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Pharmacognosy and HPTLC profiling of the stem bark of *Vachellia nilotica* (L.) P.J Hurter & Mabb. subsp. *indica* (Benth.) Kyal. & Boatwr

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Abstract
Pharmacognosy helps to authentify and standardise natural drugs. The present study attempts to characterise the bark of *Vachellia nilotica* (L.) P.J Hurter& Mabb. Subsp. *indica* (Benth.) Kyal. & Boatwr using microscopic characters, histochemical localisation, powder microscopic features, physico chemical parameters and HPTLC profiling. Bark showed the presence of cork, parenchymatous cortex, pericycle with lignified bands of stone cells, wide lignified phloem with prismatic calcium oxalate crystals and starch grains. Presence of tannin, starch and lignin were localised in the bark tissue. The HPTLC fingerprint of bark samples extracted in water, methanol + water (1:1), methanol, chloroform, acetone and petroleum ether using cold maceration technique and soxhlet method were obtained. Maceration method of extraction revealed maximum number of peaks in methanol+ water (1:1) extract while soxhlet method showed maximum peaks in acetone extract. Pharmacognostic parameters studied can be useful to lay down standards for the authentification of *V. nilotica* bark.

Keywords: *Vachellia nilotica*, anatomy, histochemical localisation, HPTLC, physicochemical localisation, powder microscopy

Introduction
The demand for of herbal medicines and botanicals have increased worldwide. This increasing demand have led to usage of poor quality raw materials and adulterants. Herbal medicines prepared from such raw materials can cause adverse effects on health [1]. In herbal medicinal products almost care should be taken to ensure that the selected plants are authenticated and free of adulterents or contaminants. High-quality herbal medicinal products can be generated only by the use of correctly authenticated raw materials which are rich in bioactive phytoconstituents [2]. Pharmacognosy helps to authentify and standardise natural drugs using morphology, histology, physico chemical parameters, phytochemical and molecular studies.
Vachellia nilotica which belongs to Fabaceae family is commonly known as babul, karuvelam, kikar or Indian gum Arabic tree. It is a medium sized tree and is distributed in tropical and sub tropical regions. Bark and gum exudates of the plant is of medicinal use. The bark is acrid, astringent, aphrodisiac, diuretic, expectorant, alexeteric, styptic, emollient, cooling, and anthelmintic**,** emetic and nutritive. It is useful in vitiated conditions of cough, bronchitis, haemorrhages, wound, ulcer, chronic dysentery, skin diseases and odontopathy [3]. Bark and pods were found to contain various secondary metabolites such as alkaloids, flavanoids, tannins and saponins. The objective of the present work was to evaluate microscopic characters, histochemical localisation, powder microscopic features, physico chemical parameters and HPTLC profile to characterise the plant material [4].

Materials and methods
Plant collection and extraction
The stem bark sample from a large sized tree of *V. nilotica* was collected from Chittur, Palakkad (district), Kerala, India (Figure 1). The sample was identified, authenticated and submitted (voucher specimen -Accession No:16689) in the Herbarium of Kerala Forest Research Institute, Thrissur, Kerala. The plant sample was washed thoroughly, dried under shade and ground to a fine powder in an electrical blender. The powder as such was used for microscopy and physicochemical characterization. For phytochemical study, two types of extracts in six different solvents were prepared using maceration technique and soxhlet method. The crude extracts in different solvents using maceration technique were prepared by extracting 10 g of dried plant powder in 100 ml solvent and kept on a rotary shaker for 24 hour [5]. The solvents used were water, methanol + water (1:1), methanol, chloroform, acetone and petroleum ether (W, MW, M, C, A, PE). The hot aqueous extracts of the powder were prepared in W, MW, M, C, A, PE using a soxhlet apparatus by continuous heating till decolourisation. The extracts were further concentrated in a rotary evaporator (Hanvapor HS-2005V, Hahnshin Scientific) and lyophilized.

Morphology
The macroscopic study was carried out by naked eye placing the plant material on a white paper surface. Distinct observable characters like colour, structure, texture and shape were noted. Simple microscope was used to confirm the characters noted by naked eye visual.

Microscopy
For anatomical study freehand sections of bark were taken and stained by safranin. For histochemical localisation sections were stained with sudan red, ferric chloride, iodine and phloroglucinol to detect the presence of oils, tannin, starch and lignin respectively. Powder microscopy was carried out to know about the inclusions and detailed

characters of the powdered material [6]. Microphotographs were taken using Trinocular 'Leica DM 3000' microscope attached with 'Leica DFC 295' digital camera connected to the computer and Leica Application Suite software was used for the observation and transferring microscopic images of the samples.

Physico chemical properties

Physicochemical parameters like total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive, ether soluble extractive and moisture content were determined as mentioned in Indian Pharmacopoeia[7].

HPTLC

For HPTLC fingerprinting analysis, 5µL of the plant samples were loaded in pre coated HPTLC plates [Silica gel 60F 254 (E. Merck KGaA) and plate size 5 cm x 10 cm].The sample loaded plates were kept in TLC twin trough a developing chamber (after saturated with solvent vapour) with respective mobile phases (flavonoids and phenols), solvent system (toluene: ethyl acetate: formic acid, 5:4:1, v/v/v), solvent front position 50.0 mm and volume 10 ml. The developed plate was dried by hot air at 60^0 C to evaporate solvents from the plate. The plate was kept in a photodocumentation chamber (CAMAG TLC Scanner 3) and the images were captured at UV 254 nm and UV 366 nm. The retention factor (Rf) values of fingerprint data were recorded by WINCAT software.

Results

Morphological characters

The outer surface of stem bark of *V. nilotica* was dark brownish black in colour both in fresh and dried form while the inner surface was off white, when fresh and caramel brown when dried. Thickness varied from 1-3 cm in thick. Outer surface of stem bark showed longitudinal fissures while the inner surface was fibrous (Figure 1).

Anatomical characters

T.S of stem bark of *V. nilotica* showed well-developed cork consisting of numerous layers of thin-walled, slightly flattened mostly rectangular cells with varying size and shape, arranged in radial rows filled with dark brown coloured contents. Cortical region was very narrow, parenchymatous. Pericyclic region was characterized with continuously running lignified bands of stone cells of various size and shapes. Phloem was very wide, traversed with uni to multiseriate elongated medullary rays. Phloem consisted of lignified phloem fibres and phloem parenchyma. Phloem tissues were filled with reddish or brown contents and were embedded with prismatic crystals of calcium oxalate, simple and compound starch grains (Figure 2).

Histochemical characters

In pericyclic region groups of lignified bands of stone cells were found. Phloem fibers were lignified and showed pink colour. Some parenchymatous cells in cortex, phloem region and multiseriate medullary ray cells showed lignification. Phloem parenchyma cells and medullary ray cells were filled with simple and compound starch grains which appeared blue with the stain. Bluish black and greyish black coloration indicated the presence of tannin (Figure 3).

Powder microscopy

Powder microscopy showed fragments of cork cells in surface and sectional view. Fragments of lignified fibres containing prismatic crystals of calcium oxalate were present. Lignified stone cells of various sizes and shapes, with narrow and wide lumen were seen. Fragments of cortical parenchyma cells filled with colored contents were found. Simple and compound starch grains with hilum were seen scattered throughout and embedded inside the parenchymatous cells (Figure 4).

Physico chemical parameters

The total ash content was found to be 6.8 %± 0.07.Acid insoluble ash was 1.9%± 0.08. Ethanol soluble extractive value was 18.25 % and water soluble extractive value was 8.73 %.

HPTLC

Different solvent compositions of the mobile phase for HPTLC analysis were examined to obtain high resolution and reproducible peaks. Distinct spots were visualised under UV 366 nm, 254 nm exposure and under day light after derivatisation (Figure 5 & 6). Chromatograms of the extracts were developed under chamber saturation conditions using toluene: ethyl acetate: formic acid (5:4:1, v/v/v) as the mobile phase. The HPTLC fingerprinting of *V. nilotica* using maceration method revealed several peaks. 4, 13, 8, 10, 11& 2 spots were observed at 5 µl of plant sample in W, MW, M, C, A & PE extracts respectively. Number of peaks obtained were maximum in methanol+ water (1:1) and minimum in petroleum ether. The peak area (%) in W, MW, M, C, A & PE extracts were 63.07, 67.67, 26.18,19.55, 23. 74 and 91.76respectively. The highest peak area (%) was found to be at Rf of 0.79 (Figure 7).

The HPTLC fingerprinting of *V. nilotica* using soxhlet method revealed several peaks. 5, 8, 7, 9 & 5spots were observed at 5 µl of plant sample in W, MW, M, C & A extracts respectively whereas no spots were observed in the petroleum ether extract. Number of peaks obtained were maximum in acetone. The peak area (%) in W, MW, M, C & A extracts were 66.56, 32.89, 19.92,19.64 and 48.30 6respectively. The highest peak area (%) was observed at Rf of 0.34 (Figure 8).

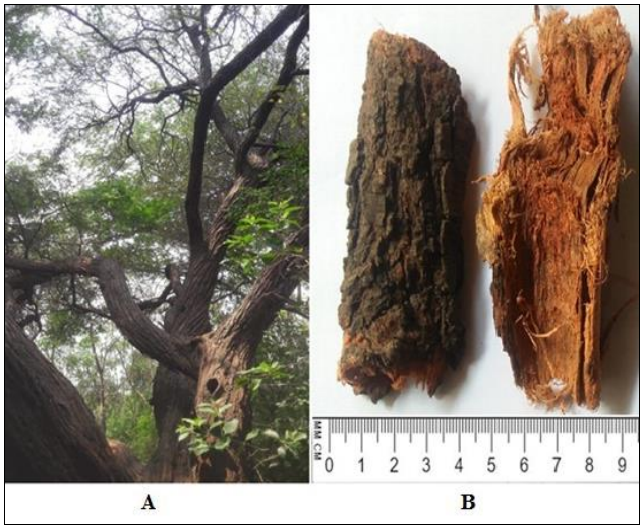


Fig 1: A. *V. nilotica habit* B. Outer and inner view of *V. nilotica* bark

Fig 2: Microphotographs of *V. nilotica* stem bark: A & B. TS of bark showing cork and phloem region; C. Stone cells; D. Phloem region.

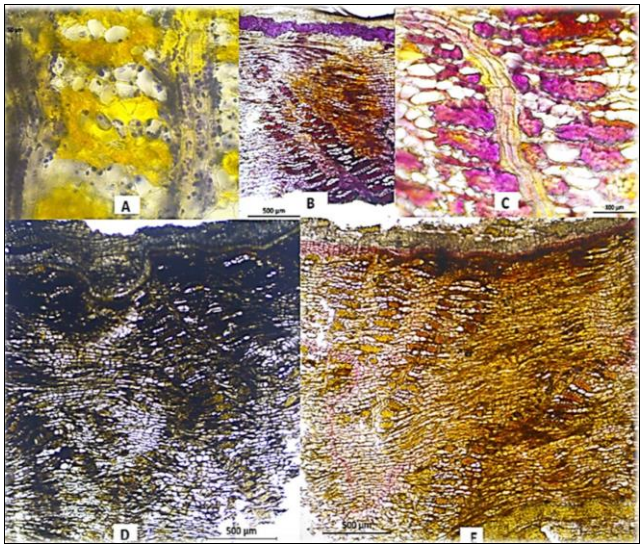


Fig 3: Histochemical test for *V. nilotica* stem bark: A. Histochemical test for oil globules; B. Histochemical test for tannin deposits; C & D. Histochemical test for starch; E & F. Histochemical test for lignin.

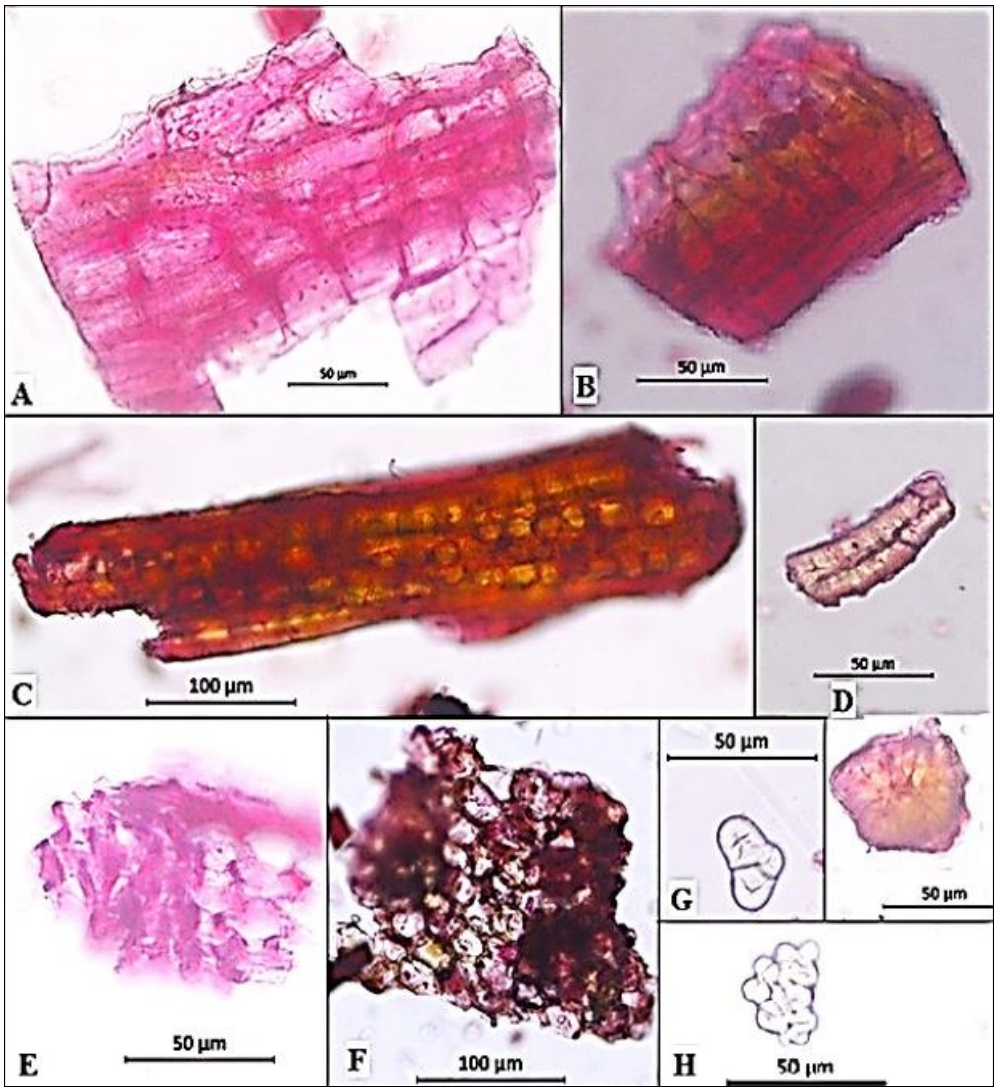


Fig 4: A. Cork cells in sectional view; B. Cork in sectional view with underlying cells; C. Fragments of fibres containing prismatic crystals of calcium oxalate; D. Stone cells; E. Parenchyma cells with coloured deposition; F. Parenchyma cells with starch grains and depositions; G. Groups of starch grains with hilum; H. Groups of starch grains

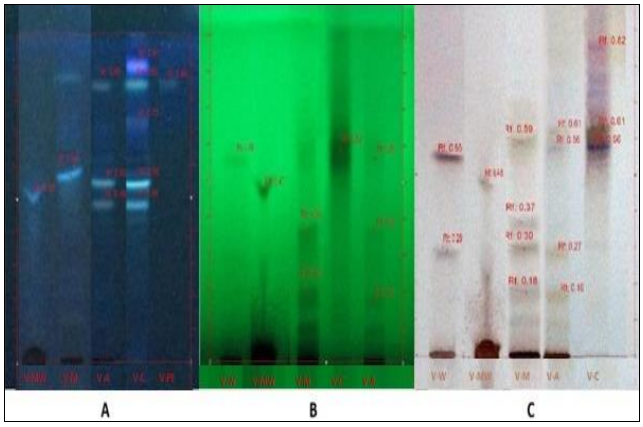


Fig 5: HPTLC profile of *V. nilotica* extracts (maceration) A: under UV 366; B: under UV 254; C: under daylight after derivation

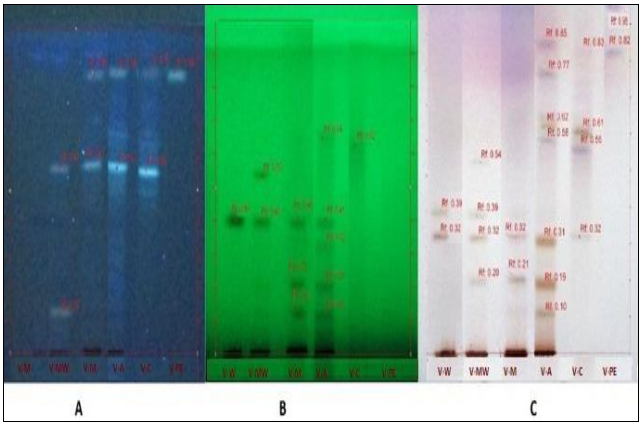
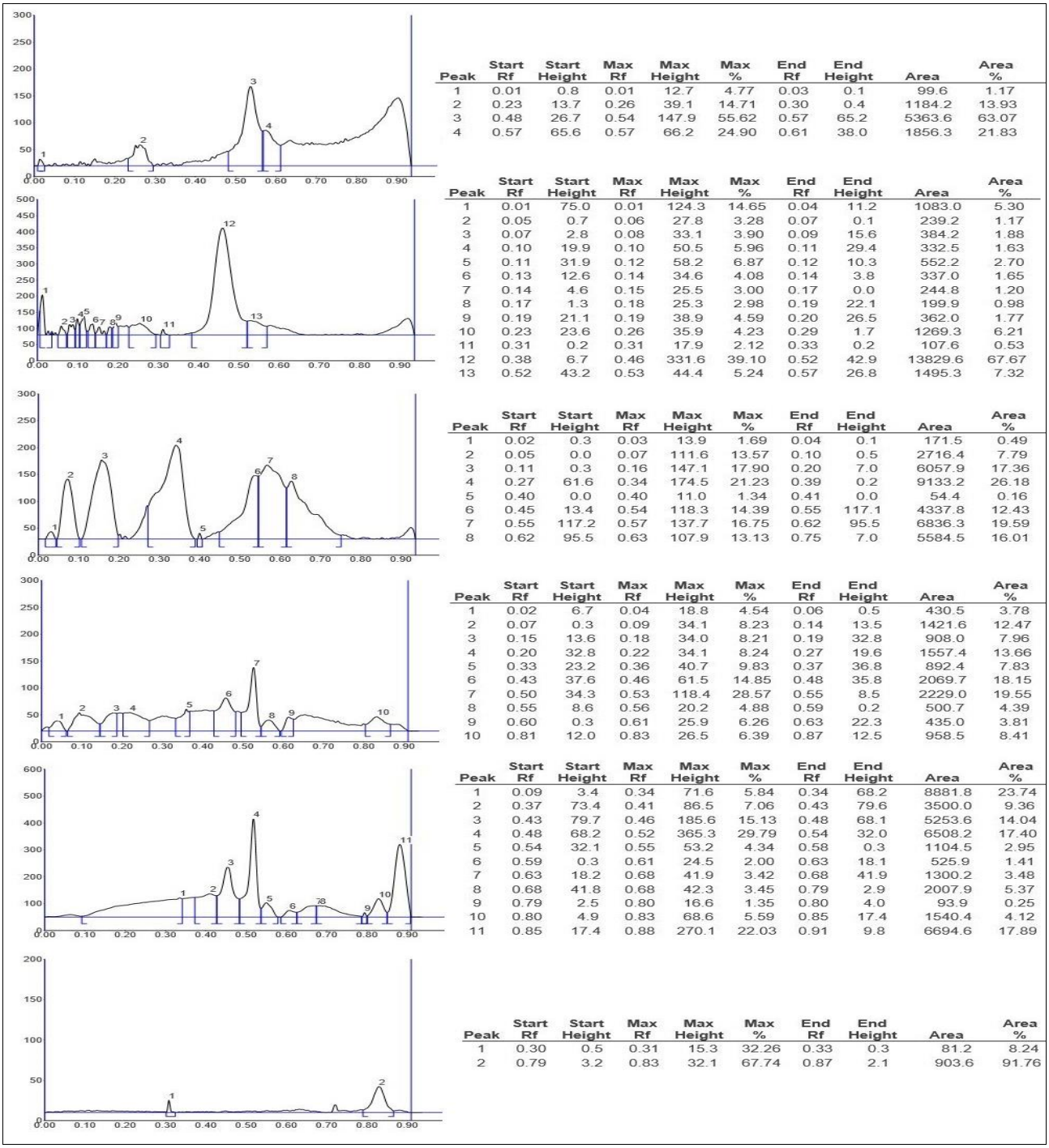


Fig 6: HPTLC profile of *V. nilotica* extracts (soxhlet) A: under UV 366; B: under UV 254; C: under daylight after derivation



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	0.8	0.01	12.7	4.77	0.03	0.1	99.6	1.17
2	0.23	13.7	0.26	39.1	14.71	0.30	0.4	1184.2	13.93
3	0.48	26.7	0.54	147.9	55.62	0.57	65.2	5363.6	63.07
4	0.57	65.6	0.57	66.2	24.90	0.61	38.0	1856.3	21.83

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	75.0	0.01	124.3	14.65	0.04	11.2	1083.0	5.30
2	0.05	0.7	0.06	27.8	3.28	0.07	0.1	239.2	1.17
3	0.07	2.8	0.08	33.1	3.90	0.09	15.6	384.2	1.88
4	0.10	19.9	0.10	50.5	5.96	0.11	29.4	332.5	1.63
5	0.11	31.9	0.12	58.2	6.87	0.12	10.3	552.2	2.70
6	0.13	12.6	0.14	34.6	4.08	0.14	3.8	337.0	1.65
7	0.14	4.6	0.15	25.5	3.00	0.17	0.0	244.8	1.20
8	0.17	1.3	0.18	25.3	2.98	0.19	22.1	199.9	0.98
9	0.19	21.1	0.19	38.9	4.59	0.20	26.5	362.0	1.77
10	0.23	23.6	0.26	35.9	4.23	0.29	1.7	1269.3	6.21
11	0.31	0.2	0.31	17.9	2.12	0.33	0.2	107.6	0.53
12	0.38	6.7	0.46	331.6	39.10	0.52	42.9	13829.6	67.67
13	0.52	43.2	0.53	44.4	5.24	0.57	26.8	1495.3	7.32

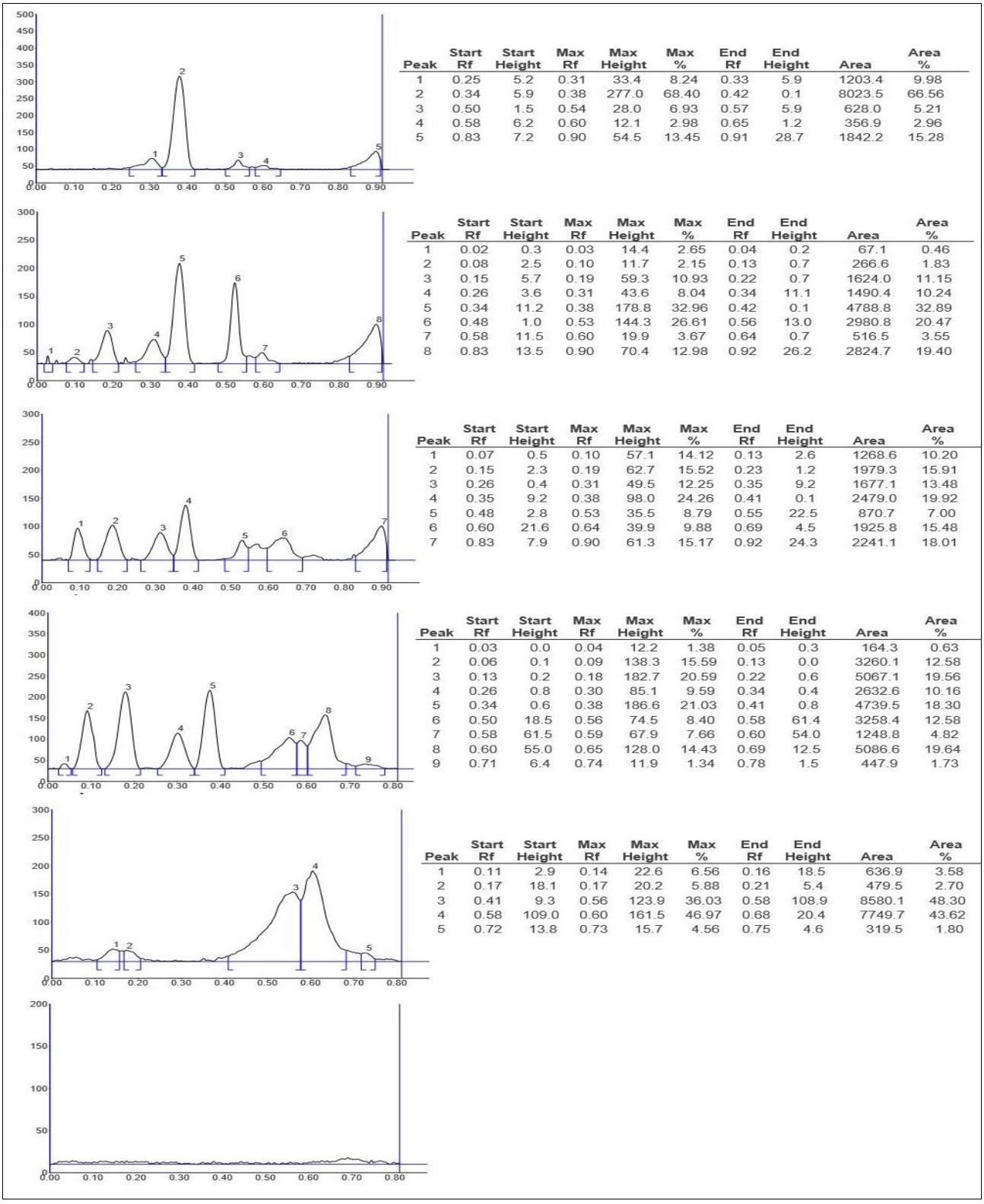
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.02	0.3	0.03	13.9	1.69	0.04	0.1	171.5	0.49
2	0.05	0.0	0.07	111.6	13.57	0.10	0.5	2716.4	7.79
3	0.11	0.3	0.16	147.1	17.90	0.20	7.0	6057.9	17.36
4	0.27	61.6	0.34	174.5	21.23	0.39	0.2	9133.2	26.18
5	0.40	0.0	0.40	11.0	1.34	0.41	0.0	54.4	0.16
6	0.45	13.4	0.54	118.3	14.39	0.55	117.1	4337.8	12.43
7	0.55	117.2	0.57	137.7	16.75	0.62	95.5	6836.3	19.59
8	0.62	95.5	0.63	107.9	13.13	0.75	7.0	5584.5	16.01

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.02	6.7	0.04	18.8	4.54	0.06	0.5	430.5	3.78
2	0.07	0.3	0.09	34.1	8.23	0.14	13.5	1421.6	12.47
3	0.15	13.6	0.18	34.0	8.21	0.19	32.8	908.0	7.96
4	0.20	32.8	0.22	34.1	8.24	0.27	19.6	1557.4	13.66
5	0.33	23.2	0.36	40.7	9.83	0.37	36.8	892.4	7.83
6	0.43	37.6	0.46	61.5	14.85	0.48	35.8	2069.7	18.15
7	0.50	34.3	0.53	118.4	28.57	0.55	8.5	2229.0	19.55
8	0.55	8.6	0.56	20.2	4.88	0.59	0.2	500.7	4.39
9	0.60	0.3	0.61	25.9	6.26	0.63	22.3	435.0	3.81
10	0.81	12.0	0.83	26.5	6.39	0.87	12.5	958.5	8.41

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.09	3.4	0.34	71.6	5.84	0.34	68.2	8881.8	23.74
2	0.37	73.4	0.41	86.5	7.06	0.43	79.6	3500.0	9.36
3	0.43	79.7	0.46	185.6	15.13	0.48	68.1	5253.6	14.04
4	0.48	68.2	0.52	365.3	29.79	0.54	32.0	6508.2	17.40
5	0.54	32.1	0.55	53.2	4.34	0.58	0.3	1104.5	2.95
6	0.59	0.3	0.61	24.5	2.00	0.63	18.1	525.9	1.41
7	0.63	18.2	0.68	41.9	3.42	0.68	41.9	1300.2	3.48
8	0.68	41.8	0.68	42.3	3.45	0.79	2.9	2007.9	5.37
9	0.79	2.5	0.80	16.6	1.35	0.80	4.0	93.9	0.25
10	0.80	4.9	0.83	68.6	5.59	0.85	17.4	1540.4	4.12
11	0.85	17.4	0.88	270.1	22.03	0.91	9.8	6694.6	17.89

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.30	0.5	0.31	15.3	32.26	0.33	0.3	81.2	8.24
2	0.79	3.2	0.83	32.1	67.74	0.87	2.1	903.6	91.76

Fig 7: HPTLC chromatogram of *V. nilotica* using maceration method with Rf value of peak, peak height and peak area



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.25	5.2	0.31	33.4	8.24	0.33	5.9	1203.4	9.98
2	0.34	5.9	0.38	277.0	68.40	0.42	0.1	8023.5	66.56
3	0.50	1.5	0.54	28.0	6.93	0.57	5.9	628.0	5.21
4	0.58	6.2	0.60	12.1	2.98	0.65	1.2	356.9	2.96
5	0.83	7.2	0.90	54.5	13.45	0.91	28.7	1842.2	15.28

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.02	0.3	0.03	14.4	2.65	0.04	0.2	67.1	0.46
2	0.08	2.5	0.10	11.7	2.15	0.13	0.7	266.6	1.83
3	0.15	5.7	0.19	59.3	10.93	0.22	0.7	1624.0	11.15
4	0.26	3.6	0.31	43.6	8.04	0.34	11.1	1490.4	10.24
5	0.34	11.2	0.38	178.8	32.96	0.42	0.1	4788.8	32.89
6	0.48	1.0	0.53	144.3	26.61	0.56	13.0	2980.8	20.47
7	0.58	11.5	0.60	19.9	3.67	0.64	0.7	516.5	3.55
8	0.83	13.5	0.90	70.4	12.98	0.92	26.2	2824.7	19.40

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.07	0.5	0.10	57.1	14.12	0.13	2.6	1268.6	10.20
2	0.15	2.3	0.19	62.7	15.52	0.23	1.2	1979.3	15.91
3	0.26	0.4	0.31	49.5	12.25	0.35	9.2	1677.1	13.48
4	0.35	9.2	0.38	98.0	24.26	0.41	0.1	2479.0	19.92
5	0.48	2.8	0.53	35.5	8.79	0.55	22.5	870.7	7.00
6	0.60	21.6	0.64	39.9	9.88	0.69	4.5	1925.8	15.48
7	0.83	7.9	0.90	61.3	15.17	0.92	24.3	2241.1	18.01

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.03	0.0	0.04	12.2	1.38	0.05	0.3	164.3	0.63
2	0.06	0.1	0.09	138.3	15.59	0.13	0.0	3260.1	12.58
3	0.13	0.2	0.18	182.7	20.59	0.22	0.6	5067.1	19.56
4	0.26	0.8	0.30	85.1	9.59	0.34	0.4	2632.6	10.16
5	0.34	0.6	0.38	186.6	21.03	0.41	0.8	4739.5	18.30
6	0.50	18.5	0.56	74.5	8.40	0.58	61.4	3258.4	12.58
7	0.58	61.5	0.59	67.9	7.66	0.60	54.0	1248.8	4.82
8	0.60	55.0	0.65	128.0	14.43	0.69	12.5	5086.6	19.64
9	0.71	6.4	0.74	11.9	1.34	0.78	1.5	447.9	1.73

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.11	2.9	0.14	22.6	6.56	0.16	18.5	636.9	3.58
2	0.17	18.1	0.17	20.2	5.88	0.21	5.4	479.5	2.70
3	0.41	9.3	0.56	123.9	36.03	0.58	108.9	8580.1	48.30
4	0.58	109.0	0.60	161.5	46.97	0.68	20.4	7749.7	43.62
5	0.72	13.8	0.73	15.7	4.56	0.75	4.6	319.5	1.80

Fig 8: HPTLC chromatogram of *V. nilotica* using soxhlet method with Rf value of peak, peak height and peak area

Discussion

Plants are widely used in the Indian system of medicine. Utmost care should be taken to ensure that the plant used is properly identified so that the expected result is obtained. World Health Organisation (WHO) suggests use of macroscopic and microscopic characters as the initial step towards plant identification [8]. Though various sophisticated tools for modern research are available, use of microscopy is considered as an easy and cheap method for plant identification [9].

Physicochemical parameters studied can be useful to detect adulteration. Values obtained were within the permissible limit [10].Total ash value indicates the amount of inorganic matter and acid insoluble ash value indicates the amount of siliceous matter like sand present in the bark.

After derivatization light yellow colored spot observed in the chromatogram indicated the presence of flavonoid and saponin [11]. Bluish purple colored spot observed in the chromatogram Blue-violet colored zones were detected from the chromatogram after derivatization confirmed the

presence of terpenoids [12]. The result were in conformity with the earlier phytochemical screening studies [13, 14].

HPTLC fingerprinting method have been used to determine the identity, stability, and consistency of herbal medicines and also identification of adulterants [15]. The HPTLC instrumentation can be used to produce fingerprint that can act as an authentic marker of a plant material. Quantitative variations may occur in HPTLC tracks of the same species but not qualitative [16]. Bark of most of the trees when dried have almost similar morphological appearance hence chances of adulteration is more. HPTLC chemical fingerprint of *V. nilotica* can be used to confirm the authenticity of the bark sample. Similar work helped in detecting substitution of plants used in herbal products that were different from those mentioned in the label of the product [17].

Conclusions

Present study has evaluated the anatomy, powder microscopic characters, histochemical localization and physico chemical parameters that can be useful to lay down standards for the authentification of the bark of this plant. HPTLC fingerprint obtained can further confirm the identity and quality evaluation of the plant.

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References

1. Zhang J, Wider B, Shang H, Li X, Ernst E. Quality of herbal medicines: challenges and solutions. Complementary therapies in medicine. 2012; 20(1-2):100-106.
2. Pferschy-Wenzig EM, Bauer R. The relevance of pharmacognosy in pharmacological research on herbal medicinal products. Epilepsy & behavior. 2015; 52:344-362.
3. Warrier PK, Nambiar VPK. Indian Medicinal Plants: A Compendium of 500 Species. Orient Longman Privte Limited, Chennai, 2005, 26.
4. Saini ML, Saini R, Roy S, Kumar A. Comparative pharmacognostical and antimicrobial studies of acacia species (Mimosaceae). Journal of Medicinal Plants Research. 2008; 2(12):378-386.
5. Parekh J, Chanda S. In vitro antibacterial activity of the crude methanol extract of Woodfordia fruticosa Kurz. Flower (Lythraceae). Brazilian Journal of Microbiology. 2007; 38(2):204-207.
6. Khandelwal KR. Practical pharmacognosy. Pragati Books Pvt. Ltd, 2008, 49-70.
7. Anonymous. The Indian Pharmacopoeia. 4th ed, Vol. II, The Controller of Publications, Government of India, New Delhi, 1996, A-53, A-54, A-089.
8. WHO. Quality control methods for medicinal plants. Geneva, 2002, 28-31.
9. Singh S, Machawal L, Chauhan MG. Pharmacognostic study of male leaves of Trichosanthes dioica Roxb. with special emphasis on microscopic technique. Journal of Pharmacognosy and Phytotherapy, 2010; 2(5):71-75.
10. Anonymous. The Ayurvedic Pharmacopoeia of India. Part I, Government of India, Ministry of Health and Family Welfare, Department of Indian Systems of Medicine & Homoeopathy, New Delhi, 2001, 1, 24.
11. Renukadevi KP, Sultana SS. Determination of antibacterial, antioxidant and cytotoxicity effect of Indigofera tinctoria on lung cancer cell line NCI-h69. International Journal of Pharmacolology. 2011; 7:356-362.
12. Taganna JC, Quanico JP, Perono RMG, Amor EC, Rivera WL. Tannin-rich fraction from Terminalia catappa inhibits quorum sensing (QS) in Chromobacteriumviolaceum and the QS-controlled biofilm maturation and LasAstaphylolytic activity in Pseudomonas aeruginosa. Journal of Ethnopharmacolology. 2011; 134(3):865-871.
13. Banso A. Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. Journal of Medicinal Plants Research. 2009; 3(2):082-085.
14. Sadiq MB, Hanpithakpong W, Tarning J, Anal AK. Screening of phytochemicals and in vitro evaluation of antibacterial and antioxidant activities of leaves, pods and bark extracts of *Acacia nilotica* (L.) Del. Industrial Crops and Products. 2015; 77:873-882.
15. Xie P, Chen S, Liang YZ, Wang X, Tian R, Upton R. Chromatographic fingerprint analysis—a rational approach for quality assessment of traditional Chinese herbal medicine. Journal of Chromatography. A, 2006; 1112(1-2):171-180.
16. Nicoletti M. HPTLC fingerprint: a modern approach for the analytical determination of botanicals. Revista Brasileira de Farmacognosi. 2011; 21(5):818-823.
17. Gallo FR, Multari G, Giambenedetti M, Federici E. Chemical fingerprinting of *Lawsonia inermis* L. using HPLC, HPTLC and densitometry. Phytochemical Analysis. 2008; 19(6):550-559.