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HPTLC Studies on the Leaf Extract of *Hydnocarpus pentandra* (Buch.-Ham.) Oken.

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Abstract

The study aims to identify the major secondary metabolites in the leaves of *Hydnocarpus pentandra* (Buch.-Ham.) Oken., an endemic medicinal species from South India. The methanolic extract of leaf was subjected to screening for secondary metabolites by HPTLC using specific solvents and derivatizing agents. The study revealed that the leaf extract of *H. pentandra* is rich in secondary metabolites like Alkaloids, Essential oils, Steroids, Triterpenes, Flavonoids, Flavonoid glycosides, Flavonolignans, Phenolics, Tannins and Saponins.

Key-Words: *Hydnocarpus pentandra*, HPTLC, Secondary metabolites, Endemic species

Introduction

Hydnocarpus Gaertn. is an Indo-Malasian genus belonging to the family Flacourtiaceae[1]. Five species of *Hydnocarpus* viz., *H. alpina*, *H. kurzii*, *H. macrocarpa*, *H. pentandra and H. pendulus* are reported from India[2-6]. Out of the five species, *H. pentandra*, *H. macrocarpa* and *H. pendulus* are endemic to South India

The genus is well known for its use in the treatment for leprosy. The 'Chaulmoogra' oil, which is used for the treatment of leprosy, is extracted from the seeds of *H. Kurzii*.[7-8] As this species is present only in the north eastern part of India, *H. pentandra* is used as an alternative source of the oil in the southern part of India.

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Photo 1: *H. pentandra* twig with bisexual flower

Photo 2: *H. pentandra* fruit

Material and Methods

Collection and Extraction of Plant materials

Fresh and uninfected leaves from the source plant were collected from Western Ghats, Kerala, India. Prior permission was obtained for the collection of plant materials from the Department of Forest and Wild Life, Government of Kerala. The voucher specimen (Voucher No. TD 080) is deposited in the herbarium of New Udaya Pharmacy and Ayurvedic Laboratories, Kadavanthara, Kochi, Kerala, India. The collected materials were thoroughly washed, cleaned, oven dried and powdered.

The powdered leaves (20g) were extracted in 200ml 100% methanol for two weeks. The extract was filtered and was concentrated using Rotary vacuum evaporator.

HPTLC method and chromatographic conditions

The HPTLC system (Camag, Muttenz, Switzerland) has Linomat V auto sprayer connected to a nitrogen cylinder; a twin trough chamber (10 x 10 cm) and a derivatization chamber. Pre-coated silica gel 60 F_{254} TLC plates (10 x 10 cm, layer thickness 0.2 mm – E Merck KGaA, Darmstadt, Germany) were used as the stationary phase. TLC plates were prewashed twice with 10 ml of methanol and activated at 80^0C for 5 minutes prior to sample application. Densitometric analysis was carried out using a TLC scanner III with winCATS software.

Sample application

7µl of sample was spotted on pre-coated TLC plate in the form of narrow bands (8mm) with 10mm from the bottom using Linomat V spotter. Samples were applied under continuous dry stream of nitrogen gas at constant application amount 7µl

Mobile phase and migration

The spotted plates were developed using different mobile phases to detect the various classes of phytochemicals. The proportion of the chemicals in the mobile phases is as follows:

Alkaloid - Toluene: Methanol: Diethyl amine (8:1:1)
Essential oils - Toluene: Ethyl acetate (8.5:1.5)
Flavonoid glycosides - Ethyl cetate: Acetic acid: Formic acid: Water (10:1.1:1.1:2.6)
Flavonoids - Toluene: Ethyl acetate: Formic acid (7:3:0.1)
Flavonolignans - Chloroform: Acetone: Formic acid (7.5:1.65:0.85)
Phenolics - THF: Toluene: Formic acid: Water (16:8:2:1)
Saponins - Chloroform: Acetic acid: Methanol:Water (6.4:3.2:1.2:0.8)
Steroids - Toluene: Methanol: Acetone (6:2:2)
Tannin - Ethyl acetate: Acetic acid: Ether: Hexane (4:2:2:2)
Triterpenes - Toluene: Chloroform: Ethanol (4:4:1)

Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 20 minutes at 25 ± 2^0C with a relative humidity of 60 ± 5%. Ten millilitres of the mobile phase (5ml in trough containing the plate and 5ml in other trough) was used for the development and allowed to migrate a distance of 70 mm from the point of sample application. After development, TLC plate was dried and the chromatogram was viewed at 254 nm and 366 nm to visualise and detect various phytochemical constituents.

Derivatization

The TLC plates were derivatized with the following reagents to detect the various classes of phytochemicals.

Alkaloids - Dragendorff reagent
Essential oils, Saponins and **Triterpenes** - Anisaldehyde sulphuric acid
Flavonoids, **Flavonoid glycosides and Flavonolignans** - NP/PEG Reagent
Phenolics and **Tannin** - Fast blue salt B
Steroids - Vanillin sulphuric acid

Documentation

The various conditions for documentation were selected based on the recommendations given in the CAMAG TLC Scanner III manual. The plates were photographed in various conditions under UV 254 nm, UV 366 nm and UV 366 nm after derivatization. The plates were subjected to scanning prior to derivatization. Densitometric scanning was performed on CAMAG TLC scanner III in absorbance mode and operated by winCATS planar chromatography version 1.3.4. The source of radiation utilized was Deuterium lamp. The spots were analysed at a wave length of 218 nm. The slit dimensions used in the analysis were of 6 mm length and 0.30 mm width, with a scanning rate of 20 mm/s. It covers 70% -90% of the application band length. The monochromator band width was set at 20 mm. Concentration of compound chromatographed were determined on the basis of the intensity of diffusely reflected light and evaluated as peak areas against concentration using linear regression equation.

Results and Discussion

The results obtained from HPTLC analysis of the methanolic extract of *H. pentandra* with respect to the major secondary metabolites viz. Alkaloids, Essential oils, Steroids, Triterpines, Flavonoids, Flavonoid glycosides, Flavonolignans, Phenolics, Tannins and Saponins are given below.

Alkaloids

The HPTLC separation of the chloroform fraction of the acidified methanol leaf extract was carried out using specific solvent system for alkaloids [Touene : methanol : Dimethylamine (8:1:1)]. Fourteen bands with specific R_f values (0.08 to 0.82) were detected in the chromatogram. The highest concentration (29.25 %) was noticed with respect to R_f 0.41. The TLC plate on derivatization with Dragendorff reagent developed a light brown colour for prominent alkaloid bands [Plate 1 & 2, Table 1, Figure 1].

Essential oils

The HPTLC separation of the petroleum ether extract with the solvent system, Toluene: Ethyl acetate (8.5:1.5), exposed five bands with respect to essential oils. The R_f values of these bands vary from 0.08 to 0.66. The band with R_f 0.49 shows the highest (45.23 %) concentration. The prominent bands developed blue, green, purple and red colour on derivatization [Plate 3 & 4, Table 2, Figure 2].

Steroids

Four bands with specific R_f values (0.66 to 0.94) were detected, when the petroleum ether extract of the leaves was subjected to HPTLC separation using specific solvent system Toluene: Methanol: Acetone (6:2:2) for steroids. The highest concentration (42.24 %) was noticed with respect to the band at R_f 0.66. The prominent bands developed light blue colour on derivatization with vanillin sulphuric acid [Plate 5 & 6, Table 3, Figure 3].

Triterpenes

Methanol extract of the leaves of *H. pentandra* on HPTLC separation using specific solvent system of triterpene as mobile phase, developed sixteen bands with R_f values (0.09 to 0.84). The band detected with respect to R_f 0.32 (20.50 %) represent the prominent compound. The prominent bands on derivatization with anisaldehyde sulphuric acid developed blue and pink colour at 366 nm [Plate 7 & 8, Table 4, Figure 4].

Flavonoids

Thirteen bands were detected when the methanolic leaf extract was subjected to separation of flavonoid compounds. The R_f of the flavonoid bands were detected between 0.12 and 0.85. The band with highest concentration (16.83 %) was identified at R_f 0.12. On derivatization, the prominent bands developed yellow, fluorescent yellow, light green, pink, orange & blue colours [Plate 9 & 10, Table 5, Figure 5].

Flavonoid glycosides

On HPTLC separation of the methanolic leaf extract for flavonoid glycosides, ten bands were detected in the chromatogram. The R_f of these bands were found between 0.11 and 0.85. The highest concentration (30.41 %) was detected at R_f 0.40. The bands developed different colours viz., fluorescent yellow, yellow, pale green and blue on derivatization [Plate 11 & 12, Table 6, Figure 6].

Flavonolignans

Five bands were detected in the chromatogram, when the successive methanol fraction of the leaf extract was subjected to specific HPTLC separation of flavanolignans (Wagner and Bladt, 1995). The flavonolignan compound with highest concentration (38.89 %) was obtained at R_f 0.41. The prominent bands developed yellow, fluorescent yellow, fluorescent green and red colours on derivatization with NP/PEG Reagent [Plate 13 & 14, Table 7, Figure 7].

Phenolics

Eight bands were detected with respect to phenolic compounds. The R_f of these bands are found between 0.10 and 0.81 and the highest concentration (28.12 %) was noticed at R_f 0.81. The phenolic compounds on derivatization with fast blue salt B developed purple and green colour [Plate 15 & 16, Table 8, Figure 8].

Tannins

When the methanolic leaf extract of *H. pentandra* was subjected to separation of tannins using specific solvent system Ethyl acetate: Acetic acid: Ether: Hexane (4:2:2:2), eight bands were detected with R_f values that ranges from 0.05 to 0.81. The highest concentration (23.26 %) was detected at R_f 0.18. The prominent bands appeared purple and green on derivatization [Plate 17 & 18, Table 9, Figure 9].

Saponins

Ten bands were detected, when the methanol leaf extract was subjected to separation of saponins under specific chromatographic conditions using the solvent system, Chloroform : Acetic acid : Methanol : Water (6.4 : 3.2 : 1.2 : 0.8) . The R_f of these compounds differ from 0.10 to 0.83. The band detected with respect to R_f 0.32 showed the maximum concentration (31.03 %). The prominent bands developed blue, olive green, red & fluorescent blue colour on visualisation after derivatization at 366 nm [Plate 19 & 20, Table 10, Figure 10].

Phytochemicals are used as potential theraputic drugs in different systems of medicines. The importance of Flavonoids and Flavinolignans in *H. pentandra* have been reported by earlier researchers[9]. Compounds like Hydnowightin, Hydnocarpin and neohydnocarpin were effective to reduce serum cholesterol and triglyceride level[9]. These compounds are also useful against human colon adinocarcinoma and Hela S uterine/ murine L-1210 lukemia growth and are known to act as an anti-inflammatory agent[9]. The antidiabetic and antioxidant

activity of the ethanolic extract of H. *pentandra* in mice has also been reported[10]. There are also reports regarding Antimicrobial activity of Hydnocarpic acid[11]. It acts by being an antagonist of biotin[17]. In the above context, the rich diversity of phytochemical compounds in the leaves of *H. pentandra* provides ample scope for characterisation of bioactive compounds with therapeutic potential.

Conclusion

The present study revealed the presence of Alkaloids, Essential oils, Steroids, Triterpines, Flavonoids, Flavonoid glycosides, Flavonolignans, Phenolics, Tannins and Saponins in the leaves of *H. pentandra*. The diversity of phytochemicals in the leaves of *H. pentandra* makes this plant a potential drug candidate for further phytochemical and pharmacognostic investigations.

Acknowledgement

We thank the Depatrment of Forest and Wildlife, Government of Kerala for giving me the permission to collect the plant materials. Thanks are also due to Mr. Shankar Iyer and Ms. Sayana P.S. New Udaya Pharmacy and Ayurvedic Laboratories, Ernakulam, Kerala for their help in HPTLC analysis. Our sincere thanks to Dr. D. Narasimhan, Department of Botany and Biotechnology, Madras Christian College, Tambaram, Chennai for offering help regarding the taxonomic identification of the plant material.

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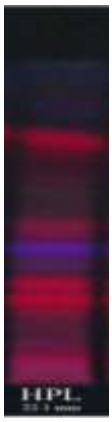

Plate 1: HPTLC profile of leaf extract of *H. pentandra* with reference to Alkaloids

Plate 2: HPTLC profile of leaf extract of *H. pentandra* with reference to Alkaloids after derivatization

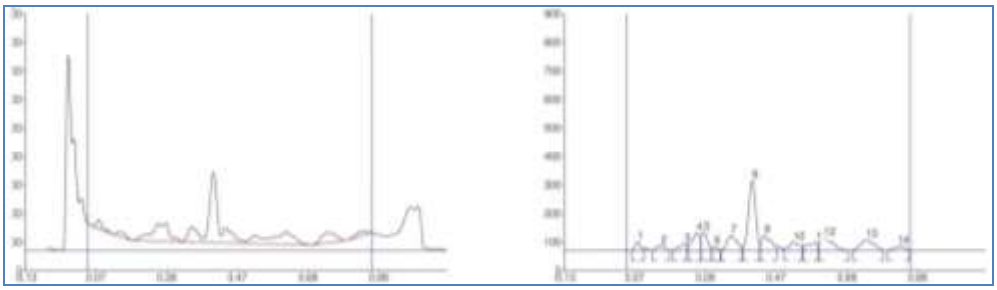

Fig. 1: Chromatogram of Alkaloids in the Methanolic leaf extract of *H. pentandra*

Table 1: Result of HPTLC scanning with reference to Alkaloids

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.07	0.4	0.08	30.5	4.28	0.10	6.7	421.0	2.81
2	0.12	0.1	0.15	16.4	2.31	0.17	0.7	358.0	2.39
3	0.18	1.2	0.22	22.8	3.21	0.22	22.0	429.8	2.87
4	0.23	21.4	0.25	61.8	8.67	0.26	52.3	1222.7	8.17
5	0.26	53.3	0.27	63.8	8.96	0.29	8.8	942.6	6.30
6	0.29	9.6	0.30	14.2	1.99	0.32	1.5	162.0	1.08
7	0.32	1.8	0.35	52.3	7.35	0.38	12.0	1291.6	8.63
8	0.38	12.5	0.41	244.9	34.37	0.43	24.0	4378.1	29.25
9	0.43	24.4	0.44	53.1	7.45	0.48	13.2	1232.2	8.23
10	0.50	4.8	0.52	27.9	3.91	0.55	17.5	733.7	4.90
11	0.55	17.4	0.59	28.4	3.98	0.59	27.1	750.5	5.01
12	0.60	26.6	0.61	44.2	6.21	0.68	0.4	1358.6	9.08
13	0.69	1.8	0.73	37.0	5.19	0.78	0.1	1268.1	8.47
14	0.79	0.3	0.82	15.1	2.12	0.85	0.9	418.0	2.79

Plate 3: HPTLC profile of leaf extract of *H. pentandra* with reference to Essential oils

Plate 4: HPTLC profile of leaf extract of *H. pentandra* with reference to Essential oils after derivatization

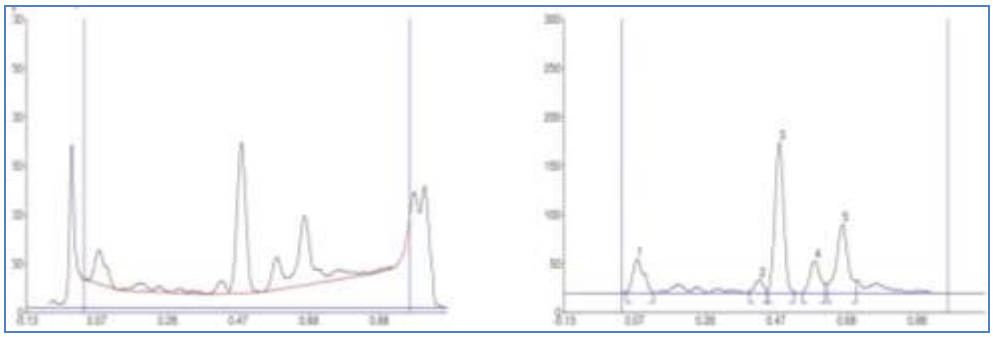

Fig. 2: Chromatogram of Essential oils in the Methanolic leaf extract of *H. pentandra*

Table 2: Result of HPTLC scanning with reference to Essential oils

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.05	0.3	0.08	34.9	11.41	0.13	0.5	935.9	12.61
2	0.40	1.5	0.43	13.4	4.39	0.45	2.1	288.8	3.89
3	0.45	2.3	0.49	153.9	50.35	0.53	1.1	3357.7	45.23
4	0.55	0.2	0.59	32.9	10.75	0.61	9.8	827.8	11.15
5	0.62	9.9	0.66	70.6	23.10	0.70	12.7	2014.2	27.13

Plate 5: HPTLC profile of leaf extract of *H. pentandra* with reference to Steroids

Plate 6: HPTLC profile of leaf extract of *H. pentandra* with reference to Steroids after derivatization

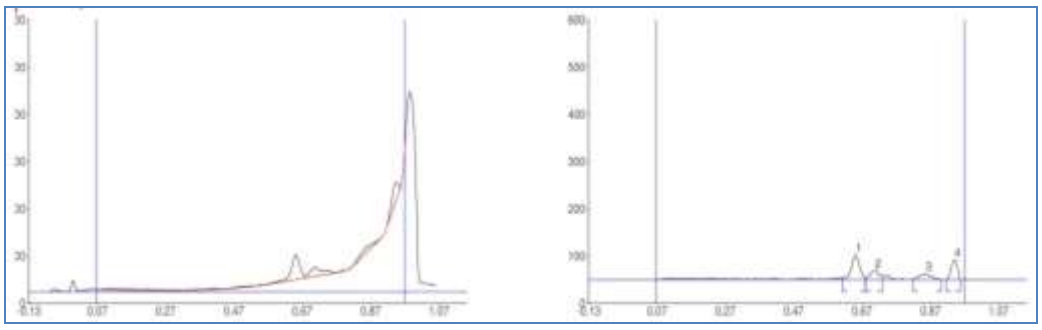

Fig. 3: Chromatogram of Steroids in the Methanolic leaf extract of *H. pentandra*

Table 3: Result of HPTLC scanning with reference to Steroids

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.62	3.6	0.66	52.2	41.45	0.68	3.3	1163.1	42.24
2	0.68	3.6	0.71	18.7	14.84	0.73	8.4	479.3	17.41
3	0.82	0.7	0.86	12.7	10.08	0.91	0.0	404.2	14.68
4	0.92	0.2	0.94	42.4	33.62	0.96	0.5	706.8	25.67

Plate 7: HPTLC profile of leaf extract of *H. pentandra* with reference to Triterpenes

Plate 8: HPTLC profile of extract of *H. pentandra* with reference to Triterpenes after derivatization leaf

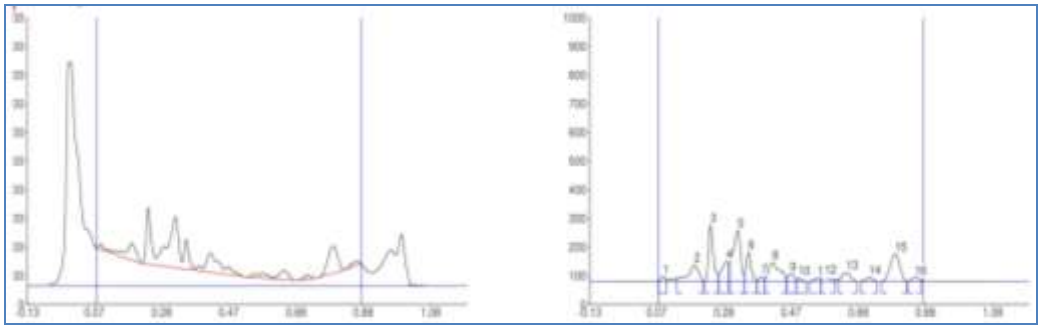

Fig. 4: Chromatogram of Triterpines in the Methanolic leaf extract of *H. pentandra*

Table 4: Result of HPTLC scanning with reference to Triterpines

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.08	0.4	0.09	19.3	2.06	0.10	7.2	214.3	1.21
2	0.13	10.6	0.19	58.4	6.23	0.21	0.7	1612.8	9.08
3	0.22	0.8	0.24	197.5	21.09	0.26	25.5	2530.2	14.25
4	0.26	25.9	0.28	70.4	7.51	0.29	65.6	1311.6	7.39
5	0.29	66.0	0.32	178.1	19.02	0.34	23.6	3639.8	20.50
6	0.34	25.0	0.35	102.7	10.97	0.37	5.3	1342.1	7.56
7	0.37	5.6	0.39	18.3	1.95	0.40	10.0	225.2	1.27
8	0.40	10.4	0.42	67.0	7.15	0.46	16.4	1945.5	10.96
9	0.46	16.7	0.47	26.5	2.83	0.49	9.5	445.2	2.51
10	0.49	9.6	0.50	12.9	1.38	0.52	0.1	162.1	0.91
11	0.53	0.5	0.55	12.4	1.32	0.56	10.0	214.9	1.21
12	0.57	10.1	0.58	16.8	1.79	0.61	3.9	354.0	1.99
13	0.62	6.3	0.64	32.2	3.44	0.67	0.4	725.9	4.09
14	0.68	0.1	0.71	14.5	1.55	0.73	0.2	272.7	1.54
15	0.75	1.3	0.79	95.9	10.24	0.82	0.2	2456.4	13.83
16	0.82	0.3	0.84	13.6	1.45	0.86	6.6	303.0	1.71

Plate 9: HPTLC profile of leaf extract of *H. pentandra* with reference to Flavonoids

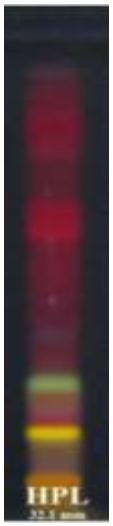

Plate 10: HPTLC profile of leaf extract of *H. pentandra* with reference to Flavonoids after derivatization

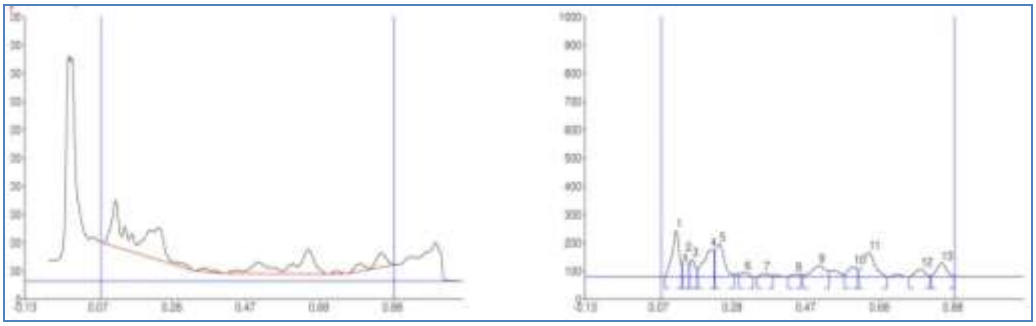

Fig. 5: Chromatogram of Flavonoids in the Methanolic leaf extract of *H. pentandra*

Table 5: Result of HPTLC scanning with reference to Flavonoids

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.09	0.6	0.12	163.5	20.57	0.14	41.5	2743.5	16.83
2	0.14	42.6	0.15	81.4	10.23	0.16	37.6	902.4	5.53
3	0.16	38.1	0.17	62.3	7.84	0.18	27.7	774.0	4.75
4	0.18	28.2	0.22	97.0	12.20	0.23	94.2	2391.0	14.66
5	0.23	94.4	0.24	114.8	14.45	0.28	7.9	2445.7	15.00
6	0.29	9.9	0.31	14.0	1.76	0.33	0.7	292.2	1.79
7	0.34	1.3	0.36	12.8	1.62	0.39	4.8	264.5	1.62
8	0.43	1.1	0.45	10.9	1.37	0.46	7.1	210.3	1.29
9	0.47	7.2	0.51	39.9	5.02	0.54	20.2	1314.9	8.06
10	0.58	7.3	0.61	36.1	4.54	0.62	25.2	741.4	4.55
11	0.62	25.2	0.65	86.2	10.84	0.70	1.0	2438.4	14.95
12	0.75	0.0	0.79	27.0	3.40	0.81	2.7	636.5	3.90
13	0.82	3.2	0.85	49.0	6.17	0.88	0.5	1151.2	7.06

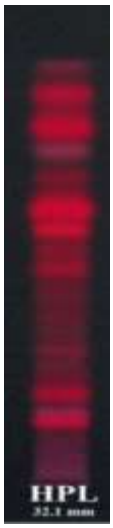

Plate 11: HPTLC profile of leaf extract of *H. pentandra* with reference to Flavonoid glycosides

Plate 12: HPTLC profile of leaf extract of *H. pentandra* with reference to Flavonoid glycosides after derivatization

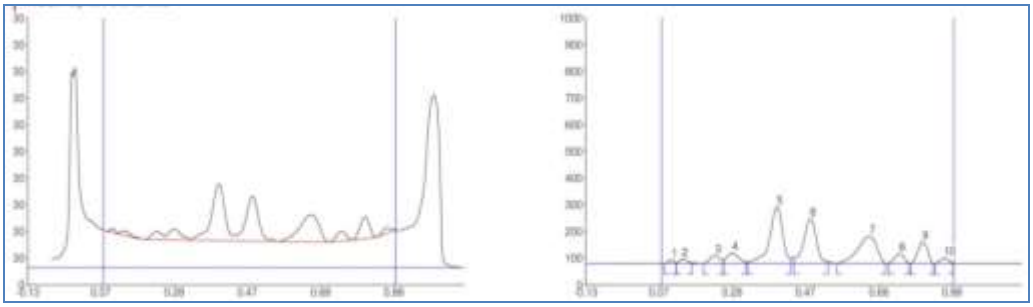

Fig. 6: Chromatogram of Flavonoid glycosides in the Methanolic leaf extract of *H. pentandra*

Table 6: Result of HPTLC scanning with reference to Flavonoid glycosides

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.09	0.3	0.11	15.9	2.20	0.12	2.5	187.4	0.85
2	0.12	2.8	0.14	17.2	2.38	0.17	3.0	338.3	1.54
3	0.20	0.2	0.23	29.7	4.10	0.25	14.5	758.8	3.46
4	0.25	14.8	0.28	40.1	5.54	0.31	6.7	1152.6	5.25
5	0.32	4.4	0.40	212.5	29.34	0.44	19.0	6674.1	30.41
6	0.44	20.1	0.49	168.4	23.26	0.54	7.4	5032.0	22.92
7	0.56	0.0	0.65	101.4	14.00	0.70	0.5	4651.2	21.19
8	0.70	1.5	0.73	36.7	5.07	0.76	0.2	829.8	3.78
9	0.76	0.4	0.80	81.1	11.20	0.83	0.0	1902.3	8.67
10	0.83	0.1	0.85	21.1	2.92	0.88	0.7	423.8	1.93

Plate 13: HPTLC profile of leaf extract of *H. pentandra* with reference to Flavonolignans

Plate 14: HPTLC profile of leaf extract of *H. pentandra* with reference to Flavonolignans after derivatization

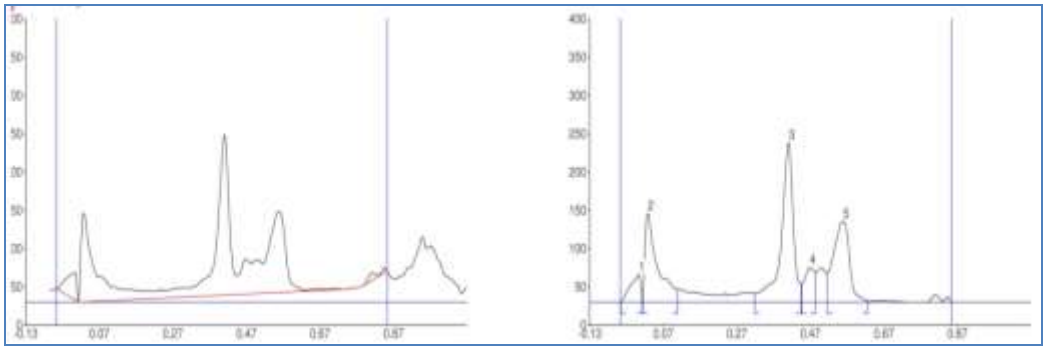

Fig. 7: Chromatogram of Flavonolignans in the Methanolic leaf extract of *H. pentandra*

Table 7: Result of HPTLC scanning with reference to Flavonolignans

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.05	0.7	0.00	36.2	7.06	0.01	2.1	857.6	5.56
2	0.01	3.9	0.03	116.0	22.66	0.11	17.0	3430.2	22.24
3	0.32	11.5	0.41	208.6	40.75	0.44	24.4	5997.5	38.89
4	0.44	24.4	0.47	45.6	8.91	0.48	39.4	1081.1	7.01
5	0.51	38.1	0.56	105.6	20.62	0.62	1.3	4054.7	26.29

Plate 15: HPTLC profile of leaf extract of *H. pentandra* with reference to Phenolics

Plate 16: HPTLC profile of leaf extract of *H. pentandra* with reference to Phenolics after derivatization

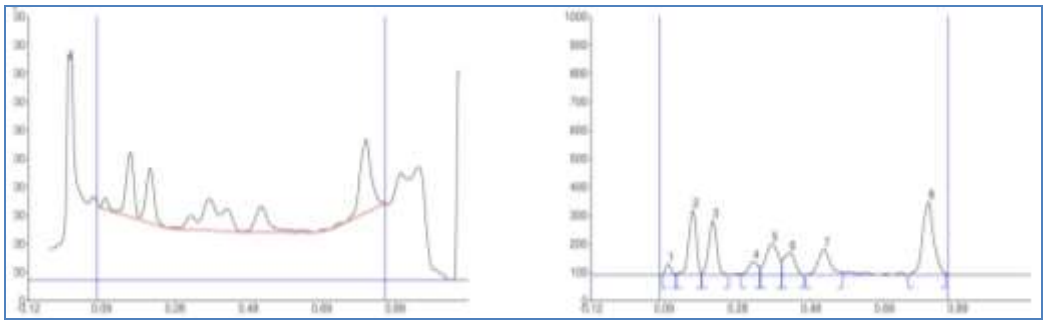

Fig. 8: Chromatogram of Phenolics in the Methanolic leaf extract of *H. pentandra*

Table 8: Result of HPTLC scanning with reference to Phenolics

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.08	0.5	0.10	39.3	3.76	0.12	2.7	583.3	2.15
2	0.12	2.8	0.16	228.5	21.88	0.19	8.3	4663.5	17.20
3	0.19	8.7	0.22	192.9	18.47	0.26	0.1	4133.9	15.24
4	0.29	3.8	0.33	47.9	4.59	0.35	28.7	1205.5	4.45
5	0.35	28.8	0.38	110.0	10.53	0.41	55.0	3640.8	13.42
6	0.41	55.1	0.43	78.3	7.49	0.47	0.6	2327.9	8.58
7	0.47	0.1	0.52	89.5	8.57	0.57	10.2	2939.1	10.84
8	0.75	0.1	0.81	258.1	24.71	0.85	6.3	7626.9	28.12

Plate 17: HPTLC profile of leaf extract of *H. pentandra* with reference to Tannins

Plate 16: HPTLC profile of leaf extract of *H. pentandra* with reference to Tannins after derivatization

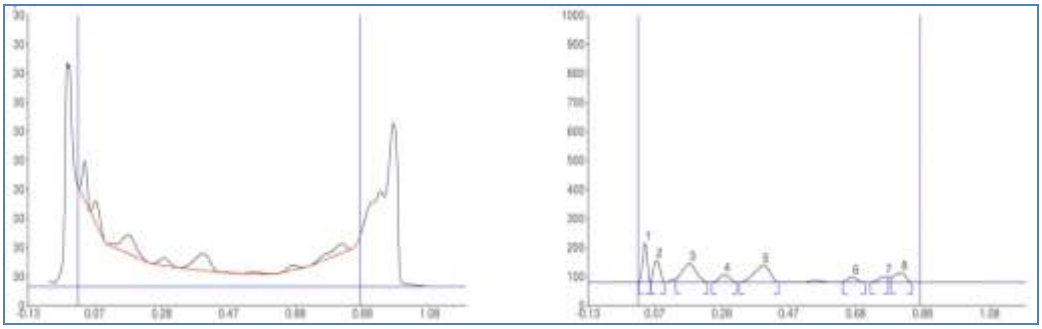

Fig. 9: Chromatogram of Tannins in the Methanolic leaf extract of *H. pentandra*

Table 9: Result of HPTLC scanning with reference to Tannins

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.03	1.1	0.05	135.1	31.53	0.06	1.3	1654.6	15.84
2	0.06	3.4	0.08	76.1	17.74	0.10	0.5	1304.5	12.49
3	0.14	11.9	0.18	64.9	15.15	0.23	1.0	2429.5	23.26
4	0.24	0.1	0.28	26.5	6.19	0.32	0.9	733.5	7.02
5	0.33	0.8	0.40	56.6	13.21	0.45	0.1	2394.7	22.93
6	0.64	1.1	0.67	17.6	4.10	0.71	0.0	455.1	4.36
7	0.72	0.2	0.77	19.5	4.55	0.77	17.9	466.9	4.47
8	0.78	17.8	0.81	32.3	7.54	0.84	0.1	1005.0	9.62

Plate 17: HPTLC profile of leaf extract of *H. pentandra* with reference to Saponins

Plate 16: HPTLC profile of leaf extract of *H. pentandra* with reference to Saponins after derivatization

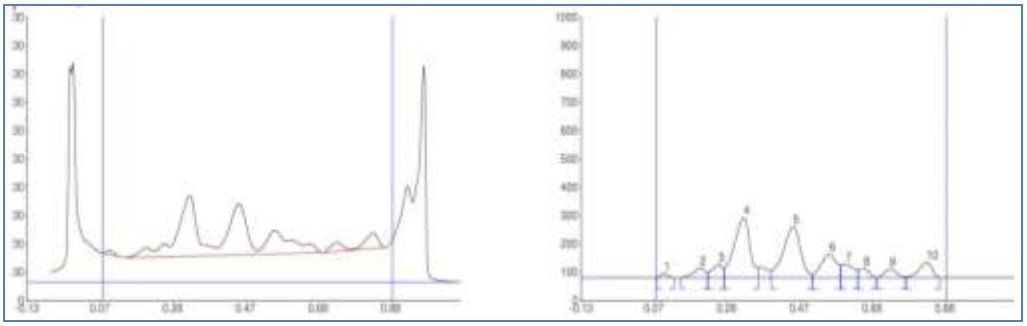

Fig. 10: Chromatogram of Saponins in the Methanolic leaf extract of *H. pentandra*

Table 10: Result of HPTLC scanning with reference to Saponins

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.08	0.4	0.10	16.0	2.17	0.13	0.0	269.5	1.03
2	0.15	0.4	0.20	34.5	4.68	0.22	18.4	1008.1	3.86
3	0.22	18.7	0.25	46.1	6.25	0.27	35.2	1163.5	4.46
4	0.27	35.6	0.32	213.3	28.93	0.36	36.2	8094.4	31.03
5	0.40	26.2	0.46	180.3	24.46	0.51	13.2	7553.1	28.95
6	0.51	13.5	0.56	83.3	11.30	0.59	44.3	3169.3	12.15
7	0.59	44.4	0.60	46.6	6.32	0.64	27.1	1379.3	5.29
8	0.64	27.1	0.65	32.8	4.45	0.69	0.3	797.2	3.06
9	0.69	0.2	0.72	30.1	4.08	0.77	3.2	936.6	3.59
10	0.77	3.2	0.83	54.2	7.35	0.86	0.1	1714.6	6.57

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