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Preliminary phytochemical and *in-vitro* cytotoxic activity of the leaves of *Symplocos cochinchinensis* (Lour.) S.Moore ssp. *laurina* (Symplocaceae)

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Abstract

The phytochemical and in vitro cytotoxic activity of various extracts of the leaves of ***Symplocos cochinchinensis*** *(Lour.) S.Moore ssp.* ***laurina*** *(Symplocaceae) were studied in the present investigation. The leaves were extracted successively with various solvents viz., n-Hexane, chloroform, ethyl acetate and methanol in a soxhlet extractor. The phytochemical tests, thin layer chromatographic studies and high performance thin layer chromatographic study were also carried out for all the extracts. All the extracts were screened for in vitro cytotoxic activity by MTT assay method using human cancer cell lines, Human breast cancer- MDA-MB-231, Colon cancer-SW 620, Liver cancer - Hep G 2. Methanol extract showed significant cytotoxic activity than all the other extracts against the above mentioned cancer cell lines.*

Key words: *Symplocos cochinchinensis*, methanolic extract, HPTLC, *in vitro* cytotoxic, MTT assay.

INTRODUCTION

The genus *Symplocos* comprises of 300-500 species of the Symplocaceae family is traditionally used to for the treatment of diarrhoea, dysentery, eye diseases, hemorrhagic gingivitis, uterine disorders, menorrhagia [1], bowel complaints, ulcers [2], snake bites, malaria, tumefaction and enteritis [3]. Recently much attention has been paid to Symplocos species due to their diverse biological activities, particularly anti HIV activity, inhibitory activities against phosphodiesterase and anti tumor applications [4].Among the Symplocos species ***Symplocos cochinchinensis*** is very important species which is otherwise known as kabli-vetti or Lodh tree is widely distributed in tropical, subtropical areas in Asia, and America.

The beneficial physiological and therapeutic effects of plant materials typically result from the combinations of the secondary products present in the plant. Therefore, a complete investigation is required to identify the phytoconstituents present in the plant. Keeping this in view, the present

study has been undertaken to investigate the nature of phytochemical constituents present in of the leaves of *Symplocos cochinchinensis (*Lour). *In vitro* cytotoxic activity was also carried for all the four extracts by MTT assay method using human cancer cell lines.

MATERIALS AND METHODS

2.1 Plant Materials

The fresh healthy leaves of *Symplocos cochinchinensis (*Lour) were collected from Nilgiri hills. It was authenticated by Botanical survey of India and Prof. P. Jayaraman, Botanist and Director, PARC, Chennai. A voucher specimen was deposited in the department for future reference. The leaves were shade dried and coarsely powdered.

2.2 Preparation of the extracts

About 2 kg of air-dried plant material was extracted in soxhlet assembly successively with n-Hexane, chloroform, ethyl acetate and methanol (order of increasing polarity). Each time before extracting with the next solvent, the powdered material is dried. Each extract was concentrated by using rotary vacuum evaporator. The extract obtained with each solvent was weighed and the percentage was calculated in terms of dried weight of the plant material. The colour and consistency of the extract were also noted.

2.3 Phytochemical tests[5-7]

The n-Hexane, chloroform, ethyl acetate and methanol extracts and the leaf powder were subjected to qualitative chemical analysis to identify the presence of various phyoconstituents present in various extracts as per the standard procedures.

2.4 Thin layer chromatogrphic studies[8,9]

All the extracts were subjected to thin layer chromatographic studies using various solvents. Several mobile phases were tried for the separation of maximum components. From the vast analysis, best solvent was selected which showed good separation with maximum number of components. The solvent system selected was Chloroform : Methanol (9:1), Methanol : Ethyl acetate: Hexane: Acetic acid (2:7:1: 0.5),Methanol : Ethyl acetate: Water (6:3:1). R_f values were noted down for each selected extracts after elution by using different detecting agents such as Dragendroff's, Ninhydrin, Libberman Burchard, Con. Sulphuric acid & Ferric chloride.

2.5 High performance thin layer chromatographic studies[10]

The extracts were dissolved in same solvent and 10 µl quantity of sample applied on the HPTLC silica merk 60F 254 graded plate sized 6cm x 10 cm as narrow bands using CAMAG Linomat 5 injector. Plates were scanned under UV at 280nm. The data's obtained from scanning were brought in to integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in the each extracts and R_f values were tabulated.

2.6 *In vitro* anticancer activity

Human breast cancer- MDA-MB-231, Colon cancer-SW 620, Liver cancer - Hep G 2. were obtained from National centre for cell science (Pune, India). Stock culture of these cells lines were cultured in RPMI -1640 or DMEM supplemented with 10% inactivated newborn calf serum, penicillin (100 IU/ml), streptomycin (100ìg/ml) and amphotericin (5 ìg/ml) under humidified atmosphere of 5% CO2 at 37°C until confluent. The cells were dissociated in 0.2% trypsin, 0.02% EDTA in phosphate buffer saline solution. The stock culture was grown in 25cm2

tissue culture flasks, and cytotoxicity experiments were carried out in 96 - well microtiter plates (Tarsons India, kolkata, India).

Cell lines in the exponential growth phase were washed, trypsinized and suspended in complete culture media. Cells were plated at 10,000 cells/well in 96 well microtiter plates and incubated for 24hr during which a partial monolayer formed. They were then exposed to various concentrations of the extract (1 - 100ìg/ml) and Doxorubicin. Control wells received only maintenance medium. The plates were incubated at 37°C in a humidified incubator with 5% CO2 for a period of 72 hr. At the end of, 72 hr, cellular viability was determined by MTT assay [11].

RESULTS AND DISCUSSION

The percentage of successive extractive values for leaves of ***Symplocos cochinchinensis Lour*** is tabulated in Table 1. Successive extractive values revealed the solubility and polarity particulars of the metabolites in the leaf powder. Percentage yield of various extracts were as follows: n-Hexane (2%), chloroform(2%), ethyl acetate (5.7%) and methanol (8.8%). Methanolic extract showed high extractive yield 8.8 %w/w among other extracts.

Qualitative preliminary phytochemical analysis was performed initially with different respective chemical detecting agent to detect the phytoconstituent nature and their presence in each extract and powder whish is tabulated in table no.2. Hexane and chloroform extracts showed the presence of steriods. Ethyl acetate extract was found to contain flavanoids, glycosides, proteins, saponins, alkaloids and carbohydrates. Methanolic extract showed the presence of carbohydrates, flavanoids, phenols, saponins, tannins, proteins, glycosides and alkaloids.

Qualitative chromatographic analysis of these extracts using thin layer chromatography was performed to separate and identify the single or mixture of constituents in each extract. The hexane extract showed 2 spots R_f values 0.62, 0.66, chloroform extract showed one spot (0.37), where as 3 spots were found with ethyl acetate extract (0.27, 0.33, 0.88) and 4 spots were found in methanol extract (R_f values 0.20, 0.45, 0.52, 0.72). TLC was performed for the identification of different components in the extracts qualitatively.

HPTLC was scanned at 280 nm with the best solvent to detect the maximum number of components and peak abundance qualitatively and quantitatively at higher resolution which is presented in fig 1. HPTLC fingerprint is one of the versatile tools for qualitative and quantitative analysis of active constituents of multicomponent sample and also a diagnostic method to find out the adulterants to check purity.

In *in vitro* cytotoxic study, both ethyl acetate and methanol extracts showed the activity, but methanol extract showed significant cytotoxic effect than ethyl acetate extract. The n-hexane and chloroform extracts did not show any activity. Methanol extract exhibits greater cytotoxic effect against colon cancer cell lines SW 620 and hepG2 (liver cancer cell) with a GI50 value of 20 mcg/ml(table 3).

Table 1: The percentage yield of successive extracts of the leaves of *Symplocos cochinchinensis Lour*.

S. No.	Extract	Method of extraction	Colour	Physical nature	Yield (%w/w)
1	n-Hexane	Successive solvent extraction in soxhlet apparatus	Green/ Sticky mass	Waxy greasy semisolid	2.0
2	Chloroform		Greenish brown/ Sticky mass	Semisolid	2.0
3	Ethyl acetate		Yellowish green/ Thick solid mass	Solid	5.7
4	Methanol		Brownish green/ Thick solid mass	Solid	8.8

Table 2: Qualitative chemical analysis of phytoconstituents of the leaf powder and various extracts of *Symplocos cochinchinensis Lour*.

S. No.	Test	Powder	n-Hexane	Chloroform	Ethyl acetate	Methanol
1.	Alkaloids	+	-	+	-	-
2.	Glycosides	+	-	-	+	+
3.	Terpenoids	+	-	-	-	-
4.	Carbohydrates	+	-	-	+	+
5.	Proteins	+	-	-	+	+
6.	Steroids	+	+	+	-	-
7.	Flavonoids	+	-	-	+	+
8.	Phenols	+	-	+	-	+
9.	Tannins	-	-	-	-	+
10.	Iridoid glycoside	-	-	-	-	-
11.	Quinones	-	-	+	-	-
12.	Anthraquinone	-	-	-	-	-
13.	Saponins	-	-	-	+	+

+ ve indicates positive result, whereas – ve indicates negative result.

Table 3: The TLC fingerprints various extracts of *Symplocos cochinchinensis Lour*.

S. No	Extract	Detection Wavelength (nm)	No. of spots	R_f values
1	n-Hexane	Chloroform:Methanol (9:1)	2	0.62 0.66
2	Chloroform		3	0.37 0.43 0.48
3	Ethyl acetate	Methanol : Ethyl acetate: Hexane: Acetic acid (2:7:1: 0.5)	3	0.27 0.33 0.88
4	Methanol	Methanol : Ethyl acetate: Water (6:3:1)	4	0.20 0.45 0.52 0.72

Table No.4 The HPTLC fingerprints various extracts of *Symplocos cochinchinensis Lour*.

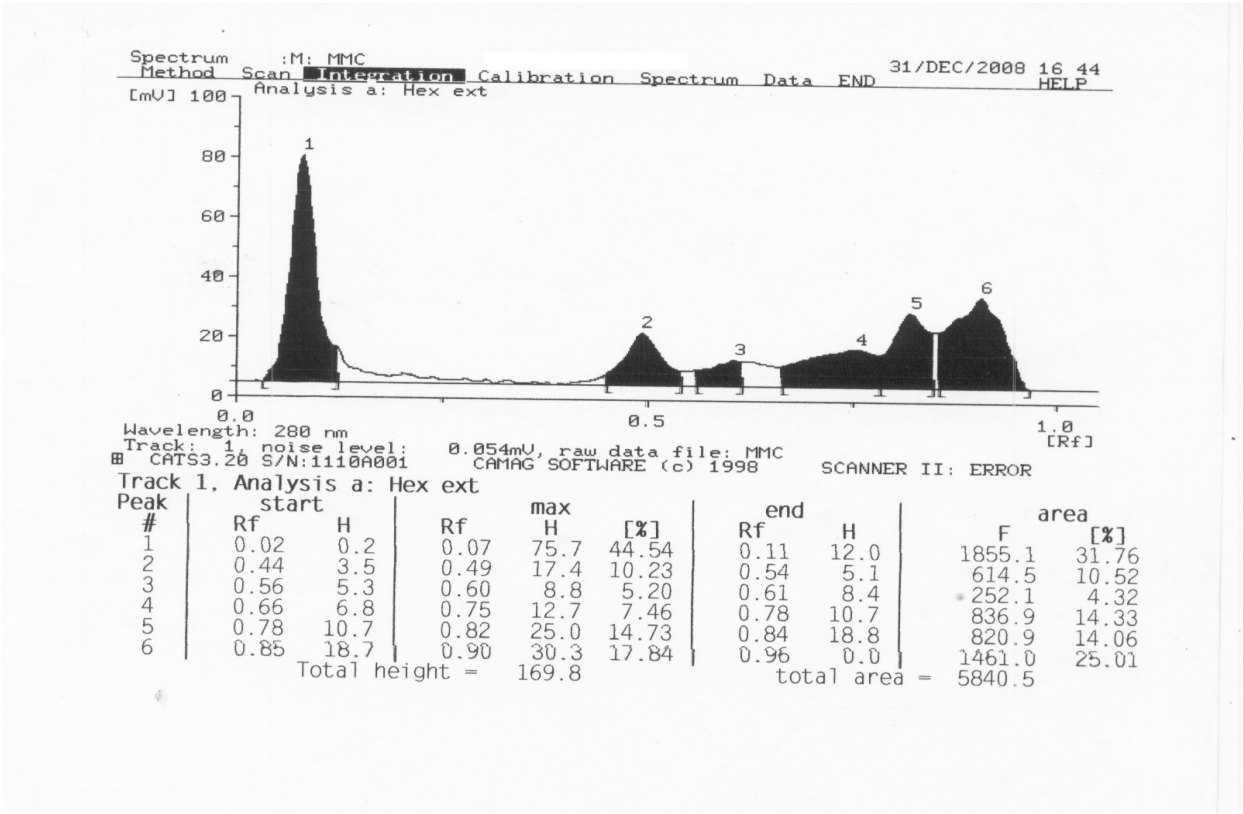
S. No	Extract	Detection Wavelength (nm)	No. of spots	R_f values
1	n-Hexane	280	6	0.07, 0.49, 0.60, 0.75, 0.82, 0.90
2	Chloroform	280	11	0.07, 0.28, 0.32, 0.42, 0.46, 0.50, 0.58, 0.76, 0.82, 0.88, 0.91
3	Ethyl acetate	280	6	0.12, 0.29, 0.36, 0.49, 0.57, 0.81
4	Methanol	280	10	0.06,0.15,0.18,0.24,0.33,0.39,0.51,0.58,0.65,0.71

Table 4: *In vitro* cytotoxic activity of various extracts of *Symplocos cochinchinensis Lour*.

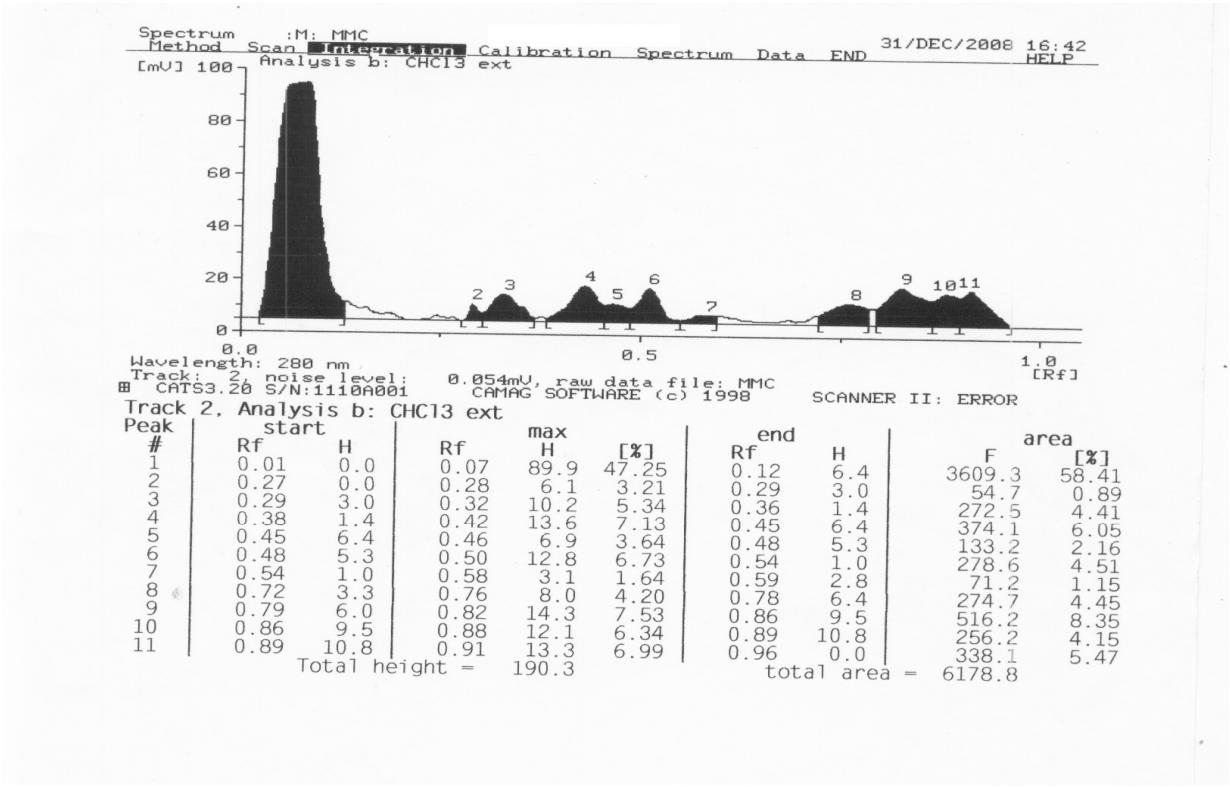
Cell lines	Hexane extract (µg/ml)		Chloroform extract (µg/ml)		Ethyl acetate extract(µg/ml)		Methanol extract(µg/ml	
	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI
MDA-MB-231	>100	>100	>100	>100	50	>100	30	70
SW 620	>100	>100	>100	>100	40	90	20	60
Hep G 2	>100	>100	>100	>100	30	>100	20	80

Average of 3 determinations, 3 replicates; GI_{50}- Drug concentration inhibiting 50% cellular growth following 72 h of drug exposure; TGI- Total cellular growth inhibition

Fig 1. The HPTLC fingerprints various extracts of *Symplocos cochinchinenis Lour*.,
a) n-Hexane extract



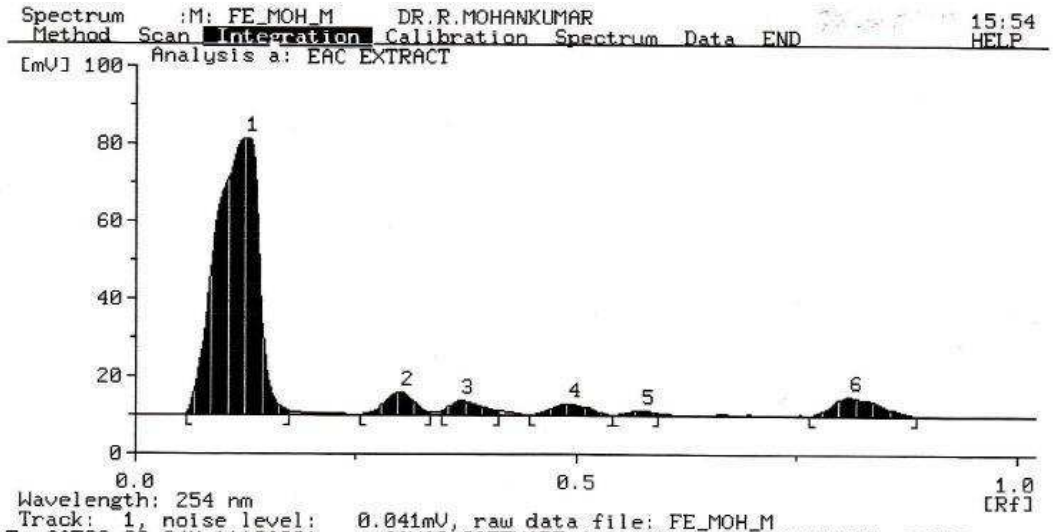
b) **Chloroform extract**



Track 2, Analysis b: CHCl3 ext

Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	0.01	0.0	0.07	89.9	47.25	0.12	6.4	3609.3	58.41
2	0.27	0.0	0.28	6.1	3.21	0.29	3.0	54.7	0.89
3	0.29	3.0	0.32	10.2	5.34	0.36	1.4	272.5	4.41
4	0.38	1.4	0.42	13.6	7.13	0.45	6.4	374.1	6.05
5	0.45	6.4	0.46	6.9	3.64	0.48	5.3	133.2	2.16
6	0.48	5.3	0.50	12.8	6.73	0.54	1.0	278.6	4.51
7	0.54	1.0	0.58	3.1	1.64	0.59	2.8	71.2	1.15
8	0.72	3.3	0.76	8.0	4.20	0.78	6.4	274.7	4.45
9	0.79	6.0	0.82	14.3	7.53	0.86	9.5	516.2	8.35
10	0.86	9.5	0.88	12.1	6.34	0.89	10.8	256.2	4.15
11	0.89	10.8	0.91	13.3	6.99	0.96	0.0	338.1	5.47
		Total height =		190.3			total area =	6178.8	

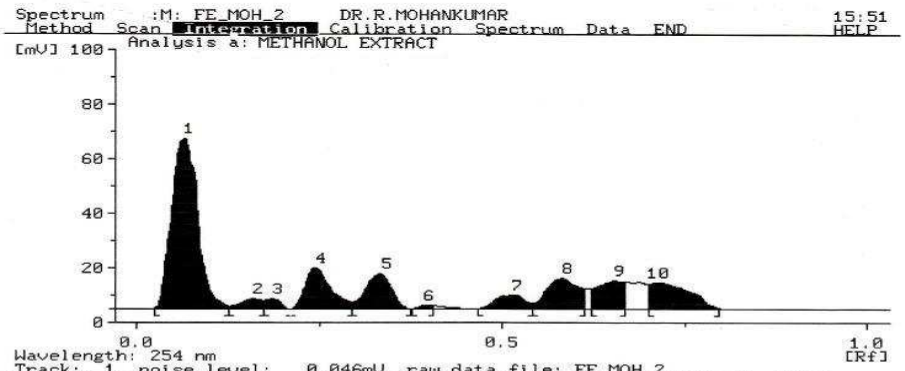
c) **Ethyl acetate extract**



Track 1, Analysis a: EAC EXTRACT

Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	0.05	0.4	0.12	71.2	79.04	0.17	1.1	1981.5	81.80
2	0.25	0.0	0.29	5.9	6.53	0.33	0.7	114.7	4.73
3	0.34	1.2	0.36	3.7	4.16	0.41	1.2	80.5	3.32
4	0.44	0.0	0.49	3.1	3.42	0.54	0.0	73.5	3.03
5	0.54	0.0	0.57	1.2	1.39	0.59	0.7	21.1	0.87
6	0.76	0.1	0.81	4.9	5.45	0.89	0.0	151.2	6.24
		Total height =		90.1			total area =	2422.5	

d) Methanol extract



Track 1, Analysis a: METHANOL EXTRACT

Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.02	0.4	0.06	62.4	46.05	0.12	0.9	1953.9	44.62
2	0.12	0.9	0.15	3.8	2.78	0.17	3.0	94.3	2.15
3	0.17	3.0	0.18	3.6	2.66	0.20	0.0	65.2	1.49
4	0.20	0.0	0.24	15.1	11.13	0.29	2.5	462.9	10.57
5	0.29	2.5	0.33	12.9	9.51	0.37	0.0	397.0	9.07
6	0.37	0.0	0.39	1.3	0.98	0.40	1.2	19.4	0.44
7	0.46	0.0	0.51	5.1	3.80	0.54	1.9	179.0	4.09
8	0.54	1.9	0.58	11.2	8.26	0.61	7.6	421.0	9.61
9	0.62	7.5	0.65	10.3	7.60	0.66	10.0	326.4	7.45
10	0.70	9.2	0.71	9.8	7.22	0.79	0.4	460.3	10.51
		Total height =		135.5			total area =	4379.3	

CONCLUSION

The preliminary phytochemical analysis and *in vitro* cytotoxic activity were studied in the present investigation. The leaf powder was extracted with different solvents and the extracts were phytochemically analysed. All the extracts were screened for *in vitro* cytotoxic activity, methanolic extract showed significant cytotoxic activity when compared with all the other extracts which may be due to the presence of one or phytoconstituents present in the extract

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