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Chromatographic Evaluation of Leaves and Stem Extracts of *Ventilago Maderaspatana* Graetn.

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Abstract

Chromatographic studies were carried out on leaves and stem extracts of *Ventilago maderspatana* Gaertn belonging to the family Rhamnaceae. It is an important and well known plant in the Indian system of medicine. Leaf and stem samples of *V. maderspatana* were subjected to maceration by using distilled water and ethanol. Aqueous and ethanolic extract were prepared from the leaf, stem of *Ventilago maderspatana*. Preliminary phytochemical analysis was carried out for all extracts. All extract were found to contain different phytoconstituents such as alkaloids, glycosides, steriods and sterols, saponins, flavonoids, carbohydrates, and tri- terpenoids and showed the absence of amino acids, proteins and acidic compounds. Extract were subjected to TLC and HPTLC analysis.

Keywords: *V.maderspatana; Leaf and Stem extract; Chromatographic evaluation*

Introduction

V. maderspatana (Rhamnaceae) is commonly known as Red creeper in English, Raktavalli in Sanskrit and pitti in Hindi [1]. It is climbing shrub and identified by it dark grey bark branch lets with brownish pubescent. It flowers in winter with an offensive odour [2,3]. It is found in Indonesia, Malaysia, Shri Lanka, Bhutan and throughout India [4,5]. The powdered root bark is carminative, stomachic, tonic and stimulant; useful in atomic dyspepsia, debility and slight cases of fever. The powdered bark (mixed with gingelly oil) is used in South India as external application for itch and other skin diseases [6]. The root bark is a valuable source of reddish dye (Ventilagin), used for colouring mordanted cotton, wool and tasar silk In combination with the root of Hedyotis umbellate, the root bark yields a beautiful chocolate colour. The bark also yields fibres, used for cordage. The pale yellow wood may be used as fuel. The long climbing stems are sometimes used by fishermen as substitute for ropes. The Seeds are eaten when cooked, and the oil from them is used for cooking [4]. Five isofuranonaphthoquinones have been isolated from the root bark [7]. Two new naphthalene derivatives and three naphthoquinones from the root bark have been isolated [8]. Antibacterial, Nitric Oxide In-Vitro and Ex-Vivo scavenging, anti-denaturation property and anti-oxidant activity of stem-bark of V. maderspatana was reported [9-11]. However, a pharmacognostic study on leaf of V. maderspatana was reported [12]. Pharmacognostic studies of the leaves and stem of Ventilago maderspatana Gaertn[13]. There are no literatures supporting antioxidant properties on leaves and stem extract of Ventilago maderspatana Gaertn. Therefore, the present study was carried out to evaluate antioxidant activity of the plant.

Materials and Methods

Materials

Petroleum ether, Chloroform, ethyl acetate, methanol etc. all the chemical were procured from sigma Aldrich (Germany).

Plant material

The Plant V. maderspatana (Stem and Leaves separately) was collected in the month of July from Tirupati district, Andhra Pradesh, India.The Plant V.maderspatana was authenticated by the botanist, Dr. K. Mahadavachetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati. Andhra Pradesh, India. Voucher No S.V.U./SC/10/26/10-11, and the specimen were deposited at the department of Pharmacognosy, JSSCP. Ooty. The plant material was subjected for garbling and cleaning processing to ensure the plant quality [14].

Extraction of selected plant material

The coarse powdered materials of the plant parts were subjected to the cold maceration. 500 gm.of leaf and stem of *V. maderaspatana* was extracted by cold maceration process in 3 liter round bottom flasks. For extraction, two solvents were used, ethanol and distilled water. The nature and yield of the extracts were noted. two extracts each of different parts of plant, were stored in a refrigerator at 4°C until further used and the extracts were labelled as *V. maderspatana* leaf ethanolic (VMLE), V.maderspatana leaf aqueous (VMLA), V. maderspatana stem ethanolic (VMSE), V. maderspatana stem aqueous (VMSA), respectively, for the purpose of convenient identification. Preliminary phytochemical analysis was carried out for all extracts [15-17]. All extract were found to contain different phytoconstituents such as alkaloids, glycosides, steroids and sterols, saponins, Flavonoids, carbohydrates, and tri- terpenoids and showed the absence of amino acids, proteins and acidic compounds, Results were mentioned in table 1.

Fractionation of aqueous and ethanolic leaves and stem extract of *Ventilago maderaspatana* Graetn.

The column chromatographic technique is for separation, isolation and purification of the natural products. The principle involved in this is the adsorption towards the adsorbent packed in the column. By changing the polarity of the mobile phase, the separation can be achieved in the column chromatography technique. Here fractions were prepared using solvent extraction techniques. Selection of the solvent was carried out based on their polarity.

1. Petroleum ether
2. Petroleum ether: Chloroform (1:1)
3. Chloroform
4. Chloroform: Ethyl Acetate (1:1)
5. Ethyl acetate
6. Ethyl acetate: Methanol (1:1)
7. Methanol
8. Water

The aqueous and ethanolic leaves and stem extracts of *Ventilago maderaspatana* Graetn, was subjected for fractionation using above mentioned solvents to separate the phytoconstituents. 8 fractions were prepared. The extracts were triturated with activated silica gel using pestle and mortar. After that the mixture was dissolved in the solvent of low polarity, stirred, heated moderately (luke warm), and filtered. The filtrated solution was collected in a lebelled conical flask, and the marc was air dried, which was used for the next solvent system of high polarity. The process was followed according to polarity of solvent system as mentioned above.

Chromatographic studies

Thin Layer Chromatography **[18]**

TLC is a very effective technique for the separation of chemical constituents of an extract and for their identification. TLC profile developed for an extract and its fractions using a defined solvent system and other parameters could be used as fingerprints in comparative qualitative evaluation of herbal drugs. The trend of evaluation by this method is becoming popular in view of its simplicity and reproducibility.

TLC is an important analytical tool in the separation, identification and estimation of different classes of natural products. In this technique, the different components are separated by the differential migration of solute between two phases – a stationary phase and a mobile phase. Here, the principle of separation is adsorption and the stationary phase acts as an adsorbent. Depending on the particular type of stationary phase, its preparation and use with different solvent, separation can be achieved on the basis of partition or a combination of partition and adsorption.

Separation of components

The extract was dissolved in respective solvents separately and spotted using a calibrated capillary tube on a prepared TLC plate 1 cm above from the bottom of the plate. The spot was equally sized

and had a diameter ranging from 2-3 mm. The method was followed for the crude extracts.

Selection of mobile phase:

The selection of solvent or mobile phase depends upon various factors as mentioned below.
Nature of substance to be separated
Nature of stationary phase (polar/non polar)
Mode of chromatography (normal/reverse phase)
Extent of separation to be achieved (analytical/preparative)

Based on the chemical tests and nature of phytoconstituents present, the solvent system was selected. The different spots developed in each system were detected by means of specific spray reagents and iodine staining. For this study, many solvent systems were used to detect the number of phytoconstituents present in the extracts. Precoated TLC plate of E – Merck was used for the study.

High performance thin layer chromatography (HPTLC) [19]

HPTLC is a modern chromatographic technique in which the principle of TLC is sophisticated and automated by which the samples are accurately and precisely estimated which can be utilized for both qualitative and quantitative purpose.

General procedures of HPTLC

Preparation of sample: The different solvent fractions of the alcoholic and aqueous crude extracts of the plant were filtered using whatman filter paper and used for study.

Application of sample: Commercially available precoated HPTLC plates (Merck) can be used for the study. The solutions of various concentrations should be applied on the respective HPTLC plates using Linomat IV applicator. The plates were dried after application. For this study Silica gel GF_{254} was used.

Application and development of plates: The 6µl sample solutions of the fractions were applied on the HPTLC plate using Linomat IV applicator. The plates were then dried after application and used.

Solvent system: The solvents were different for each fraction.

Detection: The developed plates first were observed under day light and UV light for the detection of constituents.

Densitometric scanning: The developed plates were scanned at a suitable wavelength for the qualitative analysis. Peak areas and peak heights were recorded from which the unknown concentration of the samples have been determined.

Results and Discussion

Phytochemical studies

The ethanolic and aqueous crude extracts of different parts of V.maderspatana and Z. xylopyrus were subjected to preliminary phytochemical screening for the detection of phytoconstituents. The results obtained were given below

Abbreviations used for different prepared extracts and are as follows

VMLE- *V. maderspatana* leaf ethanolic extract.
VMLA-*V. maderspatana* leaf aqueous extract.
VMSE- *V. maderspatana* stem ethanolic extract.
VMSA- *V. maderspatana* stem aqueous extract

Sr. No.	Phytochemical tests	Ventilago maderspatana			
		Leaf extract		Stem extract	
		VMLE	VMLA	VMSE	VMSA
1	Alkaloids	+	+	+	+
2	Carbohydrates	+	+	+	+
3	Steroids and sterols	+	-	+	-
4	Glycosides	+	+	+	+
5	Saponins	+	+	+	+
6	Protein and amino acids	-	-	-	-
7	Flavonoids	+	+	+	+
8	Phenolic	+	+	+	+
9	Acidic	-	-	-	-
10	Fixed oils	-	-	-	-
11	Triterpenoids	-	-	+	+

Table 1: *Preliminary phytochemical screening of the prepared extracts.*

TLC of different extracts of Ventilago maderaspatana Graetn.

HPTLC Finger print of the different extracts of Ventilago maderaspatana Graetn. Were taken and data's are recorded below in table 2

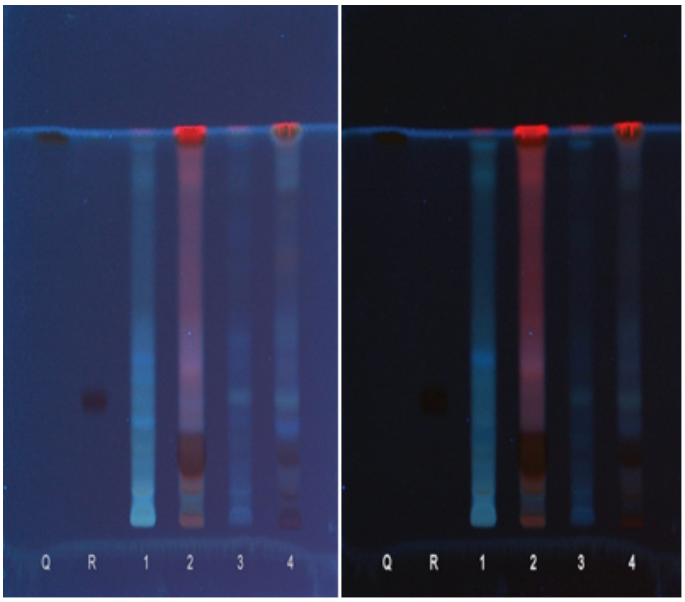
Solvent system which showed good separation in case of TLC was optimized and HPTLC work was carried out. On the basis of phytochemical tests, the solvent system of flavonoids was used

for the HPTLC fingerprinting of the different extracts of Ventilago maderaspatana Graetn.

Solvent System- Ethyl Acetate: Glacial Acetic Acid: Formic Acid: Water (100:11:11:26)

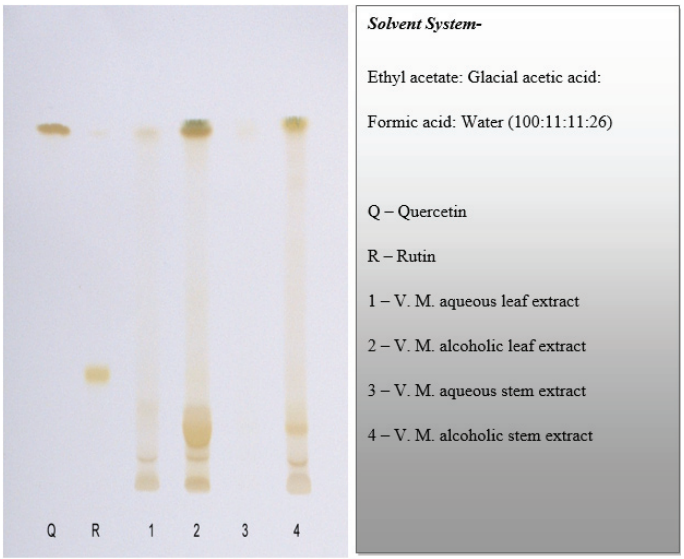
S. No.	Name of extract	No. of peaks/ spots	Rf values
1.	Alcoholic leaf extract	8	0.02, 0.07, 0.15, 0.29, 0.34, 0.52, 0.59, 0.94
2.	Aqueous leaf extract	5	0.02, 0.07, 0.20, 0.30, 0.94
3.	Alcoholic stem extract	9	0.02, 0.06, 0.15, 0.30, 0.42, 0.50, 0.75, 0.86, 0.96
4.	Aqueous stem extract	4	0.02, 0.07, 0.16, 0.30, 0.74, 0.94

Table 2: *HPTLC fingerprinting data for the different extracts of Ventilago maderspatana Graetn.*



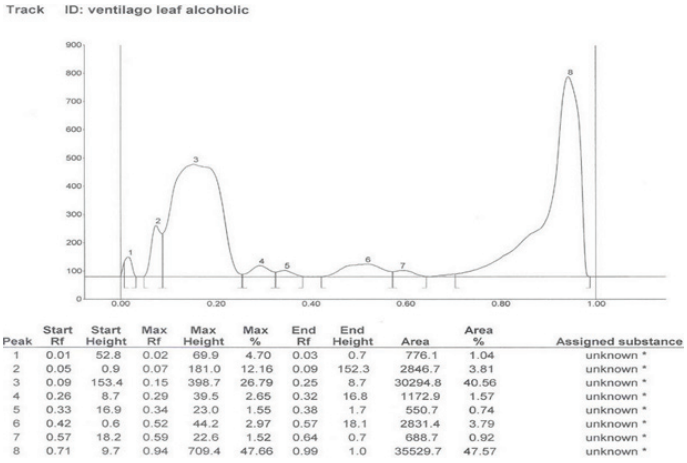
UV-254 nm UV-366 nm

Figure 1: *TLC of different extracts of Ventilago maderaspatana Graetn (In UV light).*



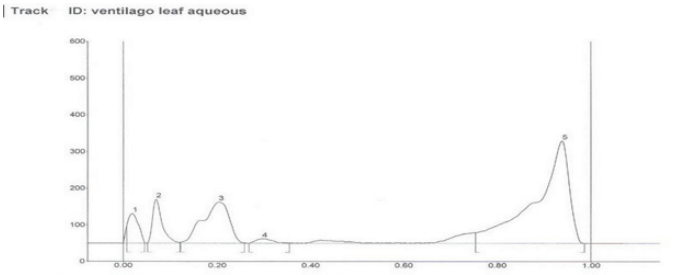
White Light HPTLC Parameters

Figure 2: *TLC of different extracts of Ventilago maderaspatana Graetn (in White light).*



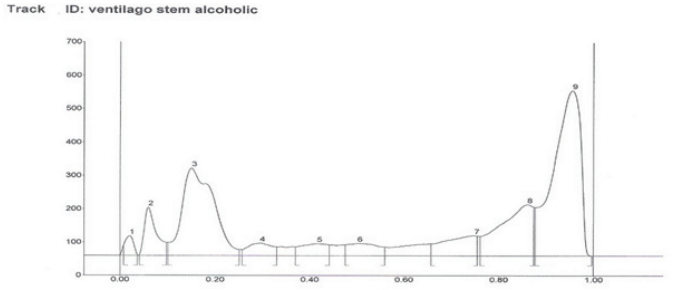
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	52.8	0.02	69.9	4.70	0.03	0.7	776.1	1.04	unknown *
2	0.05	0.9	0.07	181.0	12.16	0.09	152.3	2846.7	3.81	unknown *
3	0.09	153.4	0.15	398.7	26.79	0.25	8.7	30294.8	40.56	unknown *
4	0.26	8.7	0.29	39.5	2.65	0.32	16.8	1172.9	1.57	unknown *
5	0.33	16.9	0.34	23.0	1.55	0.38	1.7	550.7	0.74	unknown *
6	0.42	0.6	0.52	44.2	2.97	0.57	18.1	2831.4	3.79	unknown *
7	0.57	18.2	0.59	22.6	1.52	0.64	0.7	688.7	0.92	unknown *
8	0.71	9.7	0.94	709.4	47.66	0.99	1.0	35529.7	47.57	unknown *

Figure 3: *HPTLC of Ventilago maderaspatana Graetn. alcoholic leaf extract.*



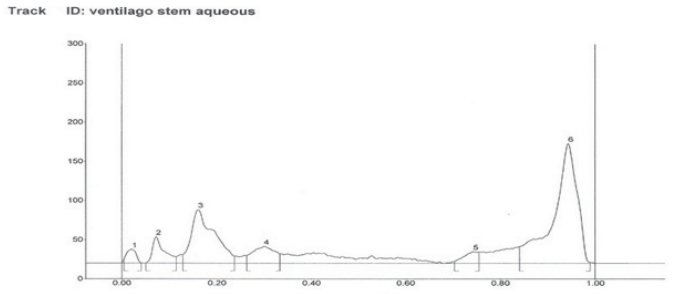
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	48.7	0.02	81.1	13.41	0.05	1.9	1395.2	5.93	unknown *
2	0.05	0.9	0.07	120.0	19.84	0.12	2.1	1847.1	7.86	unknown *
3	0.12	2.6	0.20	112.6	18.62	0.26	1.4	4814.3	20.48	unknown *
4	0.27	0.0	0.30	12.2	2.02	0.36	0.6	343.5	1.46	unknown *
5	0.75	29.0	0.94	278.8	46.11	0.99	0.2	15110.0	64.27	unknown *

Figure 4: *HPTLC of Ventilago maderaspatana Graetn. aqueous leaf extract.*



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	31.0	0.02	58.9	4.58	0.04	1.7	800.7	1.47	unknown *
2	0.04	2.3	0.06	144.2	11.23	0.10	38.8	2776.3	5.10	unknown *
3	0.10	39.0	0.15	262.5	20.43	0.25	18.0	13283.0	24.42	unknown *
4	0.26	18.4	0.30	37.3	2.90	0.33	26.5	1489.5	2.74	unknown *
5	0.37	26.5	0.42	35.7	2.78	0.44	32.2	1560.2	2.87	unknown *
6	0.48	32.4	0.50	36.4	2.84	0.56	26.5	1846.6	3.39	unknown *
7	0.66	35.8	0.75	60.3	4.69	0.76	59.1	3200.1	5.88	unknown *
8	0.76	57.8	0.86	152.9	11.90	0.88	146.0	8030.1	14.76	unknown *
9	0.88	145.1	0.96	496.4	38.64	1.00	0.2	21409.8	39.36	unknown *

Figure 5: *HPTLC of Ventilago maderaspatana Graetn. alcoholic stem extract.*



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	5.7	0.02	17.7	5.70	0.04	0.4	279.7	2.72	unknown *
2	0.05	0.1	0.07	34.0	10.96	0.11	8.2	636.0	6.18	unknown *
3	0.13	11.3	0.16	68.4	22.08	0.24	9.2	2528.0	24.57	unknown *
4	0.26	10.2	0.30	21.6	6.97	0.33	12.0	775.9	7.54	unknown *
5	0.70	2.1	0.74	14.8	4.79	0.76	14.3	349.7	3.40	unknown *
6	0.84	21.3	0.94	153.3	49.50	0.99	1.6	5719.9	55.59	unknown *

Figure 6: *HPTLC of Ventilago maderaspatana Graetn. aqueous stem extract.*

Conclusion

These parameter which reported for the first time, could be useful in isolation and identification of new lead molecule from the *V. maderspatana* plant. The results of the study can serve as a valuable source of information and provide suitable standards for identification of this plant material in future investigation and application.

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