentrations and eliminate the threat of pathological processes.[8] Phenolic compounds present in medicinal plants have

Access this article online	
Website: http://www.cysonline.org	Quick Response Code
DOI: 10.4103/2229-5186.99596	

Vijai Lakshmi, Ravi Sonkar[1], Ashok K. Khanna[1]

Departments of Medicinal and Process Chemistry Division, [1]Biochemistry Division, Central Drug Research Institute, Lucknow, Uttar Pradesh, India

Address for correspondence:
Dr. Vijai Lakshmi,
Department of Biochemistry, CSM Medical University,
Central Drug Research Institute, Lucknow, Uttar Pradesh, India.
E-mail: vijlakshmius@yahoo.com

shown powerful antioxidant property.[9] The antioxidant compounds in a typical diet are mostly derived from plant sources and polyphenolic components of higher plants which act anti-carcinogenic action.[5] Apart from antioxidant studies, the present study also involves evalutation of antihyperlipidemic activity.

Materials and Methods

Collection of plant material

Stems with leaves of *B. cylindrica* were collected from the South Andaman Coast of India in the month of march 2007 by the botany division of the Institute. The voucher specimen has been preserved in the botanydivision with a voucher specimen number 484.

Extraction and fractionation procedure

The air dried powdered plant material (1.0 kg) was extracted with 95% ethanol four times (4×4.0l) and the combined ethanol extact was filtered, concentrated under reduced pressure below 50°C to get agreen viscous mass,which was finally dried under high vacum to get the ethanol extract (30.0 g). The plant material left after the extraction with 95% ethanol, was further extracted with 50% aqueous ethanol, three times (3×4.0Lit.) and the combined extract was concentrated under reduced pressure below 50°to get viscous residual which was dried under high vacum for 2 hours toget the 50% aqueous ethanol extract (12.0 g,). Both of these extracts showed promising lipid lowering activity,therefore ethanol extract (20.0 g) was fractionated into four fractions by maceration with hexane, chloroform and n-butanol successively and each of these were concentrated under reduced pressure below 50° to get each of the residual masses. Hexane soluble (1.5 g), chloroform soluble (2.6 g), n-butanol soluble (5.6 g) respectively and the n-butanol butanol insoluble fraction (10.3 g). On chromatography of the hexane and chloroform fractions over the plates of silicagel, these two showed similar tlc pattern, therefore were mixed together and chromatographed over a column of silicagel. Only two pure compounds could be isolated in large amounts, which were bio-evaluated. These compounds were identified as lupeol (1) and β-amyrin (2) by comparision of physicochemical data with those reported in the literature[10,11] and co-tlc with the authentic samples.

Spectral data of compound (1)

White crystalline powder; mp 212°C, IR (KBr) cm-^{1} 3324, 2930, 1630, 1452, 1038, 874; EIM*Sm/z* 425 [M+ - H], 409 [M+ - OH] 218, 207-and 189, ^{1}HNMR(CDCl3, 300 MHz) δ values, 0.79, 0.89, 0.90, 0.95, 0.99, 1.07 and 1.70, (s, for six methyls) 2.42 (1H,dt, *J*= 3.5 and 9.6 Hz, H-19), 3.19 (1H, dd, *J*=4.6 and 11.6 Hz,H-3), 4.57 (1H, brs, H-29), 4.68 (1H, brs, H-29); ^{13}C NMR(CDCl3, 75 MHz) δ values, 39.4, 27.6, 79.5, 39.6, 55.3, 18.4, 34.7, 41.2, 50.7, 37.5, 21.3, 25.5,38.4, 43.2, 27.8, 35.9, 43.4, 48.3, 48.6, 151.1, 30.2, 40.4, 28.4, 15.4,16.6, 16.9, 14.9, 18.4, 109.5, 19.6.

Spectral data of compound (2)

EI-M*Sm/z* 426 [M]$^{+}$; 411[M^{+}-CH3], 408 [M^{+}-H2O] , 393 [M^{+}-H2O- CH3],^{1}HNMR(300 MHz, CDCl3) δ values: 0.79 (6H,s,Me-28, 30) , 0.84 (3H,s, Me-29), 0.86,(3H,s Me-25), 1.0, (3H,s, Me- 23)1.02, (3H,s, Me-24) 1.05, (3H s,Me-26), 1.17, (3H s,Me-27) of eight tertiary methyls 3.24 (1H,dd, *J*=5,11 Hz geminal to secondary alcoholic group). ^{13}C- δ values; 15.8, 15.9, 16.8, 18.6, 23.2,23.6, 26.2, 26.3, 26.8, 27.2, 28.4, 28.6, 31.0, 32.5, 32.8, 33.4, 34.6, 36.7, 37.2, 38.5, 38.8, 39.6, 41.4, 46.4, 47.4, 47.6, 55.3, 79.0, 121.9, 145.4.

Animals

Male adult rats of charles foster strain (150-200 g) bred in the animal house of the institute and were kept in a room with controlled temperature at 25-26°C , humidity 60 -80% and 12/12 hourslight/darkcycle, light from 8.00-20.00 underhygenic conditions. Animals were acclimatized for one week before startingthe experiment. The animal had free access to the normal diet and water adlibitum.

Lipid lowering activityina triton induced hyperlipidemic rat model

Rats were divided into 9 groups control, triton treated, triton plus extracts, fractions and compounds and a standard drug Gemfibrozil (100mg/kg) treated groups with six animals ineach group. In the 18 h acute experiment, hyperlipidemia was developed by administration of triton WR-1339 (Sigma Chemical Company, st Louis, M.O, U.S.A) at a dose of 400mg/kg b.w. intraperitoneally to animals in all groups except the control. The extracts and the fractions of *B. cylindrica* and Gemfibrozil were macerated with 0.2% w/w aqueousgumacacia and fed orally at a dose of 200mg/kg simultaneously with triton plusdrug treated groups, and diet was withdrawn. Animals in the control and triton group without treatment with extracts and fractions were given the same amount of gumacacia suspension (vehicle). After 18 h of treatment the animals were anaesthetized with thiopentone solution (50 mg/kg) prepared in normal saline and 1.0 ml blood was withdrawn from retro orbital pluxus using glass capillaryin EDTA – coated eppendorf tubes (3.0 mg/ml blood). The blood was centrifuged at 25000 psi for 10 min at 4°C and the plasma was separated. The plasma was diluted with normal saline at a ratio of 1:3 and used for the analysis of total cholesterol (TC) phospholipids (PL) and triglycerides (TG) by standard enzymatic methods[12-14] using an auto analyzer (Beckmann Coulter Synchron- CX 5 clinical system, U.S.A.) Kits were purchased from Beckmann Coulter International, U.S.A.) and post-heparin lypolytic activity (PHLA) were assayed[15] using spectrophotometer.

Antioxidant activity (Generation of free radicals)

Superoxide anions were generated enzymatically[16] by Xanthine (160 mM), xanthine oxidase (0.04 μM) and nitroblue tetrazolium (320 μM) in the absence or presence of compounds (1,2) of compounds (100μg and 200μg/ml) in 100

mM phosphate buffer. The reaction mixtures were incubated at 37°C and after 30 minutes the reaction was stopped by adding 0.5ml glacial acetic acid. The amount of formazone was measured at 560 nm on a spectrophotometer. Percentage inhibition was calculated taking absorption coefficient of formazone as 7.2×10^3 M/cm. in another set of experiment, an effect of compounds on generation of hydroxyl radicals ($OH^{\cdot}$) was also studied by nonenzymic reactants.[17] Briefly $OH^{\cdot}$ was also studied by nonenzymic system comprised of deoxy-ribose (2.0 mM) FeSO4. 7H2O (2.8 mM) sodiumcarbonate (2.0 mM) and H_2O_2 (2.8mM) in 50 mM KH2PO4 buffer, pH 7.4 toa final volume of 2.5 ml. The above reaction mixtures in the absence or presence of compounds (100 µg/ml and 200 µg/ml) were incubated at 37°C for 90 minutes. Reference samples and reagent blanks were also run simultaneously. Malondialdehyde (MDA) content in both experimental and reference samples were estimated spectrophotometrically by thiobarbituric acid method.[18]

Cholestrol induced hyperlipidemia

Hyperlipidemia was induced similarly in animals as before. Extracts and fractions were fed orally (100 mg/kg,b.w.) simultaneously with cholesterol in the drug treated groups. Control animals received the same amount of normal saline or ground nut oil. At the end of the experiments ratswere fasted overnight and blood was withdrawn. The animals were killed and their liver were promptlyexcised.

Biochemical analysis of plasma/serum

Serum from the second set of experiments with cholesterol induced hyperlypemia were analyzed for their TC, PL, TG and protein bystandard procedure.

Biochemical analysis of tissue

Liver was homogenised (10% w/v) in cold 10 mM phosphate buffer, pH 7.2 and used for the analysis of total lipolytic activity,[15] the lipid homogenate was used for the estimation of TC, PL, TG and protein.

Faecal bile acid

The rats faeces were collected from all groups over 30 days and processed for the estimation of cholicacid and de- oxycholic acid.[19]

Statistical analysis

Data was analyzed using *t*-test. Hypolipidemic groups were compared with control and extracts/fractions treated hyperlipidemic. ($P<0.05$) was considered a significant difference.

Results and Discussion

Effect of extracts/fractions in triton induced hyperlipidemia

Administration of triton WR-1339 caused a marked increase in the levels of serum lipids of TC (3.32 fold), PL (2.74 fold), TG (2.91 fold) and protein (2.07 fold). After treatment with the extracts/fractions of *B. cylindrica*, the ethanol extract and its five fractions as well as pure compounds showed a significant reversal in TC (15-31%), PL (18-31%) and TG (14-32%) and protein (12-34%) respectively against triton [Table 1]. It is obvious from this study some fractions did inhibit cholesterol biosynthesis and potentiates the activity of lypolitic enzymes toan early clearance of lipids from the circulation in triton induced hyperlipidemia. We have successfully used this model for the evaluation of the lipid lowering activity of natural products.[20-23]

Effect of extract/fractions/pure compounds in cholesterol induced hyperlipidemia

Cholesterol feedingto rats suppressed PHLA (33%) and plasma LCAT activity (51%) as well as increased their serum levels of TC (1.62 F), PL (1.56F), TG (1.48F) and protein (1.44F); a significant lowering in *B. cylindrica* crude extract and its five fractions showed reversal of TC (16-28), PL (26- 39), TG (13-24%) and protein (12-19%). These lipid and protein level followed by reactivation of plasma enzymes was observed after treatment with crude extract, fractions and pure compounds [Table 2] analysis of hyperlipaemic serum showed a marked increased in the levels of lipids and apoproteins constituting β – lipoproteins and these effects were pronounced for VLDL–TC(4.10F), VLDL-PL(2.0 F) and VLDL –TG(2.23 F). Treatment with the crude extract, fractions and purecompounds of the *B. cylindrica* significantly reduced these levels of VLDL lipids TC, PL, TGby (5-33%, 11-36%, 21-32%) respectively as well as LDL-TC (15-29%), PL (12-28%), TG (19-34%) and apo- LDL (9- 35%) respectively. In hyperlipidemic animals, lipid and apoprotein constituents of HDL were shown to be decreased (23%) and were partially recovered after marine fractions 9-26%) treatment [Tables 2a-c]. The data in [Tables 2a-c]. Show that cholesterol induced hyperlipidemia in rats caused inhibition of hepatic lipolytic activity (-47%). Treatment with *B. cylindrica* crude extract, its five fractions and its pure compounds significantly restored the enzyme activity (8-22%). The increased levels of TC, PL and TG in liver (1.62, 1.56, 1.48 fold) respectively of cholesterol fed rats were lowered by their treatment with *B. cylindrica* crude extract, its five fractions and pure compounds. These marine fractions enhances marine fractions enhances the synthesis of LDL as well as receptor protein and inhibit the oxidative modification of LDL to accelerate the turnover of LDL- in liver.

Marine fractions may also inhibit the HMG-CoA reductase, the rate limiting enzyme in the hepatic cholesterol biosynthesis. Feeding with cholesterol results in the expansion of cellular cholesterol pools within hepatocytes.[24] The abnormalities in the synthesis and the catabolism of the body lipids are closely related to LCAT deficiency and hepatic dysfunction in hypoglycemia.

Table 1: Lipid lowering activity of crude extract/fractions/pure compounds in triton treated hyperlipemic rats

Experimental Schedule	Total Cholesterol[a]	Phospholipid[a]	Triglyceride[a]	Protein[b]
Contol	90.37±6.84	80.44±7.00	85.78±6.66	5.84±0.04
Triton treated	300.88±23.11***(+3.32F)	220.84±14.88*** (+2.74F)	250.37±20.11*** (2.91F)	12.12±1.00***(2.07F)
Triton + ethanol extract	230.14±13.28*** (–23%)	170.82±13.66** (–22%)	200.19±17.33** (–20%)	8.78±0.08*** (–27%)
Triton+hexane fraction	250.33±22.17** (–17%)	176.48±17.53** (–20%)	215.58±19.19* (–14%)	10.00±0.19** (–17%)
Triton +chloroform fraction	220.78±16.16*** (–27%)	165.37±13.80*** (–25%)	184.14±13.98*** (–26%)	8.62±0.08*** (–28%)
Triton+n–butanol sol. fraction	255.37±20.18*(–15%)	179.89±13.64**(–18%)	210.11±16.87*(–16%)	10.62±1.00*(–12%)
Triton+n–butanol insol. fraction	222.18±16.92*** (–26%)	172.44±12.14** (–21%)	194.87±14.84** (–22%)	10.22±0.08* (–16%)
Triton + water sol. fraction	251.33±20.12* (–16%)	180.41±13.30** (–18%)	211.81±14.48* (–15%)	10.57±0.64* (–13%)
Lupeol (1)	225.32±1.84*** (–25%)	172.37±1.37** (–22%)	184.84±1.62*** (–26%)	9.88±0.52** (–18%)
Triton+Gemfibrozil	220.12±1.60*** 9–260	169.33±2.00***	188.77±1.89*** (–24%)	9.66±0.37** (–20%)
(std Drug)	206.11±13.79***(–31%)	153.18±12.11*** (–31%)	170.66±12.44***(–32%)	8.00±0.07*** (–34%)

Unit: a. mg/dl; b. g/dlSerum. Valuesaremean ±SD from 6 animals ***P<0.005, **P,0.01, *P,0.05 group compared with control, triton and drug treated with cholesterol

Table 2a: Effect of crude extract/fractions/pure compounds on blood serum lipids profile in hyperlipidemic rats

	TC (mg/ml)	PL (mg/ml)	TG (mg/ml)	Total Protein
Control	87.51±6.20	82.73±7.00	85.17±6.37	6.80±0.31
Cholesterolt reated	304.12±20.70*** (+3.47F)	178.56±10.47*** (+2.15F)	224.45±11.69*** (+2.63F)	11.27±0.51*** (+40)
Cholesterol and EtOHext.	220.06±9.44*** (–27)	130.94±11.33*** (–26)	169.40±10.93*** (–24)	8.11±0.12*** (–28)
Cholesterol and hexane fraction	239.46±17.97** (–21)	145.23±5.84** (–19)	171.95±11.95*** (–23)	8.37±0.14*** (–25)
Cholesterol and chlorofor fraction	254.0±19.65** (–17)	151.36±8.64* (–15)	177.27±14.29** (–21)	8.50±0.17*** (–24)
Cholesterol and n–butanolsol.fraction	225.65±20.34*** (–25)	136.42±10.47*** (–24)	172.98±12.54*** (–23)	8.62±0.13*** (–23)
Cholesterol and n–butanolinsol. Fr.	239.40±16.88** (–21)	148.98±8.15** (–17)	175.05±10.13** (–22)	8.74±0.18** (–22)
Cholesteroland watersol.fraction	238.08±15.30**(–22)	134.63±10.18*** (–24)	178.03±10.39** (–20)	9.92±0.13** (–20)
Cholesterol and compound–1	220.37±1.82*** (–27)	138.44±1.20** (–22)	175.39±1.61** (–21)	8.44±0.14*** (–25)
Cholesterol and compound–2	218.47±1.66*** (–28)	136.89±12.44*** (–23)	172.27±13.94*** (–23)	8.32±0.13*** (–26)
HF+Gemfibrozil	195.37±13.88*** (–35)	120.22±9.87*** (–32)	150.70±13.12***(–33)	7.88±0.17*** (–30)

LCAT, a key enzyme involved in body lipid metabolism,solely synthesized in liver and therefore hepatoprotective action of marine fractions may contribute to improve liver function, reactivation of LCAT and thus the regulation of lipid in experimental animals. Enhancement of faecal excretion of holicacid (7-25%) and deoxycholic acidby (13- 36%) by treatment with *B. cylindrica* crude extract and its fractions [Table 3] indicate that this drug interfered with the absorption of dietary cholesterol in the small intestine.

Effect of fractions on oxygen free radical generation *in vitro*

The Scavenging potential of *B. cylindrica* crude extract and its fractions and pure compounds (100 and 200 µg/ml) against formation of O^{-2} and $OH^{\cdot}$ in non- enzymic systems was studied [Table 4]. The significant decrease in superoxide anions by (18-77%) inhibition and hydroxylradical by (29-43%) inhibition and in microsomallipid peroxidation showed more antioxidant activity (20-39%) inhibition and in above test system the involvement of hydroxyl free radicals ($OH^{\bullet}$) hasbeen found to be a major causative factors for peroxidative damage to lipoproteins.[4] To overcome these ailments, adrughaving multifold properties such as lipid lowering, antidiabetic and antioxidant activities together is in great demand.

Conclusion

The pure compounds isolated from the active fraction were found showing lipid lowering activity similar to the fraction.

Table 2b: Effect of crude extract/fractions/pure compounds on lipoprote in metabolism in hyperlipidemic rats

	VLDL				HDL				LDL			
mg/dl plasma	TC	PL	TG	Apo–B	TC	PL	TG	Apo–B	TC	PL	TG	Apo–B
Control	8.38 ± 0.32	14.93 ± 0.28	39.69 ± 2.84	6.33 ± 0.47	13.52 ± 0.80	12.27 ± 0.36	15.40 ± 0.21	17.62 ± 1.01	46.00 ± 3.14	38.12 ± 2.41	15.27 ± 1.07	165.32 ± 1.12
Cholesterol treated	34.37 ± 3.00*** (+4.10F)	29.87 ± 2.14*** (2.00F)	88.66 ± 6.37*** (+2.23F)	13.72 ± 1.08*** (+2.16F)	65.39 ± 5.81*** (+4.83F)	45.52 ± 3.31*** (+3.70F)	36.82 ± 2.50*** (+2.39F)	27.74 ± 2.12*** (+1.57F)	35.43 ± 2.49*** (– 23)	26.63 ± 2.12*** (– 30)	12.48 ± 0.87** (– 18)	123.33 ± 11.11*** (– 25)
Cholesterol and EtOHext.	28.82 ± 2.00* (–16)	22.73 ± 1.32** (–23)	68.44 ± 5.27** (–22)	10.77 ± 1.17** (–21)	50.33 ± 3.82*** (–23)	38.42 ± 2.44** (–15)	28.11 ± 1.87*** (–23)	21.77 ± 1.62** (–21)	40.30 ± 2.89* (+12)	14.42 ± 1.20* (+13)	28.88 ± 1.76NS (+8)	143.33 ± 10.21*** (+14)
Cholesterol and hexane fraction	30.32 ± 2.41NS (–5)	25.44 ± 1.83* (–11)	66.37 ± 6.00*** (–25)	11.11 ± 1.00** (–19)	52.87 ± 4.32** (–19)	40.11 ± 3.30* (–12)	29.66 ± 2.31* (–19)	22.84 ± 2.00** (–18)	38.84 ± 3.12* (+9)	30.33 ± 2.14* (+12)	15.38 ± 1.12** (+19)	144.44 ± 10.77* (+15)
Cholesterol and chloro for fraction	29.37 ± 1.88*** (–14)	24.77 ± 2.31** (–17)	64.48 ± 6.00*** (–27)	11.00 ± 0.79** (–20)	54.73 ± 4.81** (–16)	38.72 ± 3.11** (–15)	28.66 ± 2.31*** (–22)	20.88 ± 1.69*** (–24)	37.03 ± 2.92NS (+5)	28.86 ± 2.34NS (+8)	15.00 ± 1.10* (+17)	142.00 ± 10.61* (+13)
Cholesterol and n–Butanolsol. fraction	24.14± 1.67*** (–29)	20.33 ± 1.80*** (–31)	60.66± 5.32*** (–32)	9.82± 0.62*** (–28)	46.38± 3.88*** (–29)	32.81± 2.64*** (–28)	24.12± 2.40*** (–34)	19.00± 1.21*** (–31)	42.77± 3.92* (+17)	30.92± 2.74* (+14)	16.00± 1.12 (+22)	147.77± 13.2* (+16)
Cholesterol and watersol.fraction	23.00 ± 1.62*** (–23)	66.66 ± 5.39*** (–24)	10.62 ± 1.01** (–22)	55.50 ± 4.67* (–15)	40.38 ± 3.60* (–11)	29.93 ± 2.74** (–19)	22.44 ± 2.00** (–19)	41.87 ± 3.62* (+15)	29.93 ± 2.34* (+11)	16.63 ± 1.37*** (+24)	128.73 ± 1.18NS (+4)	129.3 ± 13.1NS (+5)
Cholesterol and compound–1	31.12 ± 3.2* (–12)	62.48 ± 6.00*** (–29)	9.90 ± 0.62*** (–27)	49.33 ± 3.69*** (–24)	34.22 ± 3.00*** (–25)	27.71 ± 2.14*** (–24)	20.22 ± 1.64*** (–27)	44.88 ± 4.11** (+21)	32.88 ± 2.40** (+19)	18.17 ± 1.30*** (+31)	149.23 ± 12.81** (+17)	148.9 ± 12.6* (+16)
Cholesterol and compound–2	25.73 ± 164*** (–25)	23.00 ± 1.81** (–22)	63.77 ± 5.20*** (–28)	9.70 ± 0.77*** (–29)	50.12 ± 4.00*** (–23)	35.01 ± 3.12*** (–23)	28.33 ± 1.79*** (–23)	21.00 ± 1.37*** (–24)	44.14 ± 3.92** (+20)	31.68 ± 2.42* (+16)	17.88 ± 1.32*** (+30)	150.00 ± 14.00** (+18)
HF+gemfibrozil	22.99 ± 1.62*** (–33)	18.88 ± 1.20*** (–36)	60.10 ± 8.31*** (–32)	9.00 ± 0.32*** (–34)	46.63 ± 4.00*** (–29)	35.00 ± 2.81*** (–23)	24.17 ± 1.84*** (–34)	17.92 ± 1.10*** (–35)	48.39 ± 3.72*** (+26)	32.67 ± 2.14** (+18)	16.00 ± 1.00** (+22)	146.73 ± 12.10* (+16)

Table 2c: Effect of crude extract/fractions/pure compounds on PHLA and LCAT activity in hyperlipidemic rats

	Control	Cholesterol treated	Cholesterol and EtOHext.	Cholesterol and hexane fraction	Cholesterol and chloro for fraction	Cholesterol and n-butanol sol.fraction	Cholesterol and n-butanolinsol.Fr.	Cholesterol and watersol. fraction	Cholesterol and compound-1	Cholesterol and compound-2	HF+Gemfibrozil
PHLA (n mole free fatty acid released/h/L plasma)	17.82 ± 1.03	33.88 ± 2.53*** (–51)	50.44 ± 4.10*** (+33)	52.22 ± 4.72*** (+35)	50.48 ± 3.89*** (+32)	52.48 ± 4.70*** (+35)	42.84 ± 3.31** (+20)	40.81 ± 3.24* (+17)	54.44 ± 3.29*** (+33)	55.11 ± 3.92*** (+38)	50.11 ± 3.14*** (+32)
LCAT (n mole cholesterol released/h/L plasma)	69.33 ± 5.14	11.84 ± 0.63*** (–33)	14.37 ± 0.58* (+18)	15.33 ± 1.12*** (+23)	16.22 ± 1.32*** (+27)	16.40 ± 1.12*** (+28)	13.14 ± 1.10* (+10)	14.00 ± 1.02* (+15)	18.42 ± 1.30*** (+35)	19.12 ± 1.20*** (+38)	14.13 ± 1.00* (+16)

Each parameter represents pooled data from 6 rats/group and values are expressed as mean ± S.D. *P< 0.05; **P< 0.01; ***P< 0.001, Cholesterol treated group compared with control group and Cholesterol plus compoundsgroups compared with Cholesterol treatef group.

Table 3: Effect of crude extract/fractions/pure compounds on hepaticlipid, lipoprotein lipase and faecal bile acid activity in hyperlipemic rats

Parameters	Control	Cholesterol treated	Cholesterol + EtOHext	Cholesterol + hexane fraction	Cholesterol + chloroform fraction	Cholesterol + n-butanolsol. fraction	Cholesterol + n-butanolin sol. fraction	Cholesterol + water sol. fraction	Cholesterol + compound-1	Cholesterol + compound-2	Cholesterol +gemfibrozil
Liver[a]											
PLactivity	132.28 ± 10.21	70.14 ± 5.32*** (–4.7)	76.63 ± 4.81NS (+8)	78.89 ± 6.00* (+11)	86.16 ± 5.30** (+18)	77.74 ± 5.91* (+10)	76.33 ± 4.88NS (+8)	79.37 ± 6.12* (+12)	82.44 ± 6.89 (+15)	80.37 ± 6.74 (+13)	89.92 ± 6.84** (+22)
Total[b] cholesterol	6.82 ± 0.12	11.11 ± 0.32*** (+1.62F)	8.92 ± 0.14** (–19)	9.30 ± 0.24* (–16)	8.10 ± 0.16*** (–27)	9.00 ± 0.32** (–16)	9.11 ± 0.31** (–18)	9.30 ± 0.18** (–16)	9.00 ± 0.77 (–19)	9.10 ± 0.62 (–18)	7.90 ± 0.11*** (–28)
Phospholipid[1]	24.44 ± 2.00	38.98 ± 2.73*** (+1.56F)	24.99 ± 1.73*** (–35)	28.73 ± 1.10*** (–26)	23.77 ± 1.31*** (–39)	28.00 ± 2.00*** (–28)	26.01 ± 1.63*** (–33)	27.17 ± 1.62*** (–30)	25.28 ± 2.00 (–35)	25.00 ± 1.68 (–36)	23.61 ± 1.37*** (–39)
Triglyceride[b]											
Protein	10.87 ± 0.16	16.10 ± 1.27*** (+1.48F)	13.66 ± 0.74* (–15)	13.81 ± 1.00* (–14)	12.38 ± 0.19*** (–23)	14.00 ± 0.71* (–13)	13.77 ± 0.82* (–14)	13.83 ± 0.82* (–14)	12,20 ± 1.11 (–24)	13.11 ± 0.77(–18)	12.10 ± 1.60*** (–24)
Faecal bile cids	152.40 ± 13.17	220.14*** ± 18.61 (+1.44F)	190.77* ± 13.62 (–13)	192.66* ± 14.80 (–12)	180.11** ± 12.70 (–18)	190.77* ± 16.12 (–13)	184.88** ± 15.57 (–16)	186.66* ± 14.44 (–15)	170.33 ± 14.17 (–23)	173.22 ± 13.44(–21)	178.80** ± 12.12 (–19)
Cholic	83.72 ± 6.81	50.23 ± 3.84*** (–40)	62.39 ± 5.33** (+19)	58.77 ± 4.89* (+14)	60.11 ± 5.00* (+16)	54.40 ± 3.70NS (+8)	53.88 ± 3.77NS (+7)	58.90 ± 4.31* (+15)	62.33 ± 5.39 (+19)	64.00 ± 4.31 (+21)	67.39 ± 5.14*** (+25)
Deoxycholic acid	55.55 ± 4.31	26.69 ± 2.37*** (–52)	40.40 ± 2.66*** (+33)	38.31 ± 3.00*** (+30)	42.27 ± 3.31*** (+36)	30.38 ± 1.82* (+12)	37.50 ± 2.76*** (+29)	30.59 ± 2.61* (+13)	33.77 ± 2.84 (+21)	34.17 ± 2.81 (+22)	42.29 ± 3.00*** (+36)

Table 4: Effect of crude extract/fractions/pure compounds on superoxideion, hydroxyl radical an lipid peroxidation in microsomes

Parameters	Conc of compounds (μg/ml)	[a]Superoxide anions (O2–)	Hydroxylions[b] (OH)	Microsomal lipidper oxidation[b]
Ethanol extract	100	Control 189.32±15.66	Control 82.12±6.72	Control 90.44±8.00
	200	Exp145.33±12.37** (–18)	Exp60.22±4.14*** (–26)	Exp70.88±5.32** (–21)
		Exp120.41±10.11*** (–36)	Exp50.37±3.82*** (–38)	Exp64.12±5.11*** (–29)
Hexane fraction	100	Control 180.37±13.82	Control 85.62±5.89	Control 90.481±7.82
	200	Exp133.88±111.11*** (–25)	Exp70.38±6.11** (–181)	Exp80.22±6.39* (–11)
		Exp110.30±9.37*** (–30)	Exp60.43±4.83*** (–29)	Exp72.44±5.37** (–20)
Chloroform fraction	100	Control 190.84±17.00	Control 80.37±6.12	Control 85.78±6.99
	200	Exp140.30±10.12*** (–26)	Exp62.14±4.63*** (–23)	Exp70.12±6.11** (–18)
		Exp100.22±7.94*** (–47)	Exp48.17±3.77*** (–40)	Exp60.37±4.88*** (–30)
n–Butanol soluble fraction	100	Control 182.77±14.40	Control 92.85±7.30	Control 90.33±7.40
	200	Exp150.63±12.10** (–18)	Exp70.08±5.92*** (–24)	Exp78.30±6.14* (–13)
		Exp135.55±11.10*** (–26)	Exp60.33±4.79*** (–35)	Exp65.38±5.37*** (–27)
n–Butanol insoluble fraction	100	Control 180.91±16.10	Control 80.77±5.39	Control 85.07±7.11
	200	Exp135.82±9.88*** (–24)	Exp68.11±6.00* (–15)	Exp70.66±5.32* (–16)
		Exp100.73±8.14*** (–44)	Exp53.89±4.72*** (–33)	Exp61.88±5.22*** (–27)
Water soluble fraction	100	Control 193.87±17.39	Control 86.30±7.11	Control 91.44±6.77
	200	Exp155.67±12.81** (–20)	Exp75.18±6.38* (–12)	Exp77.77±5.70* (–15)
		Exp130.88±11.00*** (–32)	Exp60.77±5.31*** (–29)	Exp71.80±6.11** (–21)
β–Amyrin	100	Control 182.37±13.77	Control 93.77±6.84	Control 92.77±8.00
	200	Exp 155.22±12.14 (–15)	Exp 72.12±5.69	Exp 71.11±5.22
		Exp 125.11±10.22	Exp 58.77±3.84	Exp 53.37±4.00
Lupeol	100	Control 192.33±17.23	Control 87.17±8.00	Control 85.57±6.33
	200	Exp160.60±13.11** (–20)	Exp 65.33±4.81	Exp 65.22±4.70
		Exp 140.22±12.22	Exp 45.44±2.77	Exp 48.33±4.00
Standard drug		Control 26.44±1.84	Control 30.66±2.11	Control 3.88±0.05
		Exp5.88±0.09*** (–77)	Exp17.35±1.18*** (–43)	Exp2.37±0.80*** (–39)
		(Alloperinol)	(Manitol)	(α tocopherol)
		(20 μg/ml)	(100 μg/ml)	(100 μg/ml)

Further work is required to synthesize the analogs of pure compounds to enhance the activity.

Acknowledgments

We are thankful to the Director CSIR-CDRI, Lucknow for providing necessary research facilities as well as his keen interest in marine natural products. We are grateful to the Ministry of Earth Sciences, Government of India, New Delhi for providing financial assistance. Weare also thankful to HRDG, Council of Scientific and Industrial Research,Government of India, New Delhi for providing to VL emeritus scientistship which helped in compiling the work. We acknowledge to Dr. M. N. Srivastava for the collection of marine material to carry out the present studies.

References

1. Brown MS, Goldstein JL. Lipoprotein metabolism in the macrophage: Implications for cholesterol deposition in atherosclerosis. Ann Rev Biochem 1983;52:223-61.
2. Inoue T, Hayashi M, Takayanagi K, Morooka S. Lipid-lowering therapy with fluvastatin inhibits oxidative modification of low density lipoprotein and improves vascular endothelialfunction in hypercholesterolemic patients. Atherosclerosis 2002;160:369-76.
3. Wierzbicki AS, Mikhailidis DP. HDL-Cholesterol and the treatment of coronary heart disease contrasting effects of atorvastatin and simvastatin. Curr Med Res Opin 2000;16:139-46.
4. Parthsarthy S, Steinbert D, Witztum JL. The role of oxidized low-density lipoprotein in the pathogenesis of atherosclerosis. AnnuRevMed 1992;43:219-25.
5. Shoeb M. Anticancer agents from medicinal plants. Bangladesh J Pharmacol 2006;1:35-41.
6. Shetgiri PP, MelloPM. Antioxidant activity of flavonoids. A comparative study. Indian Drugs 2003;40:567-9.
7. Gopinathan N, Srinivasan KK, Mathew JE. Free radicals scavenging properties of ethanol extract of *Saccharum spontaneum*. Indian Drugs 2004;41:633-5.
8. Rajni GP, Ashok *P. In vitro* antioxidant and antihyperlipemic activities of Bauhiniavariegate Linn. Indian J Pharmacol 2009;41:227-32.
9. Kaur G, Alam MS, Jabbar Z, Javed K, Athar M. Evaluation of antioxidant activityof Cassia siamea flowers. J Ethnopharmacol 2006;108;340-8.
10. Aynilian GH, Farnsworth NR, Persinos GJ. Isolation of lupeol from *Crataeva Benthamii*.Phytochemistry1972;11:2885-6.
11. Kushiro T, Shibuya M, Ebizuka Y. β-Amyrin synthase; Cloning of oxidosqualene Cyclase that catalyzes the formation of the most popular triterpene among higherplants.Eur J Biochem 1998;256:238-44.
12. Deeg R, Ziegenhorn J. Kinetic enzymatic method for automated determination oftotal cholesterol in serum. Clin Chem 1983;29:1798-802.
13. Buccolo G, David H. Quantitative determination of serum triglyceride bythe use of enzymes.Clin Chem 1973;19:476-82.
14. Zilversmit DB, Davis AK. Micro determination ofplama phospholipids bytrichloro acetic acid precipitation. J Lab Clin Med1950;35:155-60.

15. Wing DR, Robinson DS. Cleaning factor lipase in adipose tissue. Biochem J 1968;109:841-9.
16. Halliwell B, Gutteridge JM, Arouma OI. The deoxyribose method a simple test-tube assayfor determination of rate constants for reactions of hydroxyl radicals. Anal Biochem 1987;165:215-9.
17. Schmedes A, Hølmer G. A new thiobarbituric acid(TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation.J Am Oil Chem Soc 1989;66:813-7.
18. Mosbach EH, KieniskyHJ, Hal P, Kendall FE. Determination of deoxycholic acid and cholicacid in bile. Arch BiochemBiophys 1954;51:402-10.
19. Khanna AK, Chander R, Singh C, Srivastava AK, Kapoor NK. Hypo- lipidemic activity of *Achyranthusaspera* Linn in normal and triton induced hyperlipidemic rats. Indian J Exp Biol 1992;30:128-30.
20. Ghosh S, Misra AK, Bhatia G, Khan MM, Khanna AK. Syntheses and evaluation of glucosyl aryl thiosemicarbazide and glucosyl thiosemicarbazone derivatives as antioxidant and anti-dyslipidemic agents. Bioorg Med Chem Lett 2009;19:386-9..
21. Kumar V, Singh P, Chander R, Mahdi F, Singh S, Singh R, *et al*, Hypolipidemic activity of *Hibiscusrosasinensisrootin* rats. Indian J Biochem Biophys 2009;46:507-10.
22. Sashidhara KV, Kumar A, Kumar M, Sonkar R, Bhatia G, Khanna AK. Novelcoumarin derivatives as potential antidyslipidemicagents. Bioorg Med ChemLett 2010;20:4248-51.
23. Mahley RW, Weisgraber KH, InnerarityTL, Rall SC Jr. Genetic defects in lipoprotein metabolism. JAMA 1991;265:78-93.
24. Vadivelo M, Ramakrishnan S. HDL Total cholesterol and HDL-Cholesterol ratio in liver diseases. Indian J Med Res 1986;83:46-52.

How to cite this article: Lakshmi V, Sonkar R, Khanna AK. Antihyperlipidemic and antioxidant activities of *Bruguiera cylindrinca* (L). Chron Young Sci 2012;3:236-43.

Source of Support: Ministry of Earth Sciences, Government of India, New Delhi,
Conflict of Interest: None declared