



## HPTLC Analysis of the Leaf Extract of *Hydnocarpus pendulus* Manilal, Sabu & Sivarajan

Teny David<sup>1</sup> and K.V. George<sup>2\*</sup>

1, School of Environmental Sciences, M.G. University, Kottayam, (Kerala) - India

2, Department of Botany and Biotechnology, C.M.S College, Kottayam, (Kerala) - India

### Abstract

The present study was conducted to identify the major phytochemical compounds in the leaves of *Hydnocarpus pendulus* Manilal, Sabu & Sivarajan, an endemic species reported from Silent Valley National Park, Kerala, India. The methanolic extract of leaf was subjected to phytochemical screening by HPTLC. The analysis revealed that the leaf extract of *H. pendulus* is rich in phytochemical compounds like Alkaloids, Essential oils, Flavonoids, Flavonoid glycosides, Phenolics, Saponins, Steroids, Tannins and Triterpines.

Key-Words: *Hydnocarpus pendulus*, HPTLC, Phytochemical compounds, Endemic species

### Introduction

*Hydnocarpus* Gaertn. is an Indo-Malasian genus belonging to the family Flacourtiaceae<sup>1</sup>. Five species of *Hydnocarpus* viz., *H. alpina*, *H. kurzii*, *H. macrocarpa*, *H. pentandra* and *H. pendulus* are reported from India<sup>2-6</sup>. Out of the five species, *H. pentandra*, *H. macrocarpa* and *H. pendulus* are endemic to South India. *H. pentandra* is the most widely distributed species while *H. pendulus* is a newly reported species restricted to Silent Valley National Park, Palakkad, Kerala, India<sup>7</sup> (Fig. 1 & 2).

The 'Chaulmoogra' oil, which is used for the treatment of leprosy, is extracted from the seeds of *H. kurzii*<sup>8-9</sup> and other species of *Hydnocarpus* viz., *H. alpina* and *H. pentandra*. Earlier studies have revealed the presence of biologically active flavonolignan compounds in the fruit rind of *H. pentandra* (Syn: *H. wightiana*)<sup>10-14</sup>. However, the perusal of literature has revealed that no studies have so far been undertaken to explore the phytochemical constituents in the leaves of *H. pendulus*. Hence, the present study aims to analyse the major phytochemicals in the methanolic extract of the leaves of *H. pendulus*



Fig. 1: *H. pendulus* twig with flowers



Fig. 2: *H. pendulus* twig with young fruit

### \* Corresponding Author

E.Mail: teny\_teny\_teny@yahoo.co.in,

kvgeorge58@yahoo.co.in

Mob.: 91-9447409557

### Material and Methods

#### Collection and Extraction of Plant materials

Fresh and uninfected leaves from the source plant were collected from Silent Valley National Park, Palakkad, Kerala, India. Prior permission was obtained for the collection of plant materials from the Department of

Forest and Wildlife, Government of Kerala. The voucher specimen (Voucher No. TD076) is deposited in the CMS herbarium, C.M.S College Kottayam, Kerala, India. The collected materials were thoroughly washed, cleaned, oven dried and powdered.

The powdered leaves (20 g) were extracted in 200 ml 100 % methanol in a Soxhlet apparatus for 8 hours. 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<sub>254</sub> 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 °C 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 10 mm 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 acetate: Acetic acid: Formic acid: Water (10:1.1:1.1:2.6)

**Flavonoids** - Toluene: Ethyl acetate: Formic acid (7:3:0.1)

**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 °C with a relative humidity of 60 ± 5 %. Ten millilitres of the mobile phase (5 ml 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 and Flavonoid glycosides** - 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 nm. 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. pendulus* with respect to Alkaloids, Essential oils, Flavonoids, Flavonoid glycosides, Phenolics, Saponins, Steroids, Tannin and Triterpenes are given below.

#### Alkaloids

The analysis revealed the presence of seven alkaloid bands with specific R<sub>f</sub> Values which ranges from 0.10 to 0.76. The highest concentration (41.68 %) was noticed with respect to the alkaloid band at R<sub>f</sub> 0.13 (Fig. 1, Table 1, Plate 1).

#### Essential oil

Eight bands with specific R<sub>f</sub> Values were obtained with respect to Essential oil components. The R<sub>f</sub> values

ranges from 0.03 to 0.62. The Essential oil component with highest concentration (29.67 %) was detected at  $R_f$  0.45 (Fig. 2, Table 2, Plate 2).

#### Flavonoid Glycosides

HPTLC analysis revealed the presence of six flavonoid glycoside bands in the leaf extract. The  $R_f$  values ranges from 0.10 to 0.75. The highest concentration (66.26 %) was detected at  $R_f$  0.75 (Fig. 3, Table 3, Plate 3).

#### Flavonoids

Nine prominent Flavonoid bands with specific  $R_f$  Values were identified from the leaf extract. The  $R_f$  values ranges from 0.08 to 0.83. The flavonoid band with  $R_f$  value 0.18 shows the highest (35.02 %) concentration (Fig. 4, Table 4, Plate 4).

#### Phenolics

Eight Phenolic bands were identified. The  $R_f$  values ranges from 0.13 to 0.78 in which the highest concentration (58.64 %) was noticed at  $R_f$  0.48 (Fig. 5, Table 5, Plate 5).

#### Saponins

As revealed from the analysis, five Saponin bands were detected. The  $R_f$  Values ranges from 0.14 to 0.77 and the highest concentration (68.45 %) was detected at  $R_f$  0.43 (Fig. 6, Table 6, Plate 6).

#### Steroids

Eleven Steroid compounds were detected in the present analysis. The  $R_f$  Values of these compounds range from 0.06 to 0.68. The steroidal compound with  $R_f$  0.25 shows the highest (36.67 %) concentration (Fig. 7, Table 7, Plate 7).

#### Tannins

With respect to tannin, six bands were detected with different  $R_f$  values that ranges from 0.11 to 0.63. The tannin band with highest concentration (85.06 %) was identified at  $R_f$  0.26 (Fig. 8, Table 8, Plate 8).

#### Triterpenes

Thirteen Triterpene bands with  $R_f$  values 0.08 to 0.81 were detected in the leaf extract of *H. Pendulus*. The bands detected with respect to  $R_f$  0.31 (26.92 %) and 0.22 (25.82 %) represent prominent triterpene compounds (Fig. 9, Table 9, Plate 9).

Phytochemicals are used as potential therapeutic drugs in Ayurveda, Siddha, Unani, Homeopathy, Tribal medicines and in modern medicines. Phytochemicals reported from related species of *H. pendulus* are biologically active. Earlier research studies have revealed the pharmacological importance of Flavonoids and Flavonolignan compounds reported from *H. pentandra*<sup>15</sup>. The compounds like Hydnowightin, Hydnocarpin and neohydnocarpin have shown significant biological activity which help to reduce

serum cholesterol and triglyceride level<sup>15</sup>. They also help against human colon adenocarcinoma and Hela S uterine/ murine L-1210 leukemia growth and also act as an anti-inflammatory agent<sup>15</sup>. The antidiabetic and antioxidant activity of the ethanolic extract of *H. pentandra* in mice has also been reported<sup>16</sup>. Earlier studies have shown Antimicrobial activity of Hydnocarpic acid<sup>17</sup>. It is reported that Hydnocarpic acid acts by being an antagonist of biotin<sup>17</sup>. The richness of various phytochemical compounds namely triterpenes (13 compounds), steroids (11 compounds) and flavonoids (9 compounds) offer ample scope for the characterisation of biologically active phytochemicals.

#### Conclusion

The present study reveals that the leaves of *H. pendulus* contains Alkaloids, Essential oils, Flavonoid glycosides, Flavanoids, Phenolics, Saponins, Steroids, Tannin and Triterpenes. The diversity of phytochemicals in the *H. pendulus* makes this plant a potential source for further phytochemical and pharmacological investigation.

#### Acknowledgement

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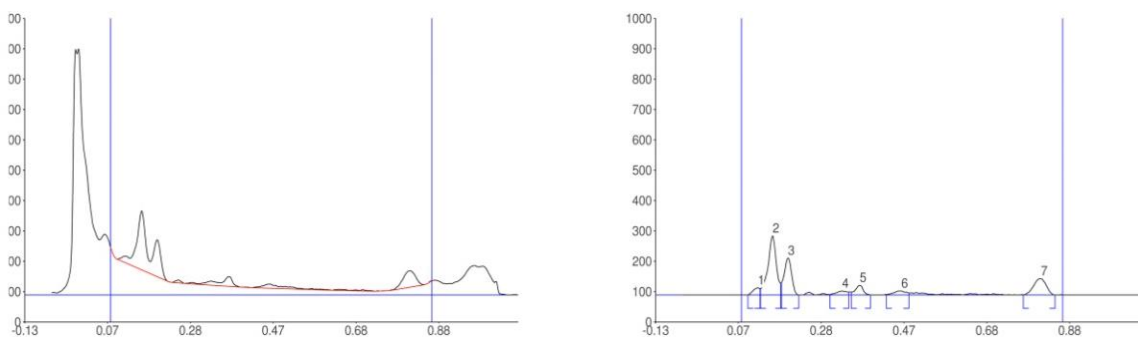


Fig. 1: Chromatogram of Alkaloids in the Methanolic leaf extract of *H. pendulus*

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	0.10	0.2	0.12	23.7	5.25	0.13	21.1	338.8	4.01
2	0.13	21.3	0.16	194.4	43.02	0.18	39.4	3518.7	41.68
3	0.18	40.3	0.19	121.4	26.87	0.22	0.0	1938.1	22.96
4	0.30	1.6	0.33	13.2	2.93	0.34	10.0	305.0	3.61
5	0.35	9.7	0.37	31.8	7.04	0.39	0.2	540.5	6.40
6	0.43	1.8	0.47	13.5	2.99	0.49	6.5	355.0	4.21
7	0.76	0.8	0.81	53.8	11.89	0.84	0.0	1446.6	17.13

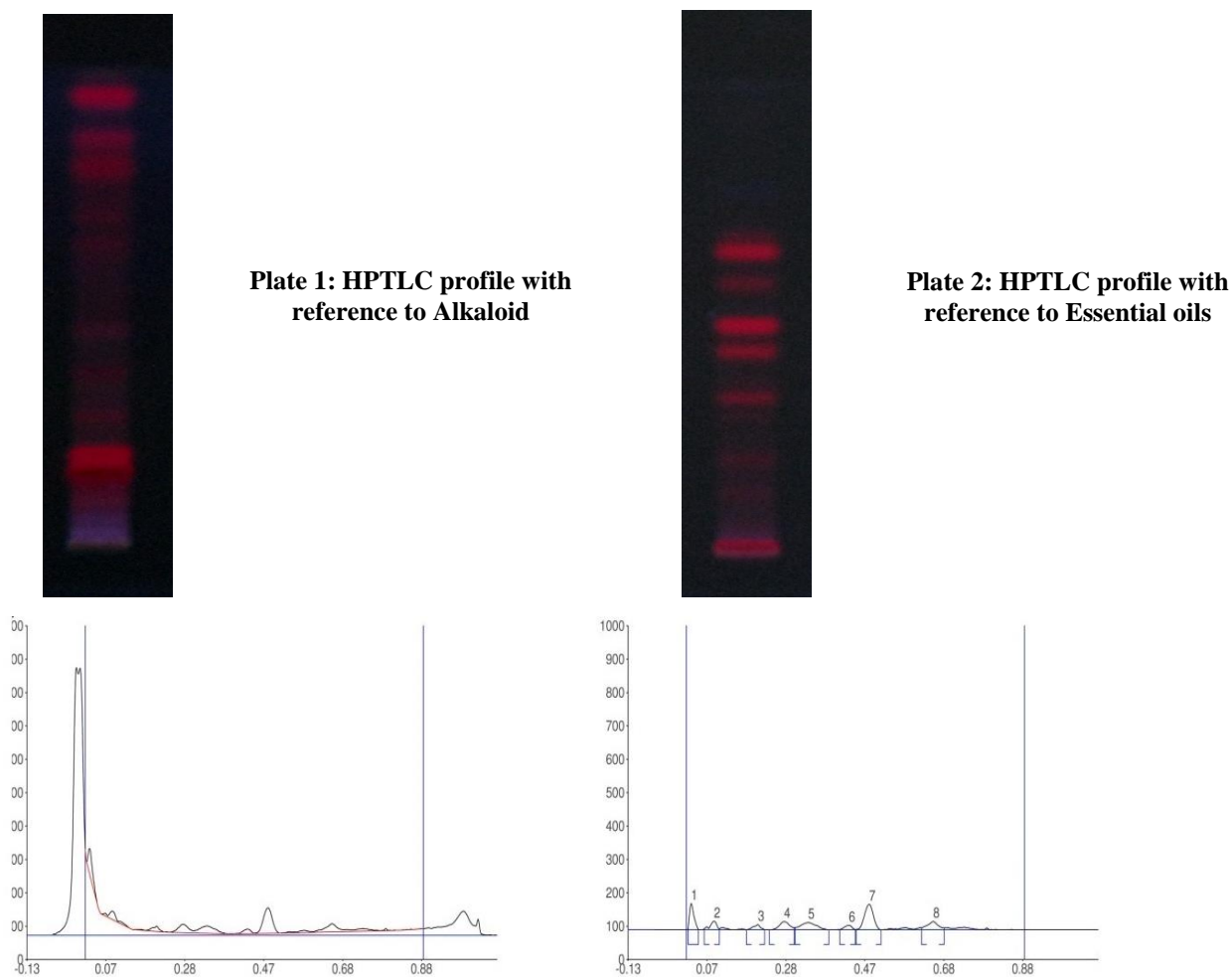


Fig. 2: Chromatogram of Essential oils in the Methanolic leaf extract of *H. pendulus*

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	0.03	4.9	0.03	78.9	27.95	0.05	1.1	783.5	15.30
2	0.07	3.5	0.09	25.8	9.12	0.11	3.4	383.6	7.49
3	0.17	0.5	0.20	16.0	5.65	0.22	0.3	246.5	4.81
4	0.23	0.1	0.27	24.7	8.76	0.30	6.6	567.4	11.08
5	0.30	6.7	0.33	21.9	7.76	0.38	0.2	768.9	15.01
6	0.41	1.0	0.43	13.7	4.85	0.45	0.9	229.9	4.49
7	0.45	1.0	0.49	76.3	27.02	0.52	0.0	1519.7	29.67
8	0.62	6.6	0.65	25.1	8.88	0.68	6.2	622.4	12.15

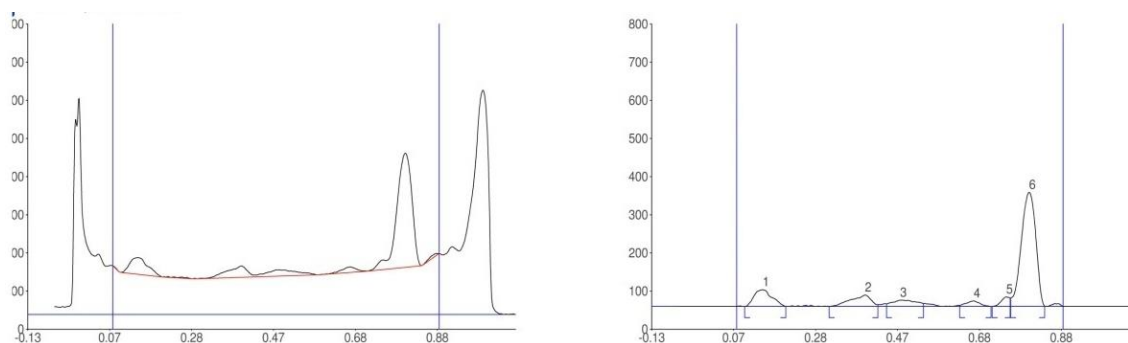


Fig. 3: Chromatogram of Flavanoid glycosides in the Methanolic leaf extract of *H. pendulus*

Table 3: Result of HPTLC scanning with reference to Flavanoid glycosides

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.10	0.2	0.15	43.9	10.25	0.20	0.0	1688.1	12.02
2	0.31	0.2	0.40	30.0	6.99	0.43	4.6	1302.9	9.28
3	0.45	7.0	0.48	16.7	3.90	0.54	8.3	868.3	6.18
4	0.63	3.0	0.66	14.3	3.34	0.70	0.0	431.1	3.07
5	0.71	0.2	0.74	24.7	5.77	0.75	22.5	449.7	3.20
6	0.75	22.5	0.80	298.9	69.75	0.84	0.7	9307.3	66.26

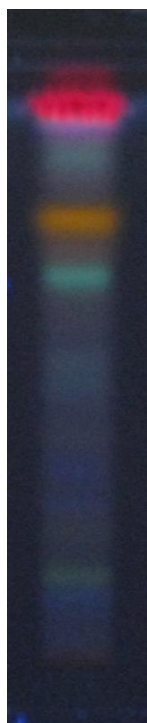


Plate 3: HPTLC profile with reference to Flavanoid glycosides



Plate 4: HPTLC profile with reference to Flavanoids

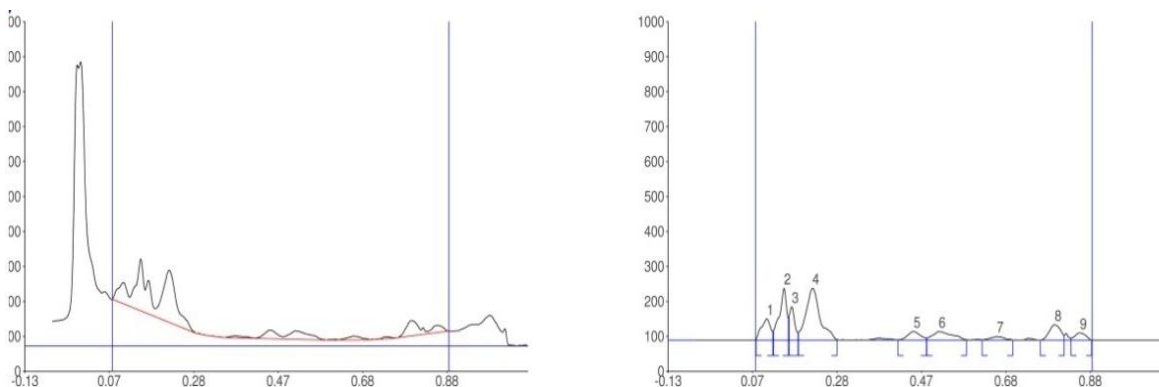


Fig. 4: Chromatogram of Flavanoids in the Methanolic leaf extract of *H. pendulus*

Table 4: Result of HPTLC scanning with reference to Flavanoids

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.08	2.9	0.11	60.6	10.49	0.12	25.9	1170.5	9.49
2	0.12	27.3	0.15	148.5	25.71	0.16	64.1	2291.5	18.58
3	0.16	67.8	0.17	95.7	16.58	0.18	22.5	1047.0	8.49
4	0.18	23.2	0.22	148.5	25.72	0.28	2.2	4319.7	35.02
5	0.42	1.0	0.46	24.8	4.29	0.49	6.6	658.8	5.34
6	0.49	6.7	0.52	24.4	4.22	0.58	1.5	1020.5	8.27
7	0.62	0.9	0.65	10.5	1.82	0.69	0.2	301.5	2.44
8	0.76	0.2	0.79	44.0	7.63	0.81	13.5	1051.3	8.52
9	0.83	6.3	0.85	20.5	3.55	0.88	0.4	473.8	3.84

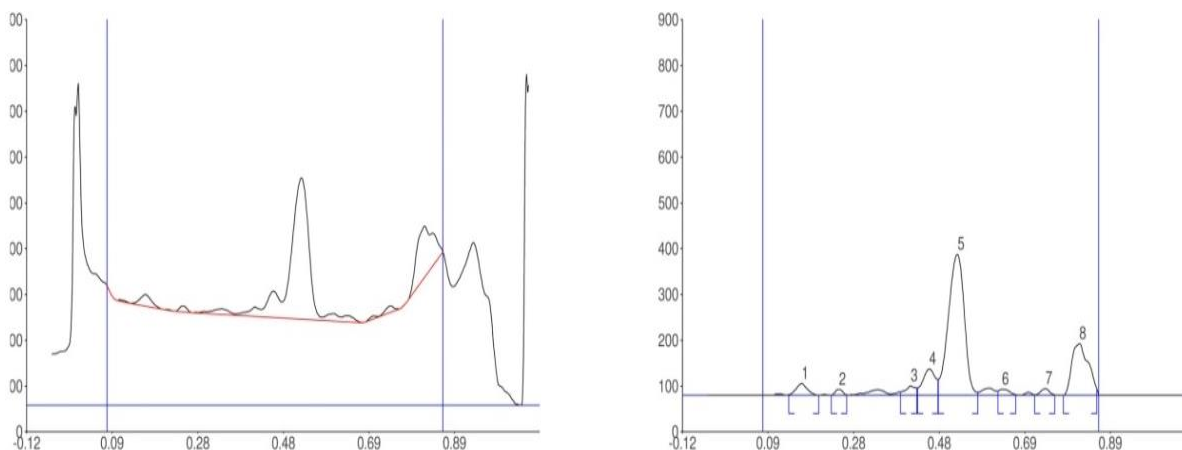


Fig. 5: Chromatogram of Phenolics in the Methanolic leaf extract of *H. pendulus*

Table 5: 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	0.13	1.4	0.16	25.5	4.52	0.20	0.3	656.9	3.47
2	0.23	0.6	0.25	13.0	2.31	0.27	1.0	218.5	1.15
3	0.40	7.4	0.42	20.7	3.66	0.43	16.0	480.8	2.54
4	0.44	16.2	0.46	57.4	10.16	0.48	34.8	1635.7	8.64
5	0.48	35.3	0.53	308.0	54.48	0.58	6.9	11096.9	58.64
6	0.62	9.2	0.63	13.2	2.33	0.67	1.0	321.5	1.70
7	0.71	1.0	0.74	14.6	2.59	0.76	0.7	290.2	1.53
8	0.78	0.1	0.81	112.9	19.96	0.85	15.2	4222.0	22.31

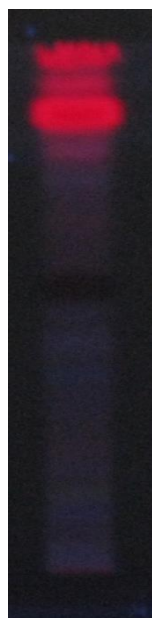


Plate 5: HPTLC profile with reference to Phenolics



Plate 6: HPTLC profile with reference to Saponins

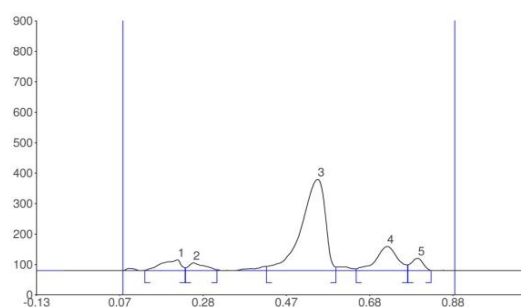
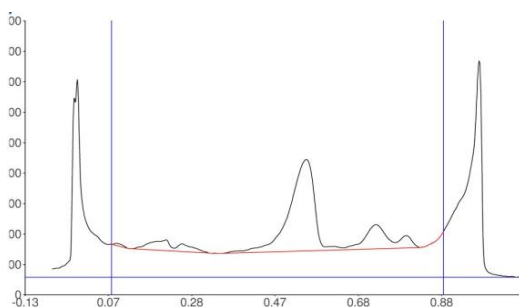


Fig. 6: Chromatogram of Saponins in the Methanolic leaf extract of *H. pendulus*



Table 6: 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	0.14	1.2	0.21	35.5	7.40	0.23	9.5	1479.3	6.76
2	0.23	9.6	0.25	25.5	5.30	0.31	3.0	869.1	3.97
3	0.43	14.3	0.55	299.8	62.39	0.59	11.7	14971.9	68.45
4	0.64	6.0	0.72	79.4	16.51	0.77	18.8	3536.0	16.17
5	0.77	19.1	0.79	40.4	8.40	0.82	0.0	1016.2	4.65

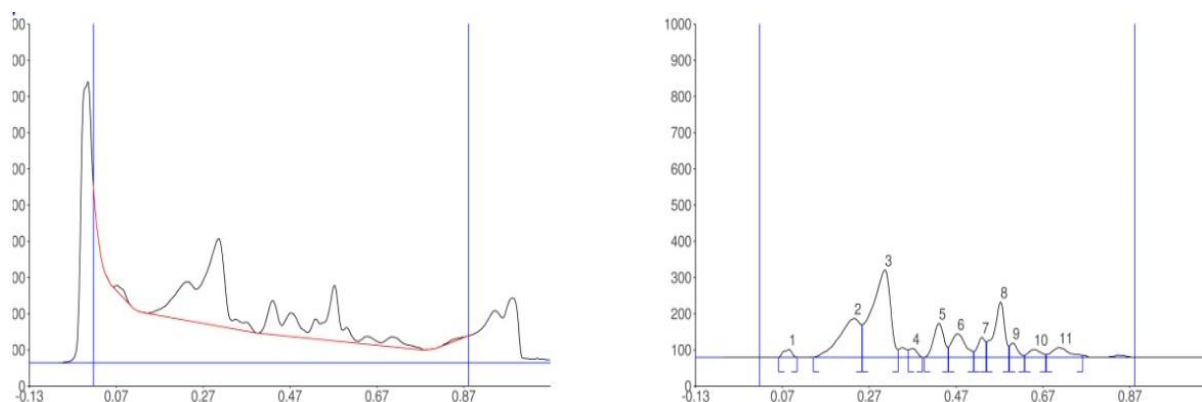


Fig. 7: Chromatogram of Steroids in the Methanolic leaf extract of *H. pendulus*

Table 7: 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	0.06	0.4	0.08	21.9	2.56	0.10	0.6	386.8	1.59
2	0.14	0.1	0.23	107.8	12.65	0.25	90.6	4582.3	18.84
3	0.25	90.6	0.30	241.9	28.37	0.33	22.4	8916.9	36.67
4	0.36	21.7	0.37	24.9	2.92	0.39	0.2	410.2	1.69
5	0.39	0.2	0.43	93.8	11.01	0.45	27.6	1959.0	8.06
6	0.45	28.3	0.47	65.0	7.63	0.51	16.2	1835.0	7.55
7	0.51	16.3	0.53	55.5	6.51	0.54	43.0	869.5	3.58
8	0.54	43.2	0.57	153.0	17.95	0.59	31.8	3207.3	13.19
9	0.59	32.0	0.60	39.6	4.64	0.63	5.7	625.1	2.57
10	0.63	5.9	0.65	21.9	2.57	0.67	8.1	549.9	2.26
11	0.68	8.1	0.71	27.3	3.20	0.76	5.8	976.6	4.02



Plate 7: HPTLC profile with reference to Steroids

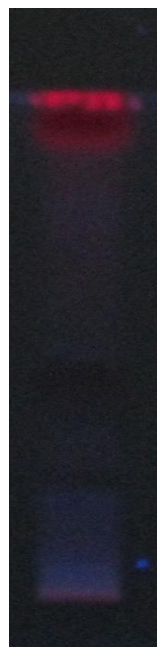


Plate 8: HPTLC profile with reference to Tannin

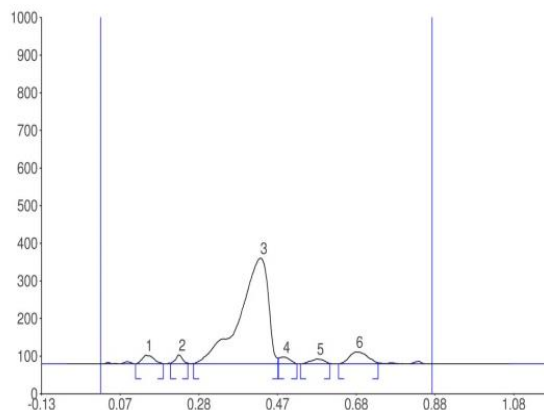
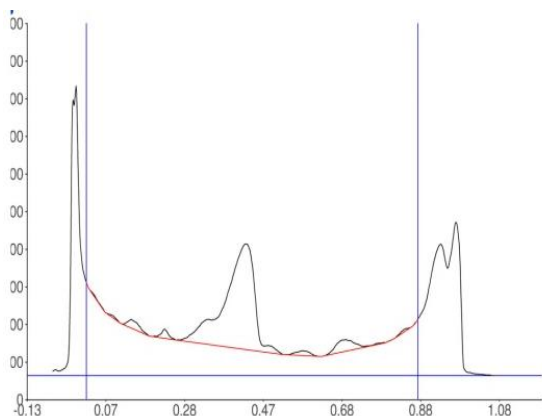


Fig. 8: Chromatogram of Tannin in the Methanolic leaf extract of *H. pendulus*

Table 8: Result of HPTLC scanning with reference to Tannin

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.11	0.0	0.14	22.9	5.85	0.18	0.2	612.4	2.89
2	0.20	1.9	0.22	24.2	6.18	0.25	2.3	385.5	1.82
3	0.26	1.1	0.43	280.9	71.82	0.48	15.5	18046.3	85.06
4	0.48	15.6	0.49	18.3	4.67	0.52	0.3	443.7	2.09
5	0.53	0.4	0.58	13.0	3.31	0.61	1.1	403.6	1.90
6	0.63	0.6	0.68	31.9	8.16	0.73	2.5	1324.1	6.24

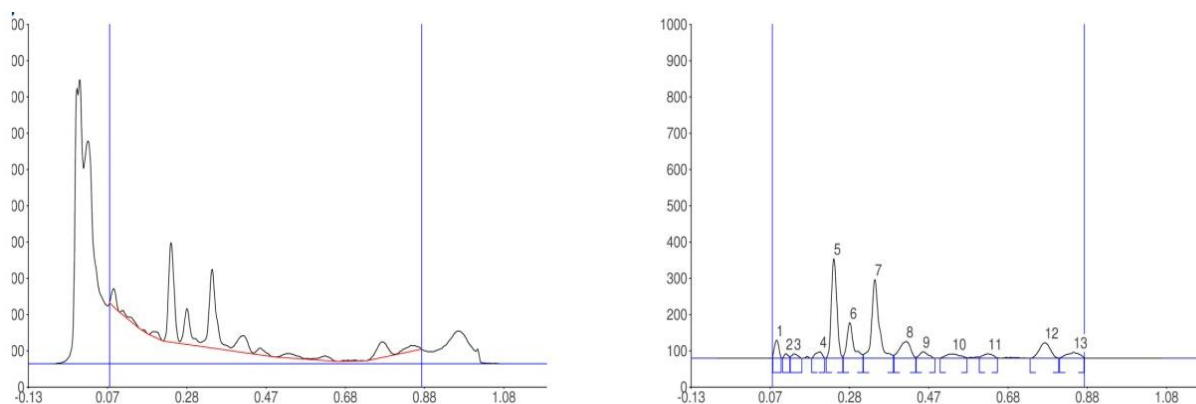


Fig. 9: Chromatogram of Triterpenes in the Methanolic leaf extract of *H. pendulus*

Table 9: Result of HPTLC scanning with reference to Triterpenes

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.08	6.9	0.09	49.8	5.99	0.10	1.9	495.6	3.78
2	0.11	1.2	0.11	12.3	1.48	0.12	5.0	114.7	0.88
3	0.13	5.2	0.13	12.2	1.47	0.15	0.1	165.8	1.27
4	0.18	0.4	0.20	17.8	2.14	0.21	0.6	270.5	2.07
5	0.22	0.9	0.24	274.3	32.99	0.26	5.1	3382.8	25.82
6	0.26	6.0	0.28	98.3	11.82	0.31	10.7	1454.2	11.10
7	0.31	10.9	0.34	216.9	26.09	0.39	8.1	3526.7	26.92
8	0.39	8.5	0.42	46.6	5.60	0.44	2.7	1135.5	8.67
9	0.44	2.8	0.46	18.5	2.23	0.49	0.0	344.3	2.63
10	0.50	0.6	0.54	12.2	1.47	0.57	1.8	385.8	2.94
11	0.60	3.8	0.63	12.9	1.55	0.65	1.0	286.5	2.19
12	0.73	0.3	0.77	43.0	5.17	0.80	1.4	1063.7	8.12
13	0.81	1.3	0.84	16.7	2.01	0.87	1.4	474.2	3.62

Plate 9: HPTLC profile with reference to Triterpenes

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