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Limonoids as antidyslipidemic from the fruits of *Xylocarpus molluccensis*

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ABSTRACT

The ocean offers a rich source of structurally unique molecules providing novel plate form for drug discovery. The 95% ethanol extract of the epicarp of the fruits of *X. molluccensis* given orally at a dose of 500mg/kg showed significant antidyslipidemic activity in hamster model. Among the four fractions tested the chloroform soluble fraction showed highly significant lipid lowering activity at 50 mg/kg. Further purification of chloroform fraction yielded four compounds. Out of four compounds bioevaluated, only one compound showed promising activity even at 25mg/kg. dose.

KEYWORDS

Xylocarpus molluccensis;
Antidyslipidemic;
Hamster model;
Limonoids.

INTRODUCTION

Dyslipidemia is elevation of plasma cholesterol, triglycerides (TGs), or both, or a low high density lipoprotein level that contributes to the development of atherosclerosis. Causes may be primary (genetic) or secondary. Diagnosis is by measuring plasma levels of total cholesterol, TGs, and individual lipoproteins. Treatment is dietary changes, exercise, and lipid-lowering drugs.

Xylocarpus molluccensis (Lamk) M.Roem. synonymous *to Carapa molluccensis* (Lamk) belongs to Natural Order Meliaceae. It is a mangrove and is commonly known as pussur and Pitakura in Hindi language. It is a tall tree ranging up to 10-12 m. tall and trunk of 60 cm diameter at the base, slightly buttressed stem. Bark is red with thick flacks. Wood red in color, leaflets7-12x3-6 cm. ovate, acute at apex, oblique at the base, flowers 2-3cm. across, white with red glands inside, staminal teeth obscure, anthers exceeding the teeth, stigma cup shaped, fruits 10-15 cm. across globose. This species of X*ylocarpus* is uncommon and grows in association with *Heritiera litteralis*. Flowering and fruiting from June to September. It is mainly reported to be found in Mahanadi deltaic region and in Andamans[1].

The bark pressings of *X. molluccensis* are used in cholera and fever in traditional system of medicine[2]. The fruits of *X. molluccensis* are also used as aphrodisiac[2]. The kernels are used in tonics and in relieving colic pains. The seeds or peels of the fruits are utilized to poultice swellings and ash of the seeds is applied to itch. The fruits are used as a cure for swellings of the breast and in elephantiasis[3]. The bark pressings are used to treat fevers including those caused by malaria. All parts are used as astringent[4], but the bark and root more so. The bark is also used in dysentery, diarrhoea, and other abdominal troubles and febrifuge[4,5]. Seed ash is mixed with sulphur and coconut oil and applied as ointment for itch[6]. The root is used to treat cholera,

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from Burma to Phillipines. The seed paste is also used for the relief of breast cancer[5,6].

Literature review revealed that some research work has been carried out on this plant to isolate the chemical constituents. Few limonoids such as Xyloccensins-A, B, C, D, E and F were isolated and characterized from the seed and timber of the plant[7] Xylomollin was isolated and characterized from the unripe fruits[8] Xyloccensins G, H, and I were isolated by Taylor group from this plant and structures were also established[9]. Few hydrocarbons, fatty acids and fatty alcohols were identified in the waxes of the plant[10]. Xyloccensins I and J were further isolated by Khisal etal[11]. Conolly and his group isolated Mexicanolide from this plant[12]. A new unsaturated aryl ketoacid and its methyl ester was isolated from this species by Bercich et al.[13]. Few more limonoids i.e. detigloyl-6-deoxyswietenine acetate, phragamalin 3,30-diacetate and phragmalin 2,3,30-triacetate were also reported from this species by African group [Mulholland].

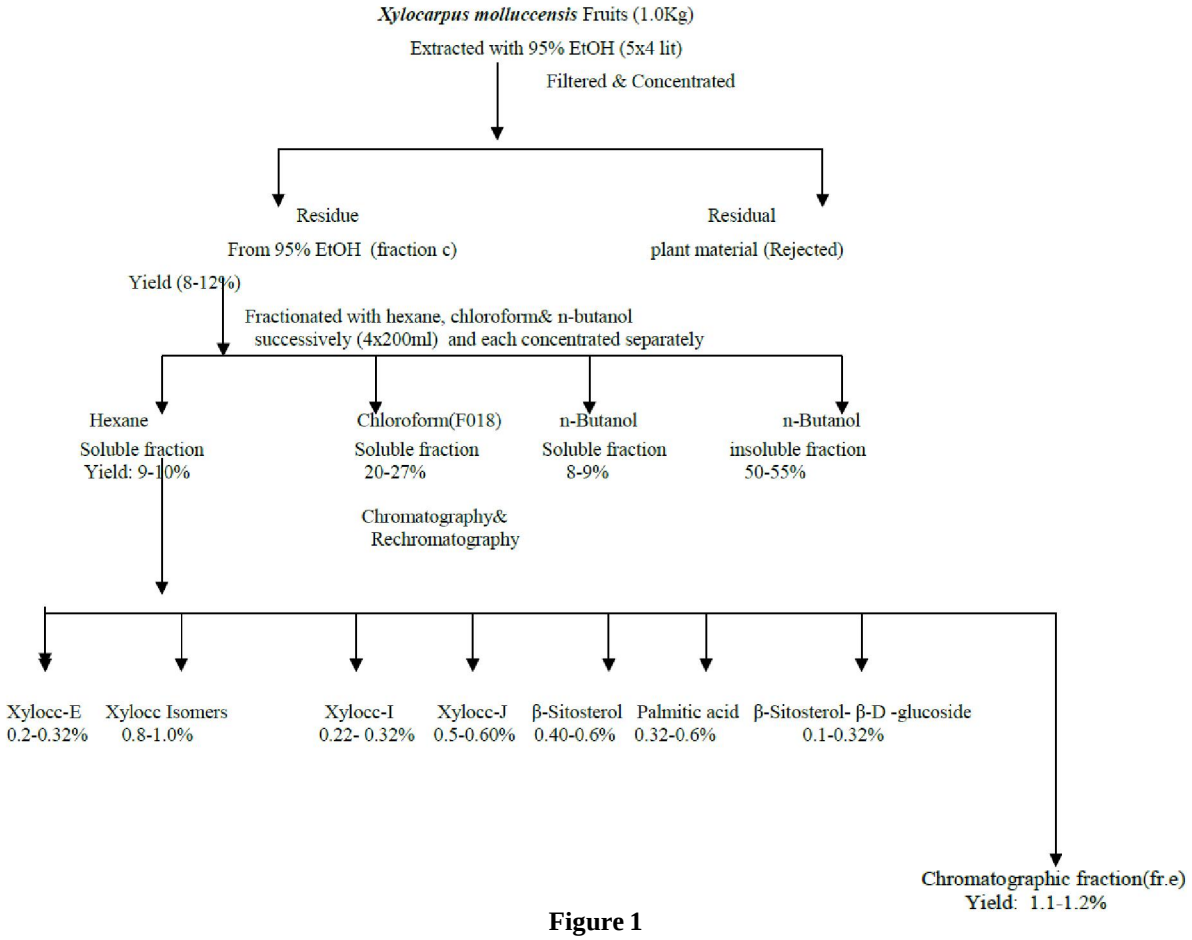

Figure 1

MATERIALS AND METHODS

Plant material

Fruits of the *Xylocarpus- molluccensis* mangrove were collected and identified by Dr. M.N.Srivastava of the Botany Division, Central Drug Research Institute from South Andaman Coast in the month of March. Specimen sample (voucher specimen number 424) has been identified and preserved in the herbarium of the Botany Division of the Institute, Lucknow, India. Fruits were shade dried and. epicarp from the fruits were separated.

Extraction fractionation and isolation of compounds

Air dried powdered epicarp (1.0Kg.) were placed in glass percolator filled with 95% ethanol 5.0 lit. and allowed to stand overnight at room temperature, the percolate was collected and the process of extraction was repeated four times. The combined extracts were filtered, concentrated under reduced pressure below 50^0C to minimum volume of 1.0 lit. It was further dried

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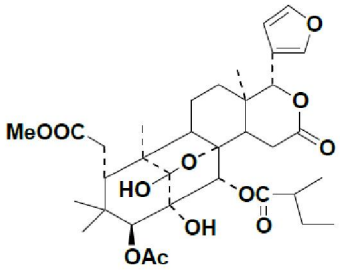
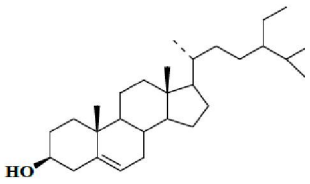
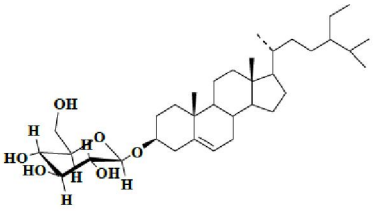
in hot air vacuum oven at 50^{0}C to brown powder (100 g) The brown powder was suspended in water (70ml) and fractionated in to chloroform soluble fraction by extraction with chloroform (5x250ml) in a separating funnel. The combined extract was concentrated under reduced pressure below 50^0 C to get brown viscous mass, which was finally dried under high vacuum for 2 hrs. to remove the last traces of solvent (chloroform soluble 25.0g). The chloroform fraction (20 g) was subjected to silica gel column chromatography (60-120 mesh) and the column (1.2 meter length and 4cm in diameter made of glass was used) was eluted with hexane-ethyl acetate (99:01-0:100) affording 48 fractions (each fractions of 200 ml volume). Fractions showing identical TLC pattern were mixed together and grouped in to 6 major groups of fractions. Initial two groups were of very non polar compounds. Rechromatography of fraction 3(grouped) over silica gel column using hexane- ethyl acetate (99:01-98:02) as eluent and 10 fractions were collectedeach of 50ml volume. Fractions showing identical TLC pattern on silica gel plates were grouped together and finally by HPLC reverse phase on C_{18} Silica columns using acetonitrile-water 6:4, v/v at 230nm using uv-detector yielded 2 pure compounds Namely xyloccensin-E[12] xyloccensin-I[14], xyloccensin-J[14], xyloccensin-X&Y[15] β-sitosterol[16], Palmitic acid[17] and β-sitosterol- β –D glucoside[18]. All these were characterized using ir, nmr, mass, derivetization and comparing the data with those given in literature for these compounds. These were also compared with authentic samples on thin layer plates as well as their spectral data.



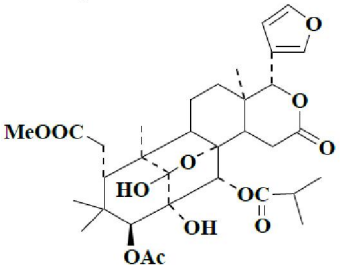
Xyloccensin-I



β-Sitosterol



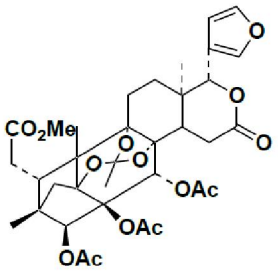
β- Sitosterol-O-β-D-glucoside



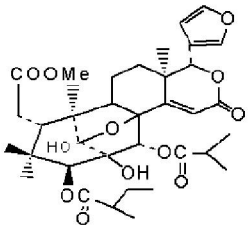
Xyloccensin-J

$CH_3(CH_2)_{14}COOH$

Palmitic acid

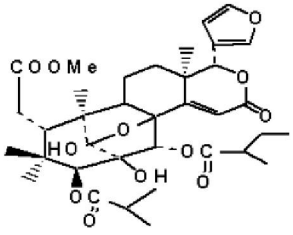


Xyloccensin E



xyloccensin –X

+



Xyloccensin-Y

Figure 2 : Structures of the pure compounds isolated and characterized.

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Test models used for the evaluation of the antidyslipidemic activity

Golden Syrian hamsters (Mesocricetus auratus), male, wt. 120±10 g were kept in a room-with temperature control (25-26^{0}C) relative humidity 60-80 % and 12:12 hours light/dark cycle light (on from 8.00 AM to 8 PM). the hygenic conditions of the room were also maintained. The animals with identification marks were acclimatized for one week before starting the experiment. The animal had free access to the normal diet and water ad libitum. Experimental protocols were approved by our Institutional Ethical Committee, which follows guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)and complies with international norms of INSA.

High fat diet

High fat diet- (HFD) was purchased from Research Diet Inc, New Brunswick USA (Product code No. D-99122211) containing fructose (9%w/w), deoxycholic acid (0.45%w/w), cholesterol (0.45%W/W) and coconut oil (26%W/W) mixed with normal chew diet.

Antidyslipidemic activity in high fat diet fed hamster model

Hamsters were fed with high fat diet- (HFD) and they were found to develop dyslipidemia. All hamsters were divided into two groups –HFD fed, HFD fed plus test samples treated group containing eight animals in each group. HFD (9.0g/animal) was given to animals of both the groups daily from day 1 to day 10. Animals had free access to the diet and the daily diet consumed by animals was calculated by subtracting the left over diet on next day from the added diet on previous day. The test samples were given orally at different doses from day 4 to 10 once a day to the drug treated groups only. The animals of HFD fed group, same amount of vehicle was given. The body weight of animals was recorded daily.

Collection of blood samples and biochemical analysis from plasma

After the day-10, the animals were kept for a overnight fasting. Blood was collected in EDTA coated tubes from the retro orbital plexus of the hamsters. The blood samples were centrifuged at 4000 rpm for five minutes and plasma was separated. The plasma of the samples were used for the assay of total cholesterol (TC)[19] triglycerides (TG)[20] HDL – cholesterol (HDL-C)[21] glucose (glu))[22] glycerol (gly)[23] and free fatty acids (FFA) (Wako Pure Chemicals Limited) were by standard enzymatic methods using auto analyzer. All assay kits except of FFA were purchased from Beckman Coulter International, USA and assay kit for FFA was purchased from Wako pure Chemical Industries Ltd, Osaka Japan[24].

At the end of the experiment i.e. on day 10th, the blood of non fasted animals were withdrawn in two sets of tubes in which one set contains 120 µl NaF (4.5mg./ml) and after 15 min. in cold, plasma was separated. Biochemical analysis of plasma with out NaF was performed on the same day for Triglycerides (TG) total Cholesterol (TC) HDL-Cholesterol using enzymatic diagnostic kits. Similarly the plasma containing NaF Glucose was assayed for glucose, glycerol and free Fatty acids (FFA) on Synchron CX-5. Clinical System Beckmann Coulter Instrument. The data was analyzed for its significance on Prism Software (TABLE 1& 2).

TABLE 1 : Antidyslipidemic activity of the EtOH ext, its chloroform fraction and pure compound in dislipidemic hamster model.

Test samples	Dose (mg/kg)	TG	CHOL	HDL	GLY	FFA	H/C
EtOH extract	500	71**	31*	+20**	59***	39**	+57
	500	79***	17	+50	68***	18	+86
	500	58***	32**	-20	26	17	+18
	500	60***	8	+18	31***	30	+29
	500	71***	2	+53***	10	12	+54
Chloroform fraction of the ethanol extract	250	-89***	-33***	+64	-69***	-33***	+152
	250	-52*	-22	+70	-40***	-30*	+122
	100	-55*	NC	+50	-27	-28*	+54
n-Butanol soluble fraction of the EtOH extract	250	-49**	-12	+29**	-48***	+9	+52
	250	-39*	-20	-9	-20	-20	+28
n-Butanol insoluble fraction of the EtOH extract	250	-50**	+21*	+43***	-37***	+36***	+19
	250	-30	-5	-6	-12	-22	NC
Fenofibrate (Standard drug)	108.24 (300µ mole)	-42*	-18*	NC	-36**	-20*	+10

RESULTS AND DISCUSSION

The ethanol extract of the fruits of *X. molluccensis* showed promising antidyslipidemic activity (TABLE 1)

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at 500mg/Kg dose in high fat diet hamsters. On further fractionation into four fractions the activity was localized to the chloroform fraction only. On chromatography of the chloroform fraction, the pure compounds isolated and tested at lower doses (TABLE 2) did not show activity except new xyloccensins X and Y mixture, it showed lowering of TG and FFA at different doses (TABLE 2). Further synthetic modification will done on these xyloccensins mixture to enhance the activity.

TABLE 2 : Antidyslipidemic activity of the column fraction and pure compounds of the chloroform fraction of the ethanol extract in dyslipidemic hamster model.

Test samples	Dose (mg/kg)	TG	CHOL	HDL	GLY	FFA	H/C
Chromatographic fraction of the chloroform fraction	10	-13	+8	-5	-3	-26	-12
	100	-75**	-37	+18	-47***	-60***	+83
Xyloccensin-E	10	-22	-18	-25	NC	-43*	-10
Novel xyloccensins Mixture (X & Y)	10	-43	-4	NC	-3	-28*	+3
	25	*-30**	*NC*	*+6*	-16	*-47***	+4
	50	-45*	-20	+21	-19	-44**	+50
Xyloccensin- I	25	NC	NC	-5	-5	-17	-7
β- Sitosterol	25	-7	NC	-5	-14	-38***	-7
Fenofibrate (Standard drug)	108.24 *(300μ mole)*	*-42**	*-18**	*NC*	-36**	*-20**	+10

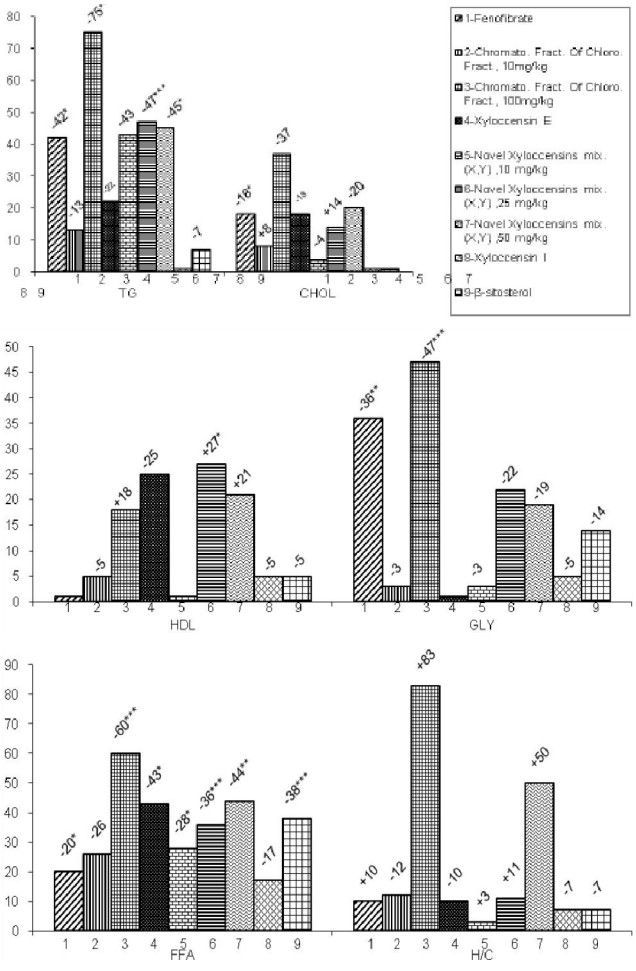

Figure 3 : Antidyslipidemic activity of the column fraction and pure compounds of the chloroform fraction of the ethanol extract in dyslipidemic hamster model.

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