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DETERMINATION OF TRITERPENOIDS AND ALKALOIDS BY HPTLC METHOD - MURVA (*MAERUA OBLONGIFOLIA)*

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ABSTRACT

Murva is an important ayurvedic drug used as one of the ingredients in many Ayurvedic preparations. *Maerua oblongifolia* (Forsk.) A. Rich. (Capparaceae) is one of the botanical sources of the Ayurvedic drug *Murva.* In present communication, an HPTLC method was developed for the standardization of *Murva* by quantitative estimation of major phytoconstituents as markers. The developed method was validated with respect to linearity, precision, accuracy, and robustness. Triterpenoids in alcohol extract and alkaloids in aqueous extract were identified by developing the plate in appropriate solvent systems.

INTRODUCTION:

Murva is an important ayurvedic drug used as one of the ingredients in many Ayurvedic preparations. *Maerua oblongifolia* (Forsk.) A. Rich. (Capparaceae) is one of the botanical sources of the Ayurvedic drug *Murva.* The accepted botanical source is *Marsdenia tenacissima*(Roxb.) Moon. *Ethanomedical survey reveals that Murva* is used to cure various diseases such as fever, stomach ache, skin infections, urinary calculii, diabetes mellitus, epilepsy, pruritis, rigidity in lower limbs, and abdominal colic. In India, the Ayurvedic systems of medicine has been existing for over three thousand years, *Charaka* and *Sushruta*, two of the earliest Indian scholars had sufficient knowledge of the properties of the Indian medicinal plants. The Vedas are the epic poems, which contain rich material on the herbal medicine of that time[1].The medicinal treatises like the *Charakasamhita* and *Sushrutasamhita* are esteemed even to this day as the treasures of literature on indigenous medicine [2]. *Murva* is a controversial drug. Amongst the many synonyms of this plant, one is *'Dha-nurgunopayogya'* meaning 'the plant whose bark is being used for the bow-strings'. These synonyms have also contributes to the existing confusion. The plant which has tough fibres is the *Murva*? There are many such fibre yielding plants are found in the veagetable kingdom. *Murva* is an important controversial drug used in diseases like anaemia (*pandu*); fever (*jwara*); diabetes (*prameha*); stomach disorders (*udara roga*); typhoid (*visama jwara*); urinary infection (*asmari*) and cough (*ksaya*) [3].

METHODOLOGY:

Collection of plant material *Maerua oblongifolia* (Forsk.) A. Rich

The plant material was collected from Kallakad forest in Tirunelveli district of Tamil Nadu on November 2007. The plant is a climber and occurs in hedges. They were washed thoroughly in running water, segregated from the extraneous material; subsequently preserved in small polythene bags. Two to three roots measuring about 5-9 cm were cut and deposited in a polythene bag. Sufficient quantity of 2% Formalin was poured into this bag to keep the specimen in fresh condition. The field data of the plant like its height, flower colour and soil condition were noted. The collection number was tagged on to the specimen.

Identification

The plant material collected was identified at the herbarium of Mother Theresa Post Graduate & Research Institute of Health Sciences, Puducherry and authenticated by DR.V.GOPAL Taxonomist and Research Guide. The taxonomic identification was carried out following Flora of Hassan (1976), Flora of Coorg (1990), Flora of Presidency of Madras (2005) and Flora of presidency of Bombay (2006)[4,5,6 &7].

HPTLC STUDIES: [8,9,10]

HPTLC is a technique used for separation and identification of components. The separation is based on the difference in adsorption coefficients of the individual components of a mixture and identification is based on the comparison of R_f values. Components which are strongly adsorbed on the stationary phase move up less readily than those which are adsorbed to a lesser extent, leading to the separation of the compound.

In the present work Camag HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar, with 12bit CCD camera for photo documentation, controlled by WinCATS- 4 software was used. All the solvents used were of HPLC grade obtained from MERCK. All weighing were done on Precisa XB 12A digital balance.

Preparation of alcohol extract solution: A solution of the alcohol extract in methanol was prepared by accurately weighing 100mg of the alcohol extract into a 10ml volumetric flask, dissolved and volume made up to 10ml with methanol.

Preparation of aqueous extract solution: A solution of the aqueous extract in water and methanol (1:1) was prepared by accurately weighing 100mg of the aqueous extract into a 10ml volumetric flask, dissolved and volume made up to 10 ml with water and methanol.

Chamber used for mobile phase: Camag twin trough chamber, (10 x 10 cm).

Preparation of mobile phase for alcohol extract: Mobile phase used for alcohol extract was toluene: chloroform: ethanol in the ratio of 28.5: 57: 14.5

Preparation of mobile phase for aqueous extract: Mobile phase used for aqueous extract was n- butanol: glacial acetic acid: water in the ratio of 7:1:3.

Stationary phase: TLC aluminium sheet pre-coated with silica gel G 60 F_{254}, 5 X 10cm (MERCK).

Preparation of iodine chamber: The chamber was taken and few pieces of iodine was added into it and cotton was kept inside. The chromatogram containing alcoholic extract after

development was derivatised with iodine by keeping it in iodine chamber and scanned at 425nm.

Procedure:

The TLC plates were activated by heating at 115˚C for about 30min prior to use. The alcohol extract solution (4µl and 8µl) were applied as 8mm bands on a coated silica gel G 60 F_{254} TLC plate, as tracks 1 and 2, with Linomat V applicator using a Hamilton syringe. Similarly the spots of 2µl and 4µl for aqueous extract were also applied on separate TLC plate. The mobile phase used for alcohol extract and aqueous extracts was toluene: chloroform: ethanol (28.5: 57: 14.5) and n- butanol: glacial acetic acid: water (7:1:3) respectively. No prewashing of the plates was done. Chamber saturation time was 1h. The TLC plates were kept for development, to a migration distance of 74mm and 66mm for alcohol extract and aqueous extract respectively. The developed plates were dried with hot air and scanned at wavelengths, 254nm, 366nm and at 425nm; band width 8mm, slit dimension 6.00 X 0.45nm, scanning speed 20nm/sec and source of radiation was deuterium, mercury and tungsten lamps respectively. The Rf and peak area were interpreted by using the software. The developed plates were photo documented under 254nm, 366nm and 425nm, using Camag Reprostar-3.

RESULTS :

HPTLC profile of the alcohol and aqueous extracts of *Maerua oblongifolia* was carried out. In this study the alcohol extract revealed 8 phytoconstituents under 254nm at R_f 0.10, 0.15, 0.19, 0.23, 0.29, 0.46, 0.53, 0.75 (fig. 1) out of these, spots at the R_f 0.09, 0.29, 0.53 are pronounced where as spots at the R_f 0.15, 0.19, 0.23, 0.46, 0.75 were less pronounced. The alcohol extract revealed 3 phytoconstituents under 366nm at R_f 0.12, 0.20, 0.42 (fig. 2). Out of these spot 0.20 is more pronounced where as 0.12, 0.42 are less pronounced. The alcohol extract revealed 3 phytoconstituents under 425nm at R_f 0.08, 0.20, 0.26 (fig. 3). Out of these spot 0.08 is more pronounced where as 0.20, 0.26 are less pronounced. The alcohol extract after kept in iodine chamber revealed 4 phytoconstituents under 425nm at R_f 0.12, 0.22, 0.26, and 0.31. Out of these 0.31 is more pronounced whereas 0.12, 0.22, 0.26 were less pronounced.

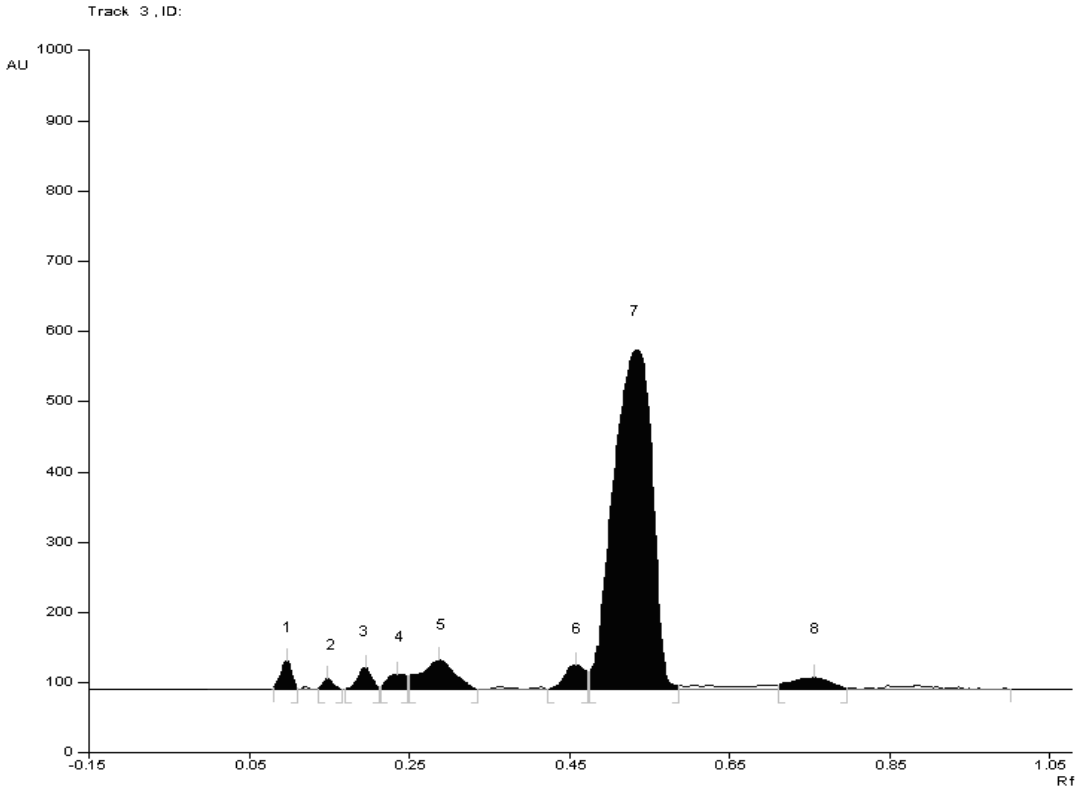
The aqueous extract revealed 8 phytoconstituents under 366nm were revealed at R_f of 0.12, 0.15, 0.20, 0.36, 0.49, 0.66, 0.77, 0.93 (fig. 4). Out of these spots at the R_f of 0.12, 0.36, 0.93 are pronounced where as spots at the R_f of 0.15, 0.20, 0.49, 0.66, 0.77 were least pronounced.

While 6 phytoconstituents under 425nm at R_f 0.232, 0.31, 0.49, 0.62, 0.70, 0.85 (fig. 5). Out of these spots at the Rf of 0.62, 0.70, and 0.85 were more pronounced where as spots at the Rf of 0.23, 0.31, 0.49 were less pronounced.

All spots in aqueous and alcohol extract were showing, black colour at 254nm, blue colour fluorescence at 366nm and light brown fluorescence under visible light.

Fig. 1: Chromatogram showing HPTLC profile of alcohol extract of *M.oblongifolia*

Under 254nm

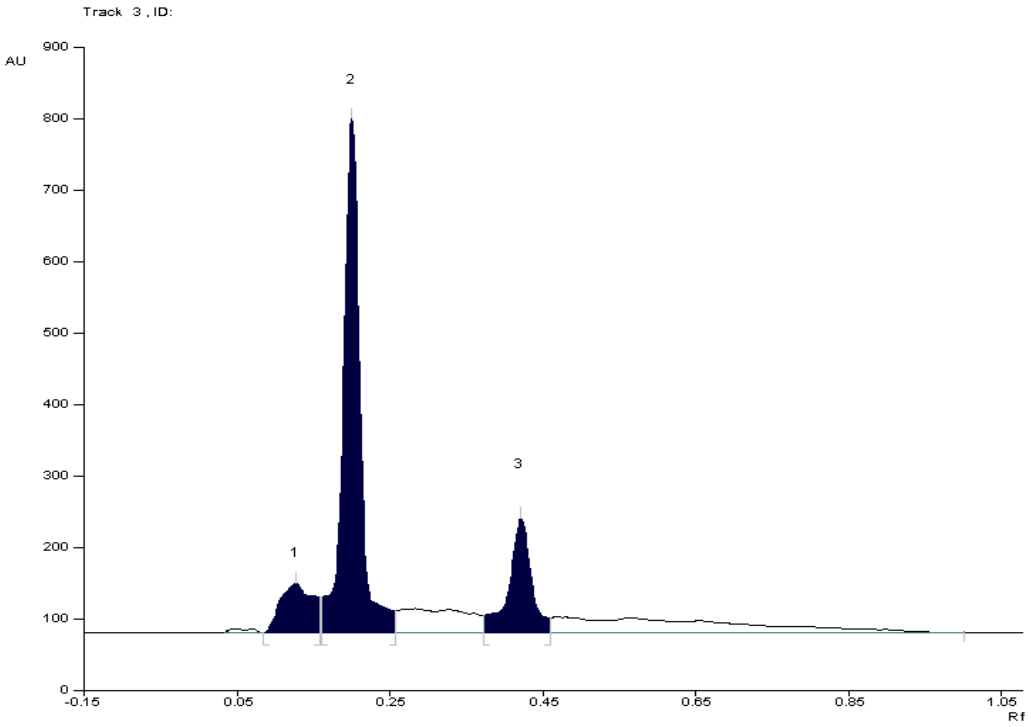


Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.08 Rf	2.5 AU	0.10 Rf	40.5 AU	5.00 %	0.11 Rf	1.8 AU	422.0 AU	1.07 %	unkrown *
2	0.13 Rf	0.5 AU	0.15 Rf	15.1 AU	2.20 %	0.16 Rf	0.2 AU	145.9 AU	0.68 %	unkrown *
3	0.17 Rf	0.2 AU	0.19 Rf	31.4 AU	4.57 %	0.21 Rf	3.3 AU	405.0 AU	1.89 %	unkrown *
4	0.21 Rf	3.5 AU	0.23 Rf	21.6 AU	3.14 %	0.25 Rf	19.6 AU	385.7 AU	1.80 %	unkrown *
5	0.25 Rf	19.7 AU	0.29 Rf	41.4 AU	6.02 %	0.33 Rf	0.0 AU	1318.4 AU	6.14 %	unkrown *
6	0.42 Rf	0.7 AU	0.46 Rf	35.4 AU	5.15 %	0.47 Rf	25.0 AU	686.0 AU	3.20 %	unkrown *
7	0.47 Rf	25.5 AU	0.53 Rf	485.1 AU	70.61 %	0.58 Rf	5.4 AU	17469.1 AU	81.41 %	unkrown *
8	0.71 Rf	5.7 AU	0.75 Rf	16.6 AU	2.42 %	0.80 Rf	2.1 AU	625.6 AU	2.92 %	unkrown *

Fig.2: Chromatogram showing HPTLC profile of alcoholic extract of *M.oblongifolia*

Under 366nm

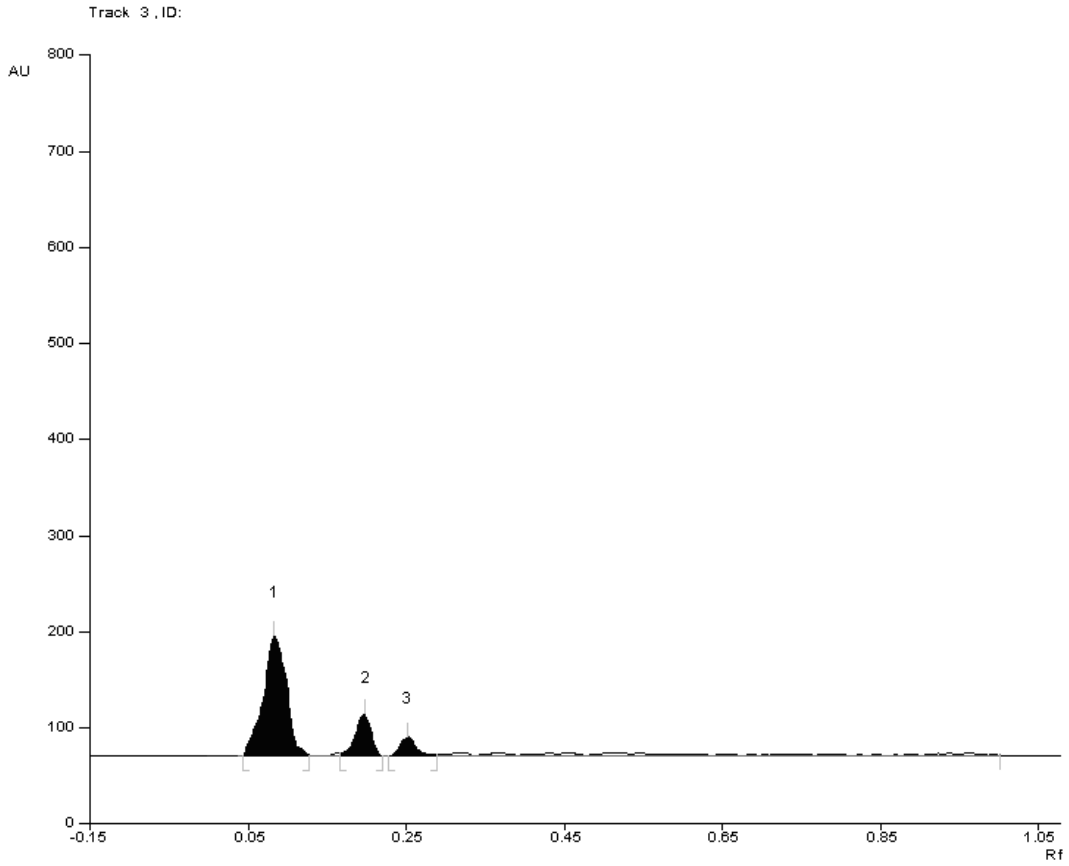


Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.08 Rf	0.0 AU	0.12 Rf	69.8 AU	7.35 %	0.16 Rf	50.5 AU	2213.9 AU	12.51 %	unknown *
2	0.16 Rf	50.5 AU	0.20 Rf	719.8 AU	75.84 %	0.26 Rf	30.8 AU	11940.7 AU	67.49 %	unknown *
3	0.37 Rf	24.6 AU	0.42 Rf	159.5 AU	16.81 %	0.46 Rf	21.2 AU	3539.1 AU	20.00 %	unknown *

Fig. 3: Chromatogram showing HPTLC profile of alcoholic extract of *M.oblongifolia*

Under 425nm

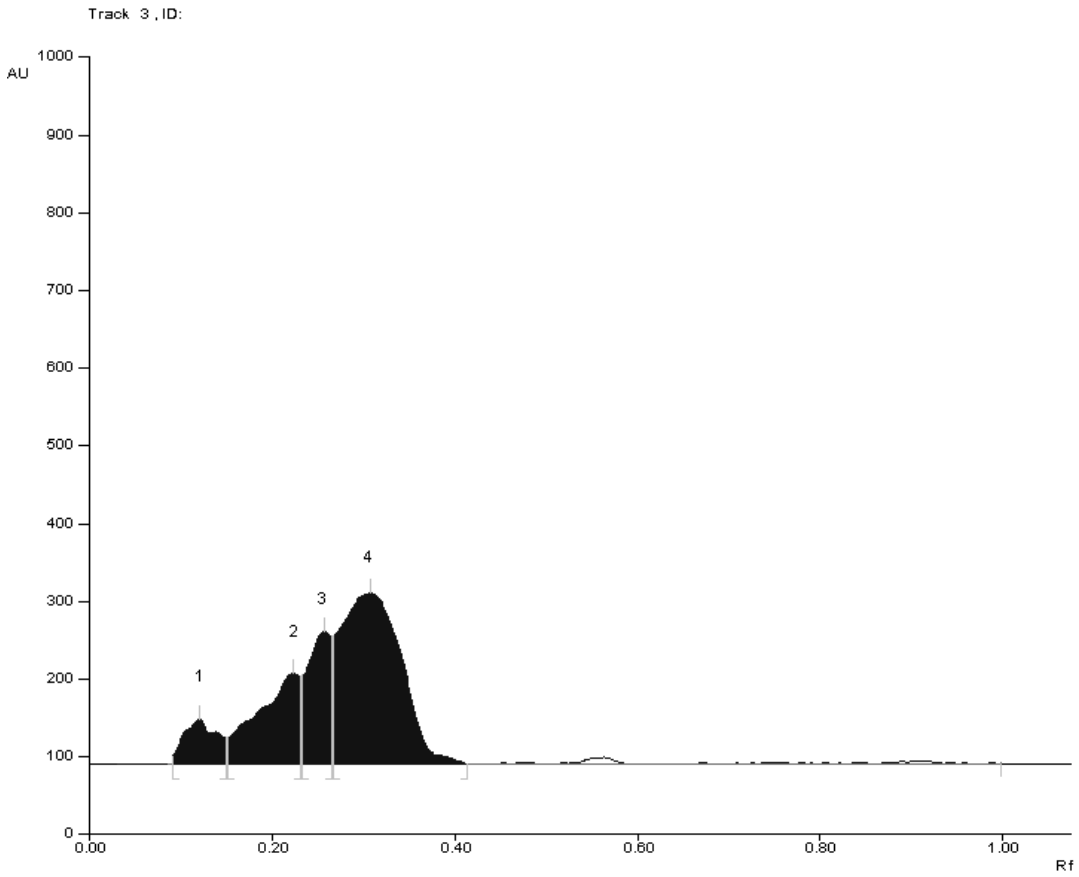


Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.04 Rf	0.7 AU	0.08 Rf	125.4 AU	66.32 %	0.13 Rf	0.3 AU	2718.5 AU	74.19 %	unknown *
2	0.16 Rf	2.7 AU	0.20 Rf	43.7 AU	23.12 %	0.22 Rf	0.1 AU	642.6 AU	17.54 %	unknown *
3	0.23 Rf	0.2 AU	0.25 Rf	20.0 AU	10.56 %	0.29 Rf	1.4 AU	303.4 AU	8.28 %	unknown *

Fig. 4: Chromatogram showing HPTLC profile of alcoholic extract of *M.oblongifolia*

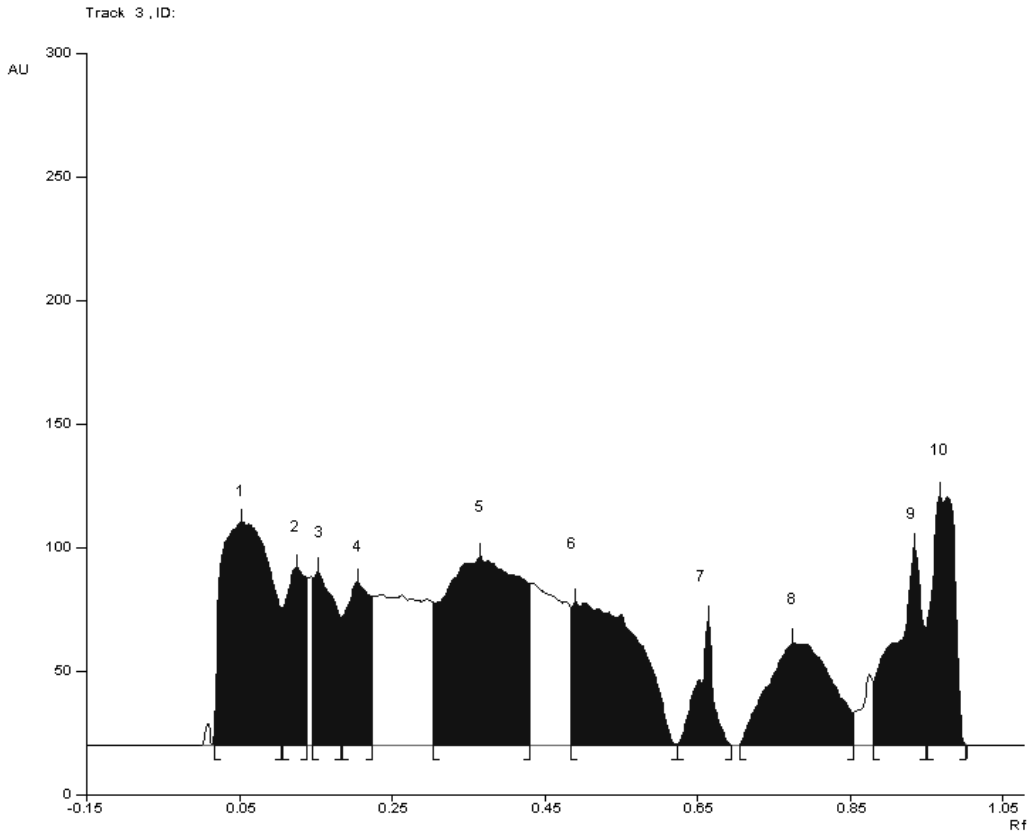
Under 425nm after derivatisation in iodine chamber



Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.09 Rf	9.9 AU	0.12 Rf	57.8 AU	10.24 %	0.15 Rf	33.6 AU	1558.5 AU	7.79 %	unknown *
2	0.15 Rf	33.7 AU	0.22 Rf	116.3 AU	20.62 %	0.23 Rf	13.7 AU	3989.3 AU	19.93 %	unknown *
3	0.23 Rf	114.1 AU	0.26 Rf	170.6 AU	30.22 %	0.27 Rf	35.0 AU	3427.6 AU	17.13 %	unknown *
4	0.27 Rf	165.5 AU	0.31 Rf	219.7 AU	38.92 %	0.42 Rf	0.1 AU	11037.3 AU	55.15 %	unknown *

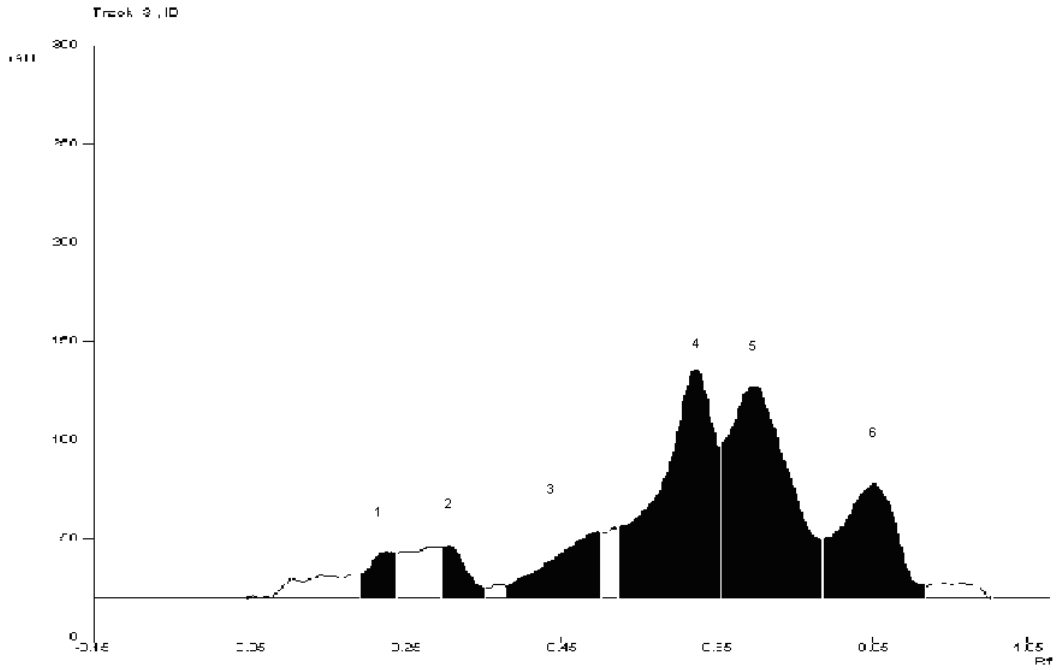
Fig. 5: Chromatogram showing HPTLC profile of aqueous extract of *M.oblongifolia* under 366nm



Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.01 Rf	7.3 AU	0.05 Rf	90.1 AU	12.75 %	0.10 Rf	55.9 AU	4435.0 AU	16.79 %	unknown '
2	0.10 Rf	56.0 AU	0.12 Rf	71.8 AU	10.16 %	0.14 Rf	37.8 AU	1458.9 AU	5.52 %	unknown '
3	0.14 Rf	67.6 AU	0.15 Rf	70.6 AU	9.99 %	0.18 Rf	51.9 AU	1563.0 AU	5.92 %	unknown '
4	0.18 Rf	52.0 AU	0.20 Rf	65.9 AU	9.32 %	0.22 Rf	50.3 AU	1635.4 AU	6.19 %	unknown '
5	0.30 Rf	57.5 AU	0.36 Rf	76.3 AU	10.79 %	0.43 Rf	55.1 AU	5730.7 AU	21.70 %	unknown '
6	0.48 Rf	55.7 AU	0.49 Rf	58.3 AU	8.24 %	0.62 Rf	0.3 AU	3785.0 AU	14.33 %	unknown '
7	0.62 Rf	0.6 AU	0.66 Rf	51.0 AU	7.22 %	0.69 Rf	0.1 AU	769.1 AU	2.91 %	unknown '
8	0.70 Rf	0.4 AU	0.77 Rf	41.7 AU	5.90 %	0.85 Rf	13.1 AU	2303.3 AU	9.86 %	unknown '
9	0.88 Rf	25.8 AU	0.93 Rf	80.2 AU	11.35 %	0.95 Rf	47.3 AU	2148.7 AU	8.14 %	unknown '
10	0.95 Rf	48.6 AU	0.96 Rf	101.0 AU	14.29 %	1.00 Rf	0.2 AU	2282.3 AU	8.64 %	unknown '

Fig.6: Chromatogram showing HPTLC profile of aqueous extract of *M. oblongifolia* under 425nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.19 Rf	12.6 AU	0.23 Rf	23.7 AU	6.51 %	0.24 Rf	22.5 AU	604.6 AU	3.29 %	unknown *
2	0.29 Rf	25.0 AU	0.31 Rf	26.6 AU	7.30 %	0.35 Rf	5.2 AU	642.1 AU	3.50 %	unknown *
3	0.38 Rf	6.0 AU	0.49 Rf	33.1 AU	9.08 %	0.50 Rf	32.6 AU	1626.5 AU	8.86 %	unknown *
4	0.52 Rf	35.0 AU	0.62 Rf	116.0 AU	31.85 %	0.65 Rf	75.7 AU	6101.3 AU	33.25 %	unknown *
5	0.65 Rf	76.2 AU	0.70 Rf	107.0 AU	29.38 %	0.78 Rf	29.4 AU	6294.8 AU	34.30 %	unknown *
6	0.78 Rf	29.4 AU	0.85 Rf	57.8 AU	15.88 %	0.92 Rf	5.7 AU	3082.1 AU	16.80 %	unknown *

DISCUSSION:

HPTLC profile showed 8 clear peaks for aqueous extract at 366nm and 6 peaks at 425nm and for alcohol extracts 8 clear peaks at 254nm, 3 peaks at 366nm and 425nm and post derivatisation with iodine was done for alcohol extract revealed 4 peaks at 425nm. Triterpenoids in alcohol extract and alkaloids in aqueous extract were identified by developing the plate in appropriate solvent systems.

CONCLUSION:

HPTLC studies of the alcohol and aqueous extracts showed the presence of 8 peaks, which correspond to different phytoconstituents. The R_f values are useful for identifying the species of the genus *Maerua*, and to establish biomarker compound. The qualitative HPTLC

investigation provides information about different phytoconstituents found in various extracts, which help future investigators regarding selection of particular extract for further investigation.

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