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Quality control analysis, phytochemistry, and pharmacognosy of botanical source plants for Murva [*Chonemorpha fragrans* (Moon) Alston and *Marsdenia tenacissima* (Roxb.) Moon]: A comparative assessment

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Ayurveda extensively documents numerous medicinal plants and their therapeutic properties, but the lack of precise botanical descriptions often results in regional substitutions without scientific validation. *Marsdenia tenacissima* is identified as the botanical source of *Murva* in the Ayurveda Pharmacopoeia of India (API), while *Chonemorpha fragrans* is commonly used as its substitute in Southern India. This study evaluates the suitability of *C. fragrans* as an alternative to *M. tenacissima* through pharmacognostic, physicochemical, and preliminary phytochemical analyses of their aqueous and hydroalcoholic extracts. High-performance thin-layer chromatography (HPTLC) and Gas Chromatography-Mass Spectroscopy (GC-MS/MS) were employed for fingerprint profiling. The study revealed distinct morphological differences: *M. tenacissima* roots were yellow-buff with a complex xylem structure, while *C. fragrans* roots were brown with white latex. Both plants exhibited glycosides and saponins, but alkaloids and phenols were exclusive to *C. fragrans*. Hydroalcoholic extracts of *C. fragrans* displayed a richer array of phytoconstituents. HPTLC analysis identified gallic acid in the aqueous extract of *C. fragrans* and the hydroalcoholic extract of *M. tenacissima*, with quercetin present in all extracts and beta-sitosterol exclusive to hydroalcoholic extracts. These findings suggest that *C. fragrans* could potentially substitute *M. tenacissima* in Ayurvedic formulations, but further pharmacological studies are necessary to confirm their therapeutic equivalence.

Keywords: Apocynaceae, Ayurveda, GC-MS/MS, HPTLC, Murva

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Introduction

Ayurveda, the ancient Indian medicinal system, heavily relies on medicinal plants for therapeutic applications. However, most Ayurvedic literature in Sanskrit uses polynomial nomenclature to describe plants, often causing controversies over their precise botanical identity[1]. The texts typically emphasize the therapeutic attributes of plants rather than providing detailed botanical descriptions. In India's diverse linguistic and geographical landscape, this has led to regional variations, where plants abundant in specific areas, bearing similar vernacular names or sharing therapeutic properties, are used as substitutes for the originally identified drug. Challenges such as plant unavailability, limited botanical knowledge, and the emergence of parallel knowledge systems have further contributed to the use of multiple botanical sources for the same Ayurvedic drug name across different regions[2].

The Ayurveda Pharmacopoeia of India (API) provides standardized monographs for specific plant species corresponding to classical herbs mentioned in Ayurvedic texts. However, regional interpretations of botanical drugs often use heterogeneous terms, leading to potential misidentification or inappropriate use of plant species[3-4]. Considering the growing concerns about the safety and acceptability of medicinal plants, the use of substitutes must be guided by rigorous scientific validation to ensure their efficacy and safety in therapeutic applications. No genuine phytochemical/pharmacological or clinical evidence-based guidelines have been formulated for substituting a plant species with another variety[5-7].

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Thus, rather than evidence-based substitution, there is more often, the practice of adulteration. Nair *et al*. have discussed the controversial nature of certain plants and their botanical sources. They highlighted the importance of intertextual referencing, emphasising that the number of ingredients in the same formulations can vary, and sometimes, different botanical sources are associated with the same Sanskrit name[8].

Murva is a drug widely used in Ayurveda in the management of pain and inflammation related to various conditions. *Marsdenia tenacissima* (MT) (Family - Apocynaceae) root is the botanical source of the plant in API. In various regions of India, different plant species are used as *Murva*; for instance, in West Bengal, *Sansevieria roxburghiana* Schult. & Schult.f. is used as a substitute for *Murva*. Similarly, other plants such as *Helicteres isora* (Sterculiaceae) from Punjab and *Maerua amneria* (Capparaceae) from Bihar are also used. In South India, *Chonemorpha fragrans* (Apocynacea), *Wattakaka volubilis* (Asclepiadaceae) and *Salvadora persica* (Salvadoraceae) serve as substitutes. In contrast, in other regions, plants like *Argyreia nervosa* (Convolvulaceae), *Maerua oblongifolia* (Capparaceae), and *Dregea volubilis* (Apocynaceae) are also employed[9-13]. These regional substitutes are utilised based on their local availability and similar therapeutic properties, and the practice has been passed down through generations, rooted in practical experience and Ayurvedic tradition.

In Kerala, numerous Ayurvedic formulations are prepared based on local classical texts such as *Sahasrayoga* and *Chikitsa Manjari*. *Murva*, often sourced from *Chonemorpha fragrans* (CF), is a key ingredient used in the preparation of several widely used polyherbal formulations, including *Manjistadi Kashaya*, *Maharasnadi Kashaya*, *Varunadi Kashaya*, *Sudarshana Choorna*, *Kumaryasava*, *Mahatiktaka Ghritha*, *Ayaskriti* and *Marma Gutika*. Most of these formulations have a long history of safe usage and have also demonstrated clinical effectiveness. The Kerala Forest Research Institute (KFRI) survey between 2004-2009 on the raw drugs requirement of the Ayurvedic medicine manufacturing industry in Kerala highlighted a significant demand for CF, locally referred to as *Perukurumba veru*. According to the findings, this raw drug had an estimated annual requirement of 25-50 tonnes in the Ayurvedic pharmaceutical industry of Kerala[14].

In preclinical studies, MT exhibits immunomodulatory[15], anti-inflammatory[16], cardioprotective[13], antipyretic[17], anti-tumor[18], and anti-HIV activities[19]. CF root extract has also demonstrated hepatoprotective activity[20], antioxidant properties[21], and antibacterial effects[22]. However, there is a lack of comparative pharmacognostic and phytochemical analyses of the different botanical sources of *Murva*, particularly focusing on these two plants. This study aims to evaluate the substitution of MT with CF by analysing their aqueous (AE) and hydro-alcoholic (HE) extracts. A comprehensive approach involving comparative pharmacognostic studies, quality control analysis, preliminary phytochemical evaluation, and fingerprint profiling using HPTLC and GC-MS/MS methods will be employed. The findings aim to establish scientific validation, provide quality control parameters, and develop a chemical fingerprint and chemo profile, ensuring the quality and safety of these plants. This research is expected to support their potential substitution in Ayurvedic formulations and their application in herbal medicine preparation within the pharmaceutical industry.

Materials and Methods

Plant material

Roots of MT were collected from Gandhamardan Hills, Bargarh District, Odisha (20.91251, 82.822642) in December 2022 (Fig. 1a,b). CF was collected from forest areas near Mangalam Dam, Palakkad, Kerala (10.47910, 76.5884). The botanist at NARIP Cheruthuruthy, Kerala, authenticated the collected samples in April 2023 (Fig. 1c,d). Voucher specimens were preserved in the herbarium, and samples were deposited in a raw drug repository with the numbers NARIP/IMR-PSP/001 and NARIP/IMR-PSP/004 for MT and CF, respectively.

Chemicals and reagents

HPLC grade solvents (Methanol, Acetonitrile, Formic acid, Toluene) were procured. Distilled water and rectified spirit were used to extract the plants. Aluminium $60F_{254}$ TLC plates were procured from Merck. Reagents were procured from the Sigma-Aldrich.

Botanical studies

Morphological studies were carried out by close observation and through a Unilab zoom stereo microscope for micro-morphological character

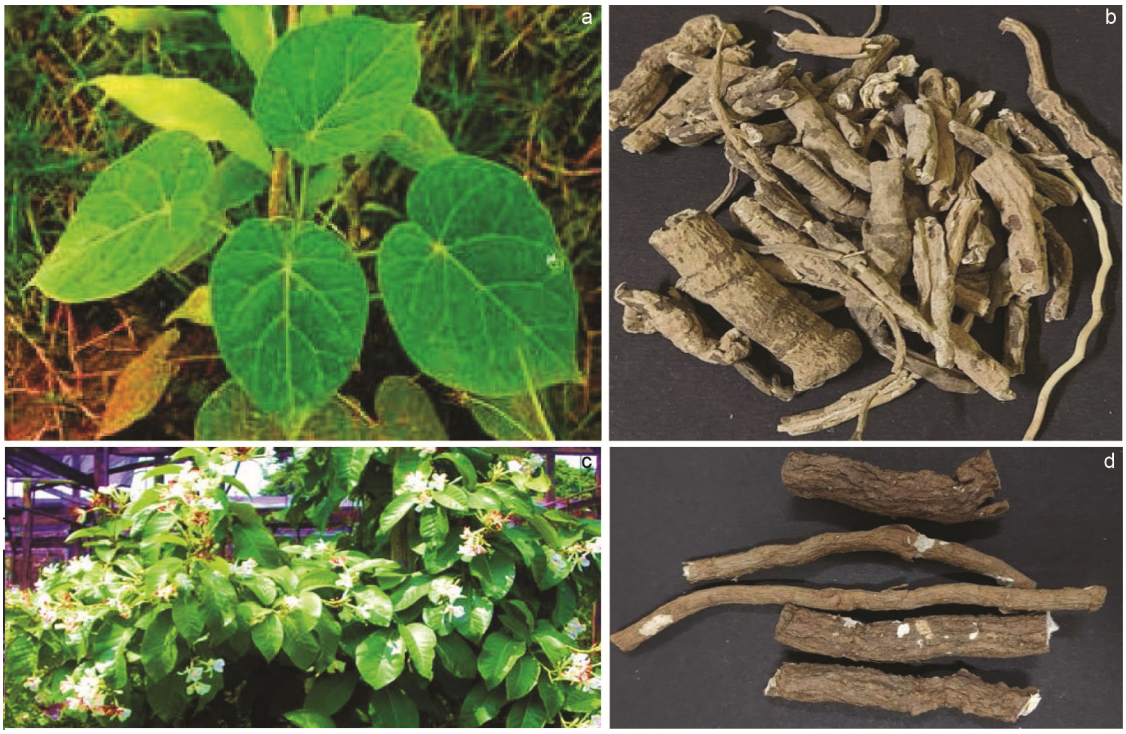


Fig. 1 — a) Images of plant, b) dried root part of *Marsdenia tenacissima,* c) plant, and d) dried root part of *Chonemorpha fragrans.*

studies. The morphological characters were analysed by referring to the standard works. Then, thin free-hand root sections were taken using a stainless-steel blade and observed under the Olympus trinocular compound microscope after staining. The powder of shade-dried root is observed under the compound microscope as per the procedure mentioned in the API[23].

Sample extraction

Initially, the root parts of both raw drugs were botanically authenticated and then subjected to physicochemical analysis before extraction. For the extraction of CF and MT roots, 300g of each root powder was separately dissolved in 2.4 L of a hydro-alcoholic solvent (1:1 ratio) and 2.5 L of double-distilled water in conical flasks for 24 hours, performing hydro-alcoholic (HECF and HEMT) and aqueous extractions (AECF and AQMT) respectively. The mixtures were filtered using Buchner funnels with a vacuum pump, and the filtrates were stored at 10°C. This process was repeated three times with the same powders, resulting in separate filtrates for the hydro-alcoholic solvents (HAS) and aqueous solvents (AQS), which were collected and stored in containers in the refrigerator. Each filtrate was subjected to a vacuum rotavap to remove the solvents[24].

Quality control analysis of the extracts

The sticky semi-solid extracts of *C. fragrans* (HECF and AECF) and *M. tenacissima* (HEMT and AEMT) underwent preliminary phytochemical analysis. Further extracts were subjected to organoleptic examination and physicochemical analysis tests, such as water-soluble extractive, alcohol-soluble extractive, and pH of the extracts in 10% aqueous solution, etc.

Additionally, MT extracts were tested for pesticide residues (IFL C/QSP G/007), Aflatoxins (IFL C/QSP G/002), and heavy metals (IFL C/QSP G/009). *C. fragrans* extracts (HECF and AECF) were tested for pesticide residues (AOAC Official Method 2007.01), Aflatoxins (modified AOAC 991.31), and heavy metals (USP <233>). Extracts were also screened for detection of any microbial contamination.

HPTLC analysis

Sample preparation

To prepare 100 mg/mL HPTLC samples, both HEMT, AEMT and HECF, AECF were dissolved separately in HPLC-grade methanol in standard flasks. Each flask was sonicated for 15 minutes at 45°C, and the resulting solutions were filtered using a 0.22 µm PTFE membrane and a 5 mL syringe. The filtrates were stored individually in 5 mL sample vials

for HPTLC fingerprint profile analysis. Further, the Marker compounds of Gallic acid (GA), Beta-Sitosterol (BS) and Quercetin are prepared in 1 mg/mL concentration, respectively, in HPLC grade methanol[24].

Procedure

AECF, HECF and CF samples in 5 and 10 µL as duplicated samples were applied on tracks 01 to 06. Similarly, concentrations of the AEMT, HEMT and MT samples and duplicated samples were applied on tracks 07 to 12. In contrast, µL of Gallic acid (GA), Beta-Sitosterol (BS), and Quercetin (QE) were applied on tracks 13, 14, and 15, respectively, on a pre-coated silica gel $60F_{254}$ HPTLC plate 20 x 10 cm (Merck). The plates were developed in 20-minute pre-saturated 20×10 cm TLC chambers with the specified mobile phases up to a distance of 80 mm. The mobile phase was optimized as a Toluene: Ethyl acetate: Formic acid (4:5:1 v/v/v). After development, the plates were dried and visualised at 254 and 366 nm. The plates were dipped in a p-Anisaldehydesulphuric acid reagent for derivatisation and heated at 105°C for three minutes to reveal the colour spots/bands.

Visualisation

The developed TLC plates were observed under different wavelengths, including UV-254, 366, and 545 nm after derivatization. Chromatogram interpretations were made using Vision Cats 3.1 for both plants.

GC-MS/MS analysis

Sample preparation

The mixture of 0.5 g of the powdered sample (extracts) and 5 mL of methanol was sonicated for 15 minutes and then centrifuged at 5000 rpm for 5 minutes. Upon filtration by a 0.22-micron syringe filter, the resultant supernatant was used as the sample solution for GC-MS analysis.

Instrumentation

Agilent 8890 GC System coupled with 7000D GC/TQ was used in the (Gas Chromatography- Mass Spectrometry) GC-MS/MS analysis of MTHAE & MTAQE by employing a combination of two Agilent HP-5MS UI columns (5%-phenyl)—methylpolysiloxane nonpolar, 15 m x 250 µm x 0.25 µm). Phytochemicals were separated by using helium as carrier gas at a constant flow of 1.0 mL/min in Column I and 1.1 mL/min in Column II. The above samples were dissolved in methanol solvent, applied in the volume of 1 µL with a split ratio 5:1, and injected into the instrument by the autosampler (ALS). Further, during the chromatographic run, the initial oven temperature was 60°C, which further increased to 90°C at a rate of 5°C/min (hold time 0 min), which further increased to 150°C at a rate of 15°C/min (hold time 0 min); which further increased to 220°C at a rate of 10°C/min (hold time 10 min). The condition of the mass detector was source temperature at 230°C, mass detector (MSD) transfer line temperature at 280°C, ionisation mode Electron Impact (EI) at 70 eV scan time 300 ms (0.3 s), in positive and MS2 scan mode with a solvent delay of 2 min. The pressure of quench gas He and Collison gas N_2 were set at 2.25 mL/min and 1.5 mL/min, respectively. The obtained mass spectra of major peaks, i.e., major phytochemicals in GC-MS chromatograms, were compared with the database stored in the GC-MS NIST Library.

Results

Plant material authentication

The plant materials collected were analysed for their botanical features as a preliminary study, and both plants morphological, anatomical and powder features were compared. The summary of the comparison is given in Table 1.

Macroscopic characters

The root of MT was cylindrical, externally yellow to buff-coloured, with dark brown patches on the cork. It featured prominent longitudinal ridges, furrows, and transverse cracks (Fig. 1b). The bark was easily separable from the wood. The root displayed a short, granular fracture in the bark region and a fibrous fracture in the wood. The taste was slightly bitter, and the odour was indistinct. In contrast, the root of CF was also cylindrical but brown in colour (Fig. 1d), with white latex present at the cut edge. The odour of CF root was pleasant.

Microscopic characters

In MT, the microscopical examination revealed a cork composed of 15-25 layers of thin-walled, tangentially elongated, rectangular cells, some filled with reddish-brown contents. The secondary cortex was characterised by an outer region of a broken ring of stone cells of varying thickness, followed by a wide zone of oval to polygonal parenchymatous cells. Stone cells were yellow and varied in shape and size.

Table 1 — Comparative features of *Marsdenia tenacissima* and *Chonemorpha fragrans* roots

Features studied	Characters	*Marsdenia tenacissima*	*Chonemorpha fragrans*
Morphological and Organoleptic characters	Root shape	Cylindrical	Cylindrical
	Colour	Externally yellow to buff colour with dark brown patches on cork.	Brown; latex in cut edges
	Texture	Prominent longitudinal ridges and furrows; and transverse crakes; bark separable from wood.	Rough; not have prominent ridges and furrows.
	Taste	Slightly bitter	Bitter
	Odor	Indistinct	Pleasant smell
Anatomical characters	Cork	15-25 layers, thin walled tangentially elongated thin-walled cells; some cells filled with brown content.	6-8 layers, thin walled tangentially elongated thin-walled cells.
	Secondary cortex	Outer broken ring of stone cells; then parenchyma	Parenchymatous with starch grains
	Sec. phloem	Parenchymatous and strands of stone cells; resin cells present	Phloem parenchyma is major
	Sec. xylem	Wedge-shaped structures present	Not have wedge shaped structures
	Cell inclusions	Calcium oxalate crystals and starch grains	Starch grains
Powder characters	Colour	Light brown	White or creamish
	Cells and cell inclusions	Fragments of cork, calcium oxalate crystal, simple and compound starch grains	Starch grains, Parenchyma cells

The secondary phloemis composed mostly of parenchyma with small patches of sieve elements and small strands of stone cells, similar to those in the secondary cortex. The secondary xylem was segmented and showed a wedge-shaped structure with concentric bands of lignified and unlignified parenchymatous tissue, along with rosette and prismatic crystals of calcium oxalate and abundant starch grains (Fig. 2a). In comparison, the cross-section of the CF stem showed 6-8 layers of phelloderm and lenticels, followed by cork cambium. The cortical region contained parenchyma cells with starch grains, and the stele consisted of a secondary xylem and phloem (Fig. 2 b).

Powder microscopy

The powder of MT was light brown and showed many stone cells, fibres, tracheids, fibre-tracheids, vessels with pitted walls, fragments of cork, rosette and prismatic crystals of calcium oxalate, and simple and compound starch grains (Fig. 3a). On the other hand, the powder of CF was white in colour and contained starch grains and parenchyma cells (Fig. 3b).

Quality control analysis

The extract yields observed were 13.57% (w/w) for HECF, 11.45% (w/w) for AECF,12% (w/w) for HEMT, and 14% (w/w) for AEMT. HEMT and AEMT showed similar phytoconstituents, including glycosides, saponins, flavonoids, phenols, and carbohydrates (Table 2). Further extracts were subjected to organoleptic examination and physicochemical analysis tests, such as water-soluble extraction, alcohol-soluble extraction, and pH of the extracts in 10% aqueous solution. Details of the same are shown in Table 3.

HPTLC analysis

High-performance thin Layer Chromatography (HPTLC) fingerprinting showed distinct phytochemical profiles for both plants at 254, 366, and 545 nm, as shown in Table 4. The HPTLC Chromatogram at 254 nm of MT exhibited 4-7 significant phytochemical bands (Fig. 4), while CF showed 5-7 bands, indicating differences in chemical composition between the two species, with respective two plants and comparison of similar R_F values with their peak area percentage are shown in Fig. 5 and 6. Further, AECF and HECF had similar R_F values: 0.011, 0.406, 0.549 and 0.683, and only 0.051 differed from the HECF. Similarly, the same pattern observed in the AEMT at R_F 0.008, 0.403, 0.551 and 0.679, and two bands, 0.049 and 0.344, differed from the HEMT as shown in Fig. 7. Hydro-alcoholic extracts displayed a higher number of phytoconstituents compared to aqueous extracts for both plants. Respective Marker compounds of Gallic acid (GA) showed an R_F value of 0.35, and Quercetin (QE) R_F: 0.56. Fig. 8 shows similar R_F values were also observed in both plant extracts, (AECF, HEMT).

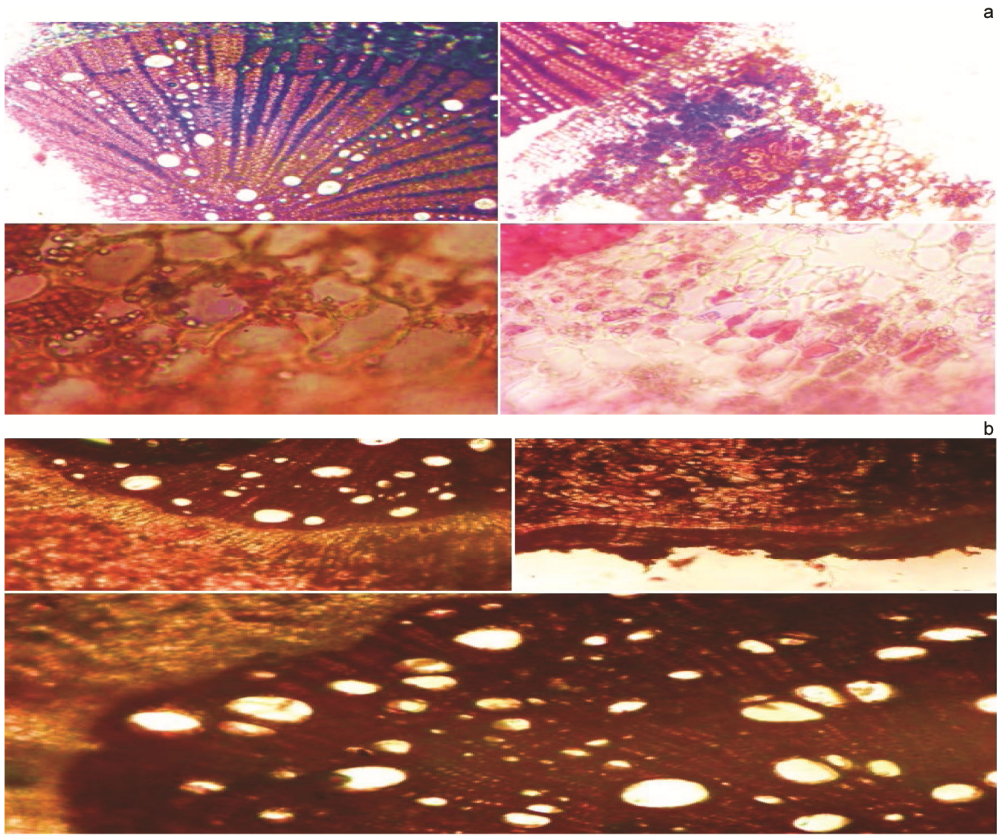


Fig. 2 — C. S of root of a) *Marsdenia tenacissima*, and b) *Chonemorpha fragrans*.

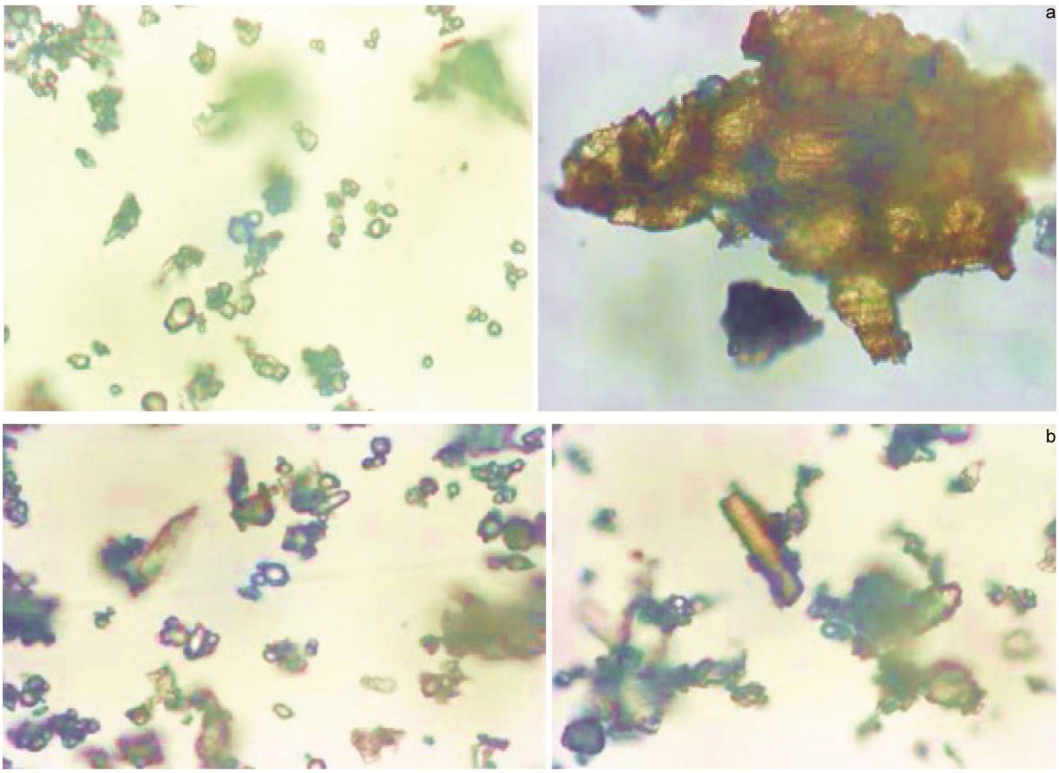


Fig. 3 — Powder Microscopyof of root of a) *Marsdenia tenacissima*, and b) *Chonemorpha fragrans*.

Table 2 — Preliminary phytochemical analysis of *Marsdenia tenacissima* extracts and *Chonemorpha fragrans* extracts

S. No.	Tests for Phytochemicals		HEMT	AEMT	HECF	AECF
1	Glycosides	Salkowski	+++	++	++	+++
2	Protein	Ninhydrin	-	-	-	+
3	Alkaloid	Wagner's test	-	-	+++	-
4	Tannin	Braymer test	-	-	-	-
5	Saponins	Foam	+++	+++	+++	++
6	Flavonoids	Lead acetate	+	+	++	-
7		Shinoda	-	-	-	-
8	Phenols	Lead acetate	+	+	++	-
9		Ferric chloride	-	-	-	-
10	Carbohydrates	Felhing's test	+	+	-	-
11		Molish's test	+++	+++		

Table 3 — Physicochemical parameters of *Marsdenia tenacissima* and *Chonemorpha fragrans* extracts

S. No.	Test parameters	Results (n=3)		Results (n=3)	
		HEMT	AEMT	HECF	AECF
1	Description	Stickysemi-solid	Stickysemi-solid	Stickysemi-solid	Stickysemi-solid
2	Colour	Dark brown	Brownish	Dark brown	Brownish
3	Odour	Pleasant smell	Pleasant smell	Pleasant smell	Pleasant smell
4	Taste	Slight magnanimous	Slight magnanimous	Slight magnanimous	Slight magnanimous
5	Loss on drying (w/w %)	6.486±0.116	18.87±0.033	7.30 ±0.12	12.23±0.33
6	Total ash (w/w%)	6.029±0.005	7.267±0.0064	7.94±0.16	12.07±0.02
7	Acid insoluble ash (w/w%)	0.542±0.009	1.450±0.008	1.35±0.10	2.14±0.26
8	Alcohol soluble extractive (w/w%)	32.748±0.03043	8.753±0.0219	8.41±0.15	38.5±0.19
9	Water soluble extractive (w/w%)	82.280±0.05044	77.696±0.003464	86.81±0.51	87.47±0.38
10	pH (10% aqueous)	7.52±0.012	6.86±0.012	4.68±0.005	4.40±0.005

Table 4 — Observed the R_F values of Aqueous and Hydroalcoholic and plant extracts of *Chonemorpha fragrans*-root & *Marsdenia tenacissima*-root

Track numbers	Name of the Plant Extract/Markers	Observed R_F bands @ Different Wavelengths		
		Developed plate @ 254 nm	Developed plate @ 366 nm	Derivative plate @ 545 nm
1 & 2	AECF	Number of bands: 05 0.011, 0.342, 0.406, 0.549, 0.683	Number of bands: 02 0.47 (light blue), 0.54 (light blue)	Number of bands: 07 0.021, 0.047, 0.111, 0.151, 0.333, 0.403 (green), 0.929 (violet)
3 & 4	HECF	Number of bands: 05 0.010, 0.051, 0.415, 0.558, 0.690	Number of bands: 03 0.47 (light blue), 0.53 (blue), 0.73 (sky blue)	Number of bands: 07 0.018, 0.050, 0.147, 0.217, 0.42 (green), 0.661 (light violet), 0.767 (purple)
5 & 6	CF root Alcoholic extract	Number of bands: 07 0.010, 0.047, 0.428, 0.557, 0.688, 0.797, 0.875	Number of bands: 05 0.43 ((light blue), 0.47 (light blue) 0.55 (light blue), 0.60 (blue), 0.73 (sky blue)	Number of bands: 10 0.018, 0.047, 0.144, 0.358, 0.424 (green), 0.544, 0.651 (light violet), 0.76 (purple), 0.819, 0.915 (violet)
7 & 8	AEMT	Number of bands: 04 0.008, 0.403, 0.551, 0.679	Number of bands: 02 0.48 (light blue), 0.74 (sky blue)	Number of bands: 07 0.011, 0.039, 0.135, 0.325, 0.393, 0.665, 0.912
9 & 10	HEMT	Number of bands: 06 0.008, 0.049, 0.344, 0.411, 0.553, 0.685	Number of bands: 05 0.43 ((light blue), 0.46 (light blue) 0.57 (light blue), 0.65 (blue), 0.72 (sky blue)	Number of bands: 11 0.010, 0.03, 0.096, 0.140, 0.171, 0.203, 0.394, 0.533, 0.640 (light violet), 0.753(purple), 0.910 (violet)
11 & 12	MT root Alcoholic extract	Number of bands: 06 0.008, 0.050, 0.547, 0.682, 0.753, 0.883	Number of bands: 05 0.43 (light blue), 0.49 (light blue) 0.54 (light blue), 0.59 (blue), 0.74 (sky blue)	Number of bands: 07 0.008, 0.037, 0.411 (green), 0.483, 0.636, 0.749(purple), 0.907 (violet)
13	Gallic acid (GA)	Number of bands: 01 0.35	Number of bands: 01 0.35	Number of bands: 01 0.35
14	Beta-Sitosterol (BS)	Number of bands: Zero ND	Number of bands: Zero ND	Number of bands: 01 0.72
15	Quercetin (QE)	Number of bands: 01 0.56	Number of bands: 01 0.56	Number of bands: 01 0.56

ND-Not detected

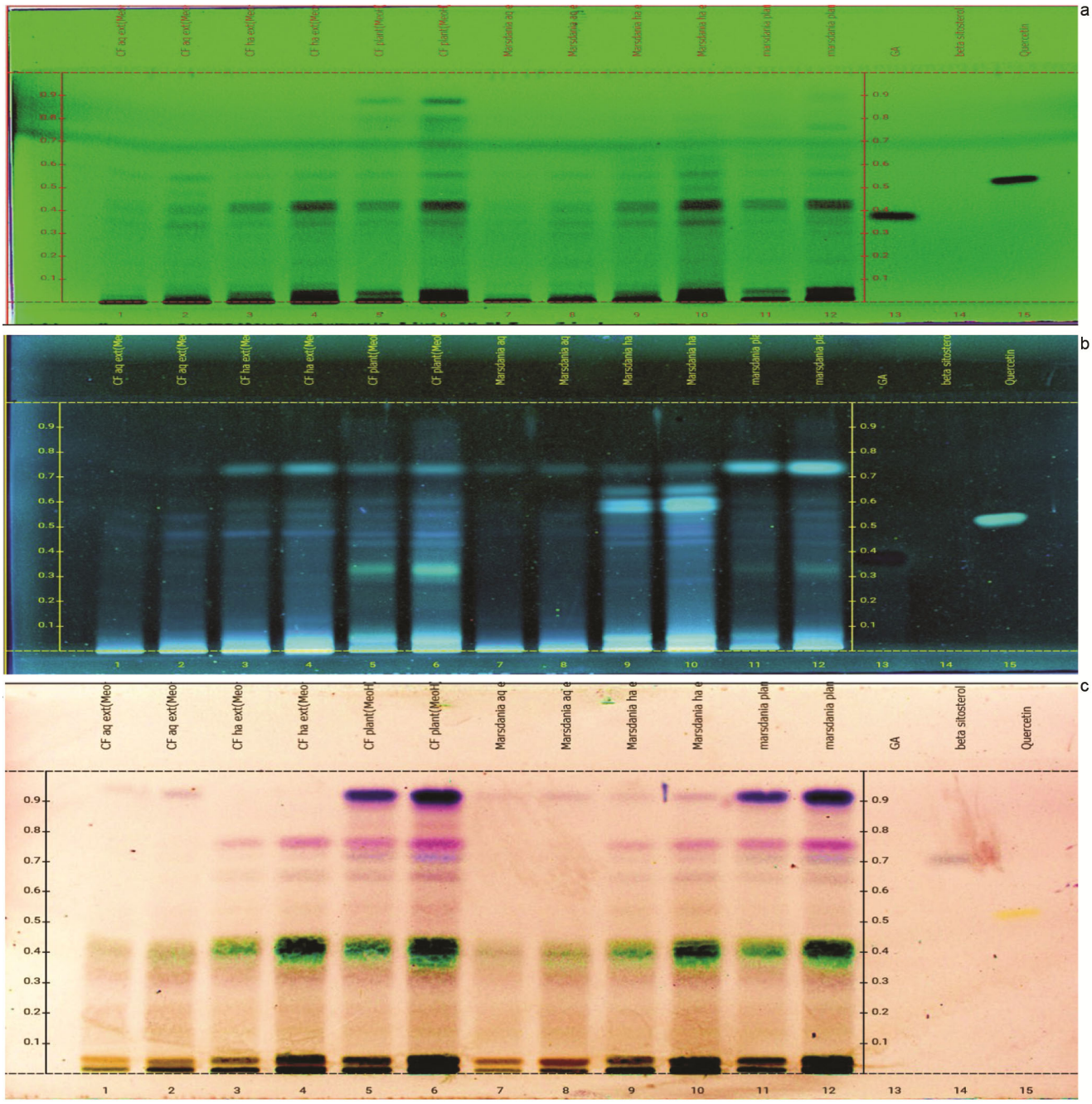
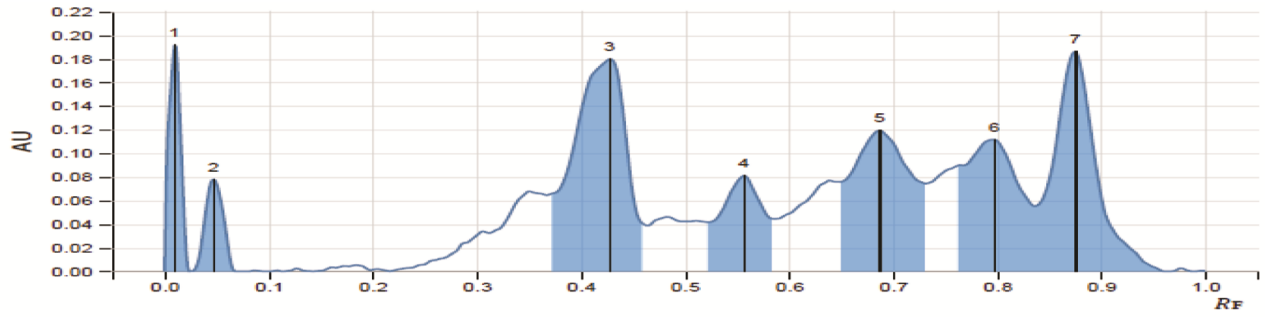


Fig. 4 — HPTLC Chromatogram {*Chonemorpha fragrans*-Aqueous extract-AECF (tracks -1,2), Hydroalcoholic extract of *Chonemorpha fragrans*- HECF (tracks-3,4), Alcoholic extract of *Chonemorpha fragrans*- CF (tracks-5,6), *Marsdenia tenacissima*-Aqueous extract-AEMT (tracks-7,8), *Marsdenia tenacissima*-Hydroalcoholic extract-HEMT (tracks-9,10), Alcoholic extract of *Marsdenia tenacissima* (tracks-11,12), Gallic acid-GA (track-13), Beta-Sitsoterol-BS (track-14), and Quercetin-QE (track-15) at a) 254 nm, b) 366 nm, and c), 545 nm}.

The HPTLC Chromatogram @ 366 nm had number bands in both plant extracts. AECF had 2 bands, with R_F 0.011 and 0.479, and HECF had 1 similar band, with R_F 0.010. AEMT and HEMT had 1 similar band, R_F 0.008, but HEMT had 2 bands with R_F 0.490 and 0.926, shown in Figs. 9 and 10.

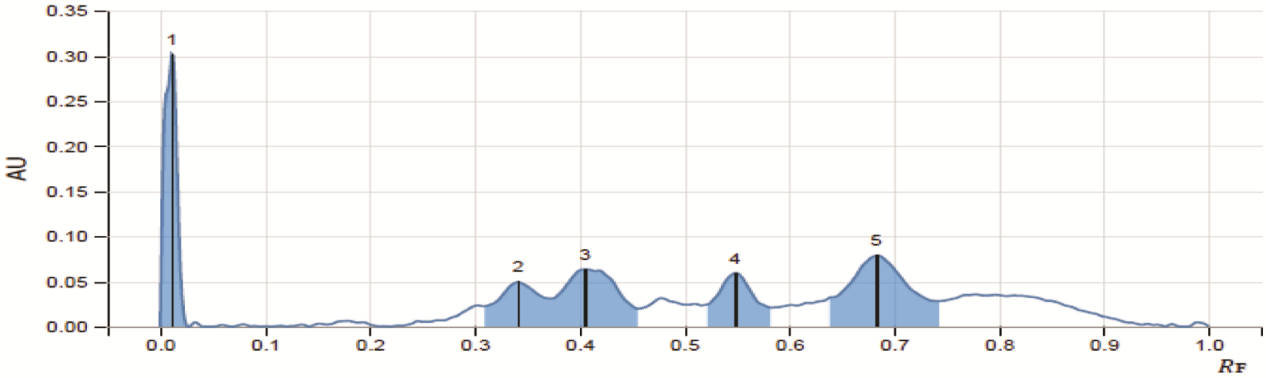
Further, in the HPTLC chromatogram @ 545 nm of AECF, HECF and AEMT, HEMT had similar bands observed with respective to R_F values. AECF and HECF have 7 bands, shown in Fig. 11; AEMT has 7 bands, and HEMT (11 bands) is shown in Fig. 12. HEMT has more bands than AEMT. HEMT had

a



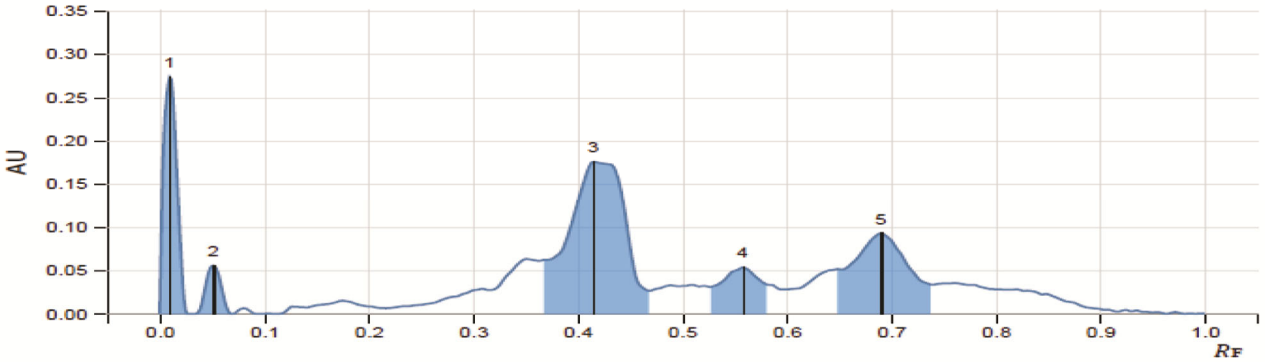
Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.010	0.1916	20.23	0.024	0.0000	0.00258	6.07
2	0.026	0.0000	0.047	0.0779	8.22	0.068	0.0000	0.00157	3.70
3	0.368	0.0645	0.428	0.1797	18.98	0.463	0.0389	0.01097	25.84
4	0.522	0.0415	0.557	0.0809	8.54	0.586	0.0443	0.00382	9.00
5	0.649	0.0757	0.688	0.1193	12.60	0.731	0.0743	0.00788	18.54
6	0.761	0.0893	0.797	0.1117	11.79	0.836	0.0551	0.00682	16.04
7	0.836	0.0551	0.875	0.1860	19.64	0.960	0.0000	0.00884	20.82

b



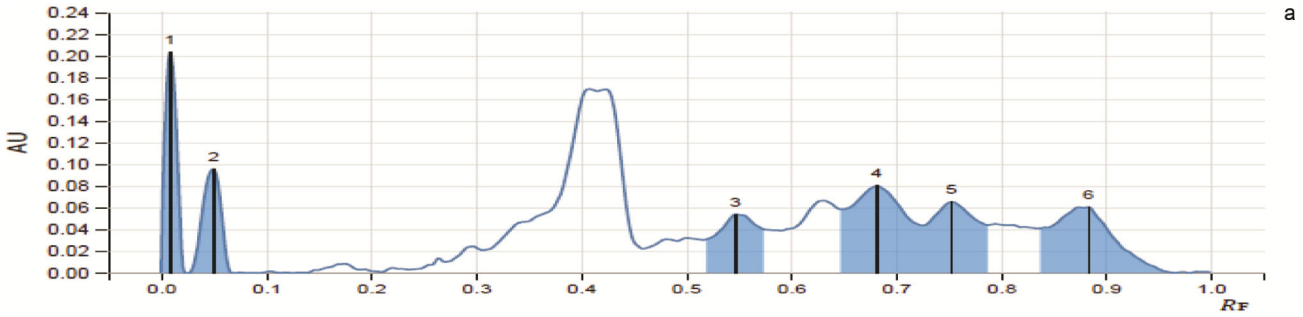
Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.011	0.3020	54.61	0.026	0.0000	0.00429	23.19
2	0.308	0.0225	0.342	0.0494	8.93	0.374	0.0312	0.00239	12.93
3	0.374	0.0312	0.406	0.0636	11.51	0.456	0.0197	0.00386	20.85
4	0.517	0.0233	0.549	0.0594	10.73	0.583	0.0210	0.00254	13.75
5	0.636	0.0303	0.683	0.0786	14.22	0.743	0.0283	0.00542	29.29

c

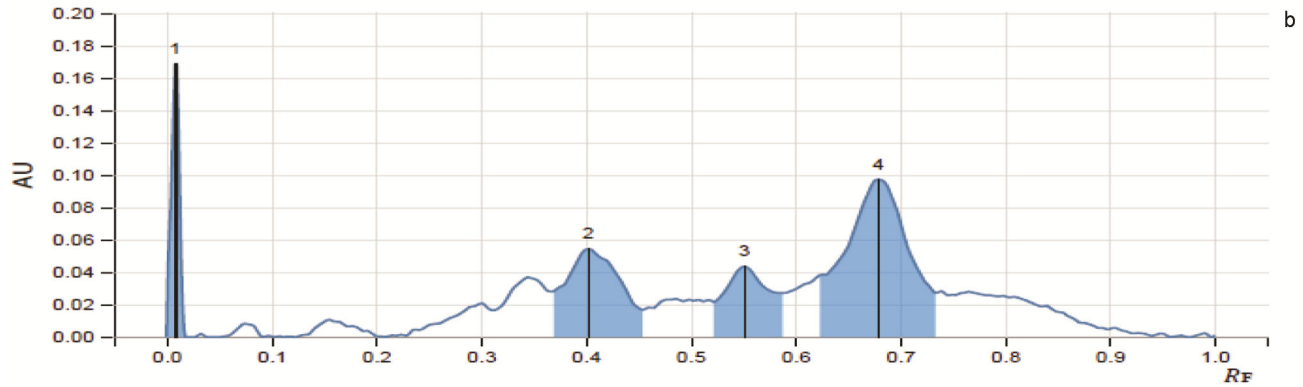


Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.010	0.2728	42.04	0.026	0.0000	0.00408	16.53
2	0.033	0.0000	0.051	0.0551	8.49	0.068	0.0000	0.00092	3.74
3	0.364	0.0607	0.415	0.1756	27.07	0.468	0.0263	0.01133	45.96
4	0.526	0.0307	0.558	0.0532	8.20	0.583	0.0330	0.00239	9.69
5	0.646	0.0506	0.690	0.0922	14.20	0.739	0.0333	0.00594	24.08

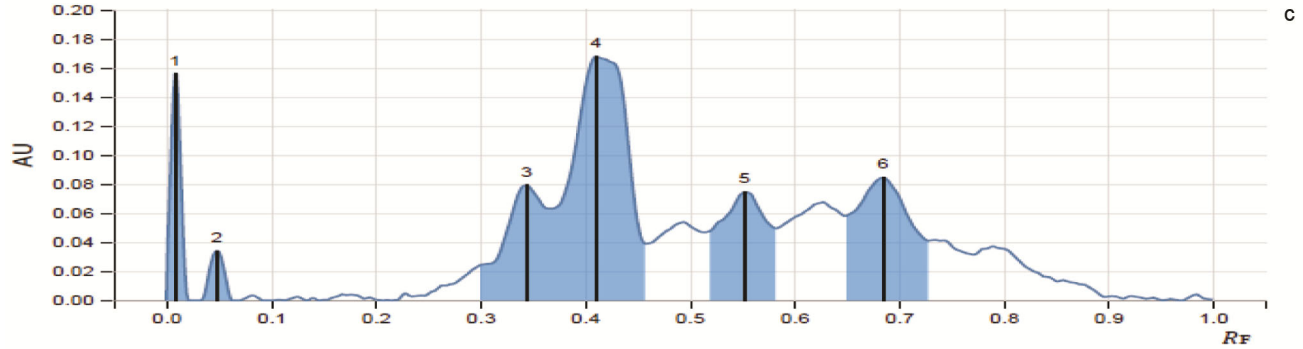
Fig. 5 — Comparison of observed R_F bands in the a) alcoholic extract *Chonemorpha fragrans*-root: CF, b) Aqueous extract of *Chonemorpha fragrans* AECF, and c) Hydroalcoholic extract of *Chonemorpha fragran* HECF @ 254 nm.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.008	0.2029	36.38	0.022	0.0000	0.00264	13.39
2	0.026	0.0000	0.050	0.0953	17.08	0.068	0.0000	0.00194	9.83
3	0.517	0.0302	0.547	0.0539	9.66	0.576	0.0398	0.00260	13.19
4	0.647	0.0585	0.682	0.0800	14.34	0.725	0.0436	0.00499	25.30
5	0.725	0.0436	0.753	0.0651	11.68	0.787	0.0438	0.00338	17.15
6	0.838	0.0409	0.883	0.0606	10.86	0.965	0.0000	0.00417	21.14



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.008	0.1685	46.35	0.018	0.0000	0.00153	10.98
2	0.368	0.0280	0.403	0.0543	14.93	0.454	0.0168	0.00331	23.86
3	0.522	0.0217	0.551	0.0435	11.98	0.588	0.0271	0.00215	15.49
4	0.622	0.0377	0.679	0.0972	26.74	0.735	0.0272	0.00690	49.67



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.008	0.1556	26.16	0.021	0.0000	0.00173	6.82
2	0.032	0.0000	0.049	0.0341	5.72	0.064	0.0000	0.00054	2.13
3	0.300	0.0243	0.344	0.0791	13.29	0.364	0.0631	0.00343	13.47
4	0.368	0.0629	0.411	0.1676	28.18	0.458	0.0388	0.01042	40.97
5	0.515	0.0467	0.553	0.0742	12.48	0.583	0.0495	0.00408	16.06
6	0.649	0.0580	0.685	0.0843	14.17	0.728	0.0410	0.00523	20.56

Fig. 6 — Comparison of observed R_F bands in the a) alcoholic extract of *Marsdenia tenacissima*- -root: MT, b) Aqueous extract of *Marsdenia tenacissima* AEMT, and c) Hydroalcoholic extract *Marsdenia tenacissima* HEMT @ 254 nm.

Substance 0.35 (R_F 0.344 +/- 0.023):

Track	R_F	X (mm)	Y (mm)
2	0.340	31.4	32.5
4	0.349	54.2	33.1
6	0.349	77.0	33.1
8	0.344	99.8	32.8
10	0.343	122.6	32.7
12	0.346	145.4	32.9

Substance 0.42 (R_F 0.419 +/- 0.037):

Track	R_F	X (mm)	Y (mm)
2	0.419	31.4	38.2
4	0.418	54.2	38.1
6	0.428	77.0	38.8
8	0.403	99.8	37.0
10	0.411	122.6	37.6
12	0.424	145.4	38.5

Substance 0.56 (R_F 0.536 +/- 0.048):

Track	R_F	X (mm)	Y (mm)
2	0.550	31.4	47.6
4	0.551	54.2	47.7
6	0.551	77.0	47.7
8	0.550	99.8	47.6
10	0.551	122.6	47.7
12	0.547	145.4	47.4
15	0.522	179.6	45.6

Substance 0.89 (R_F 0.874 +/- 0.030):

Track	R_F	X (mm)	Y (mm)
6	0.875	77.0	71.0
12	0.874	145.4	70.9

Substance 0.77 (R_F 0.756 +/- 0.044):

Track	R_F	X (mm)	Y (mm)
8	0.797	99.8	65.4
10	0.794	122.6	65.2
12	0.754	145.4	62.3

Substance GA (R_F 0.358 +/- 0.055):

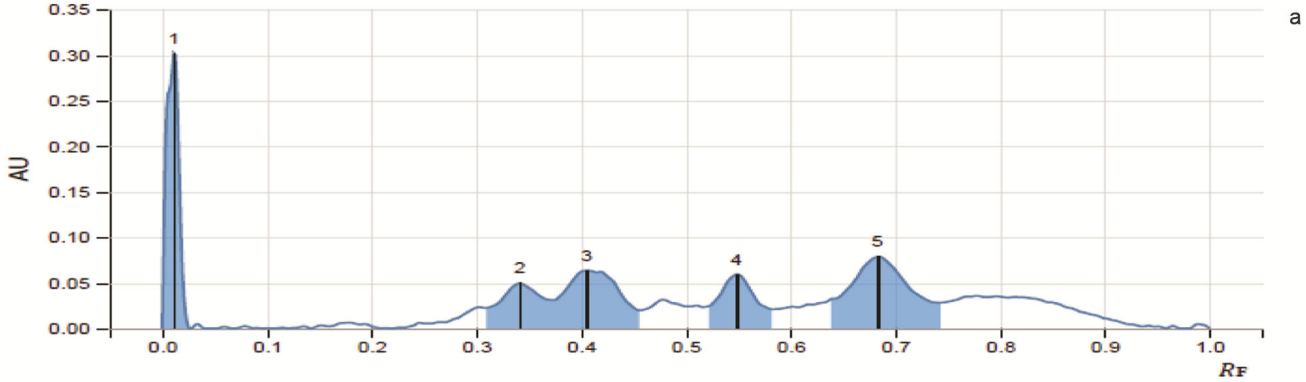
Track	R_F	X (mm)	Y (mm)
2	0.354	31.4	33.5
4	0.354	54.2	33.5
6	0.354	77.0	33.5
8	0.354	99.8	33.5
10	0.354	122.6	33.5
12	0.362	145.4	34.1
13	0.365	156.8	34.3

Fig. 7 — Observed the similar R_F bands in the CF, AECF, HECF, MT, AEMT and HEMT @ 254 nm.

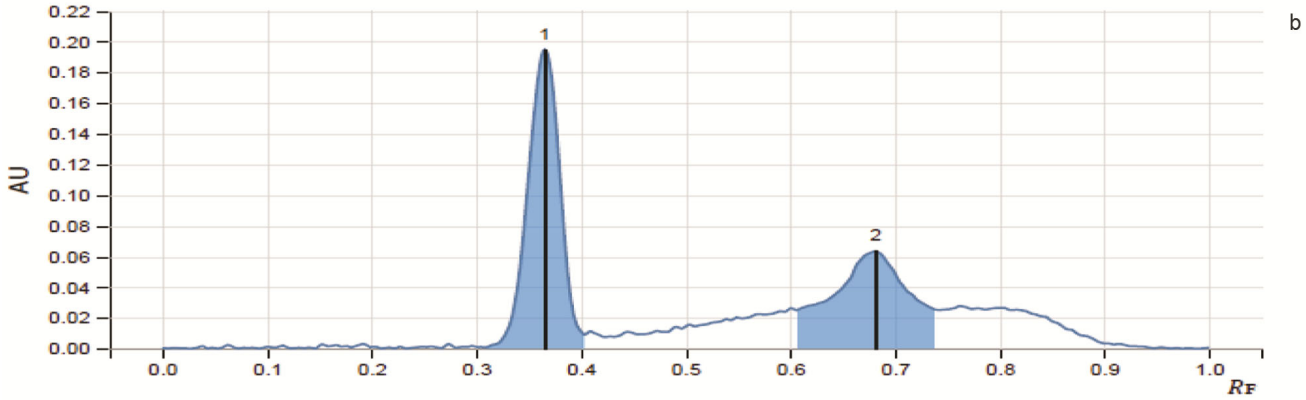
more phytochemicals with R_F: 0.147, 0.217, 0.661, and 0.767, which differed from the AEMT with RF: 0.11, 0.151, 0.333, and 0.929. However, AEMT and HEMT had similar five bands at R_F: 0.011, 0.039, 0.393, 0.665, and 0.912. AEMT had bands with R_F: 0.135 and 0.325, whereas HEMT had bands at R_F: 0.096, 0.171, 0.203, 0.533, and 0.753. Fig. 13 shows the phytochemical of Beta-Sitosterol at R_F: 0.72, which was observed in both HECF and HEMT compared to the aqueous extract of both plants; it indicated sterol chemicals are much higher in the hydroalcoholic extract of CF and MT.

GC-MS/MS analysis

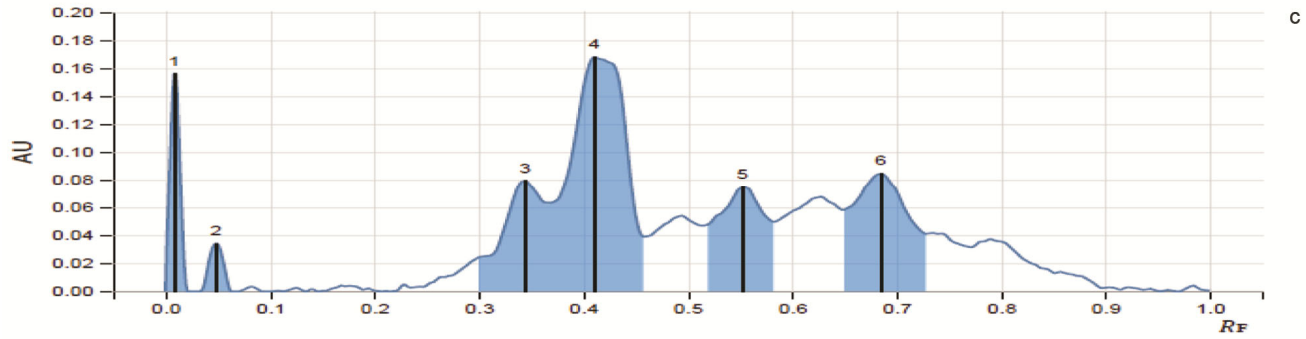
The GC-MS/MS analysis of the HEMT and the AEMT revealed distinct chromatographic profiles, as illustrated in Fig. 14a-d. In the HEMT extract, 20 chromatographic peaks were observed at varying



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.011	0.3020	54.61	0.026	0.0000	0.00429	23.19
2	0.308	0.0225	0.342	0.0494	8.93	0.374	0.0312	0.00239	12.93
3	0.374	0.0312	0.406	0.0636	11.51	0.456	0.0197	0.00386	20.85
4	0.517	0.0233	0.549	0.0594	10.73	0.583	0.0210	0.00254	13.75
5	0.636	0.0303	0.683	0.0786	14.22	0.743	0.0283	0.00542	29.29

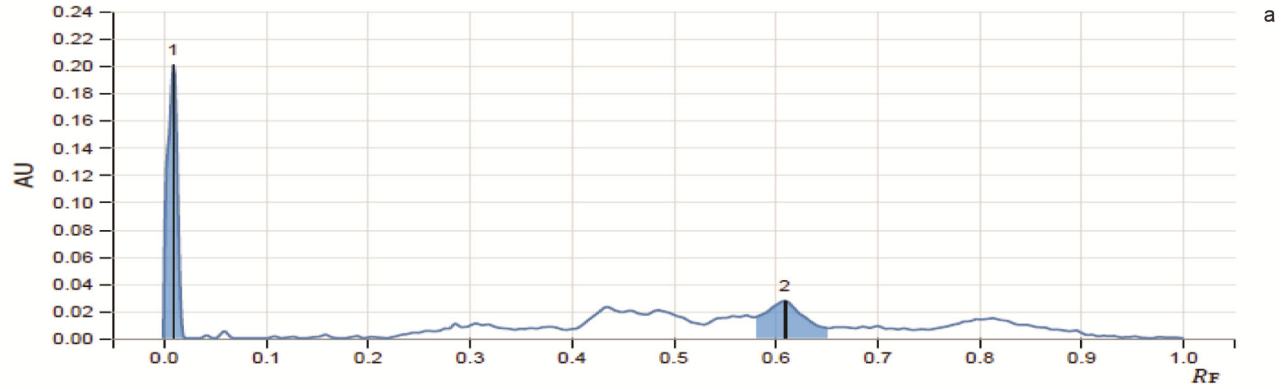


Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.308	0.0008	0.365	0.1947	75.40	0.403	0.0092	0.00696	55.84
2	0.606	0.0250	0.682	0.0635	24.60	0.740	0.0251	0.00551	44.16

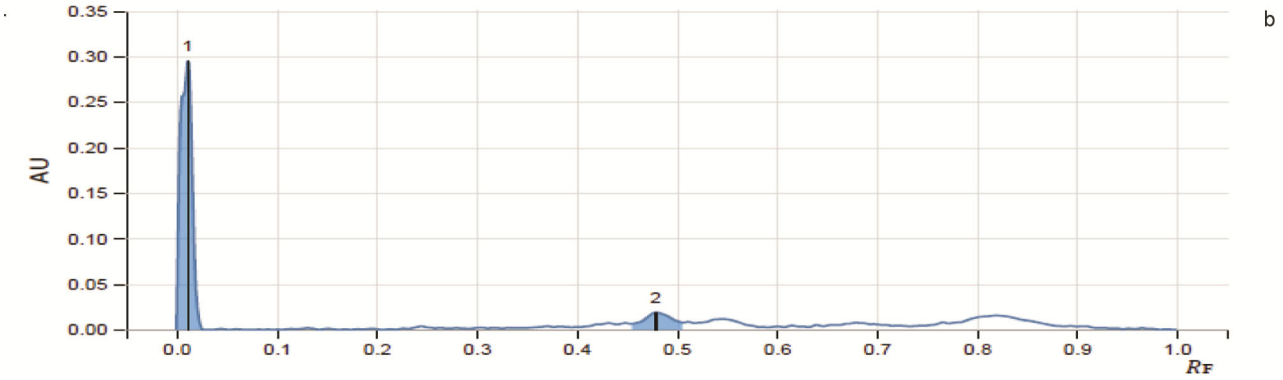


Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.008	0.1556	26.16	0.021	0.0000	0.00173	6.82
2	0.032	0.0000	0.049	0.0341	5.72	0.064	0.0000	0.00054	2.13
3	0.300	0.0243	0.344	0.0791	13.29	0.364	0.0631	0.00343	13.47
4	0.368	0.0629	0.411	0.1676	28.18	0.458	0.0388	0.01042	40.97
5	0.515	0.0467	0.553	0.0742	12.48	0.583	0.0495	0.00408	16.06
6	0.649	0.0580	0.685	0.0843	14.17	0.728	0.0410	0.00523	20.56

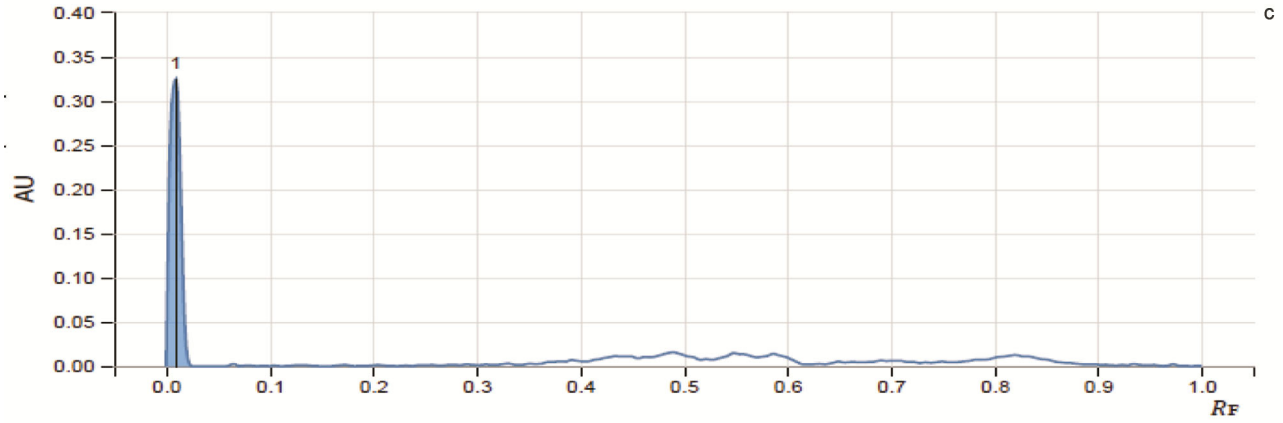
Fig. 8 — Observed R_F bands in the a) aqueous extract of *Chonemorpha fragrans*- -root: AECF, b) Gallic acid-GA, and c) hydroalcoholic extract *Marsdenia tenacissima* HEMT @ 254 nm.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.010	0.2009	87.97	0.021	0.0000	0.00225	63.36
2	0.579	0.0153	0.610	0.0275	12.03	0.653	0.0073	0.00130	36.64

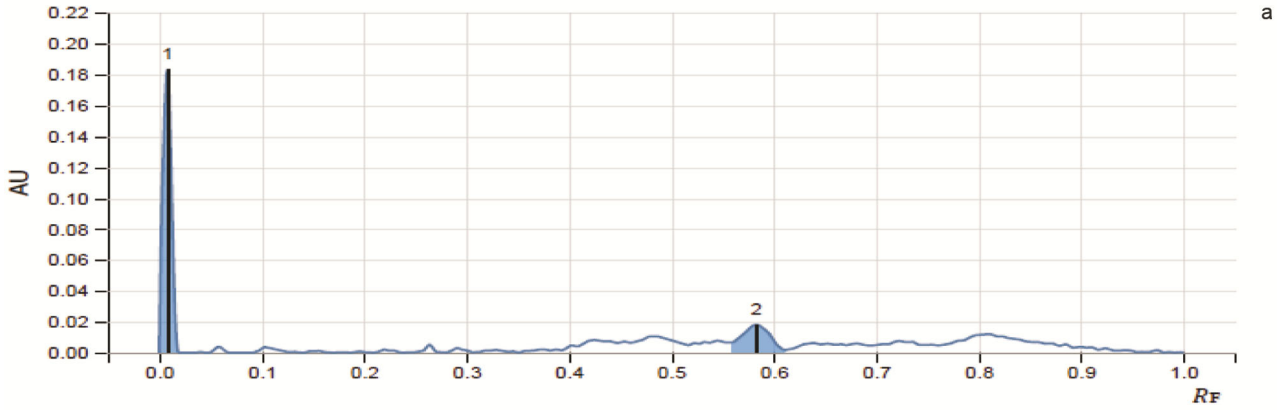


Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.011	0.2950	93.98	0.026	0.0000	0.00407	86.10
2	0.454	0.0063	0.479	0.0189	6.02	0.507	0.0077	0.00066	13.90

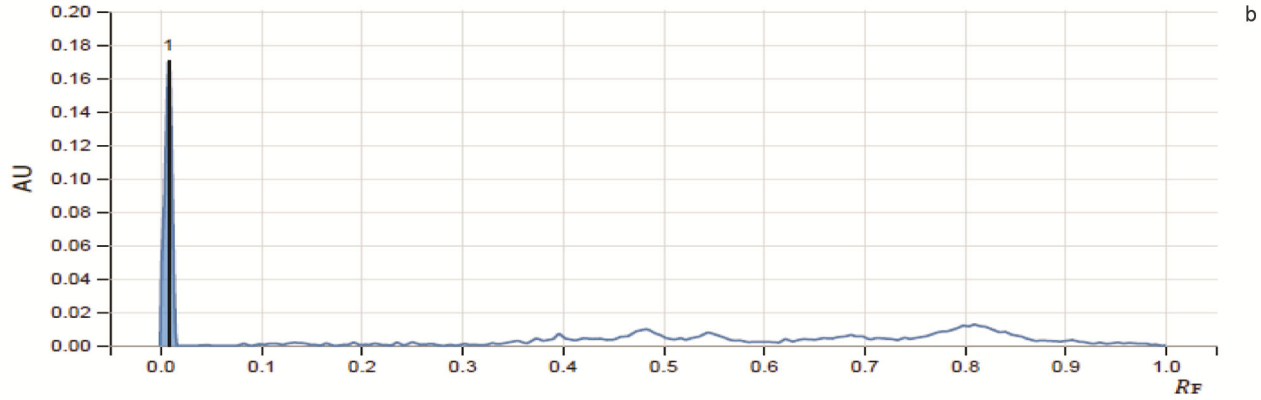


Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.010	0.3240	100.00	0.025	0.0000	0.00412	100.00

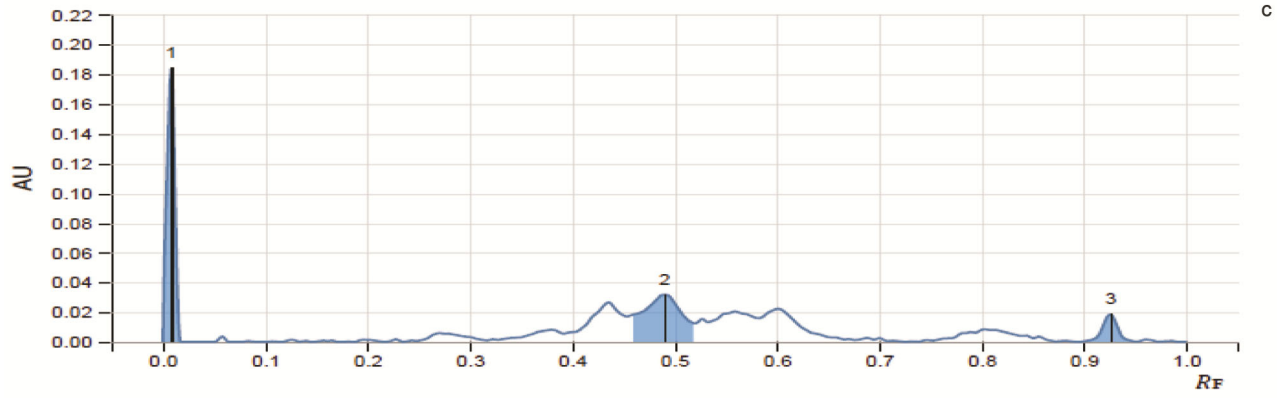
Fig. 9 — Observed R_F bands in the a) Alcoholic extract of *Chonemorpha fragrans*-root: CF, b) Aqueous extract of *Chonemorpha fragrans*: AECF, and c) Hydroalcoholic extract of *Chonemorpha fragrans* HECF @ 366 nm.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.008	0.1834	91.02	0.019	0.0000	0.00183	74.51
2	0.553	0.0065	0.583	0.0181	8.98	0.611	0.0016	0.00063	25.49

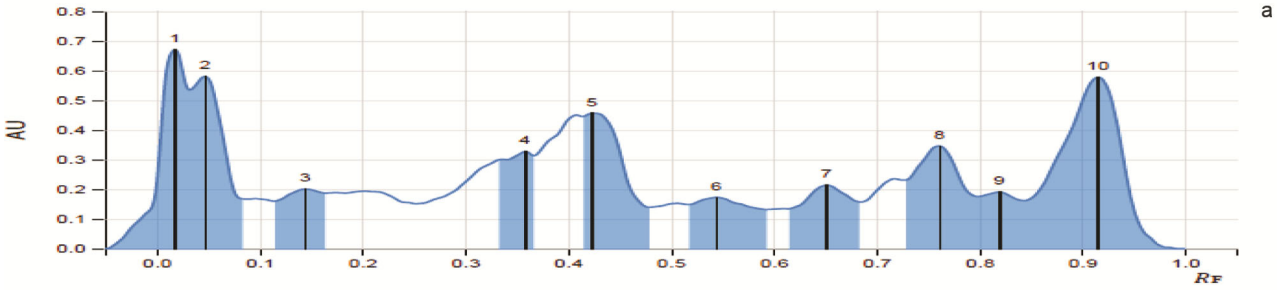


Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.008	0.1698	100.00	0.017	0.0000	0.00146	100.00

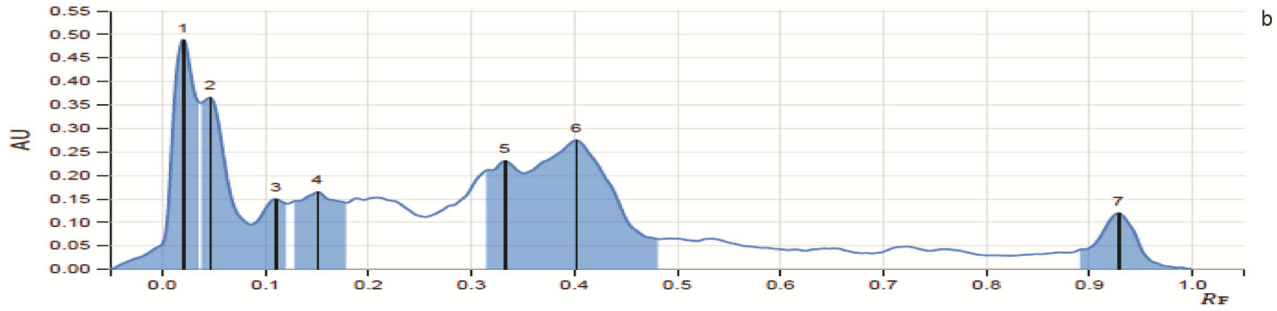


Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.008	0.1838	78.58	0.018	0.0000	0.00168	48.19
2	0.454	0.0171	0.490	0.0318	13.59	0.519	0.0125	0.00148	42.48
3	0.893	0.0000	0.926	0.0183	7.83	0.953	0.0002	0.00033	9.33

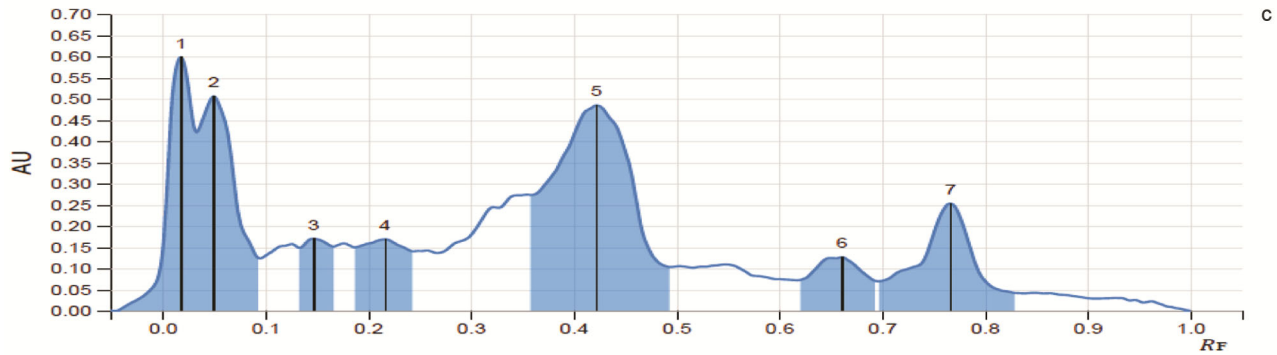
Fig. 10 — Observed R_F bands in the a) Alcoholic extract of *Marsdenia tenacissima*- -root: MT, b) Aqueous extract of *Marsdenia tenacissima*: AEMT, and c) Hydroalcoholic extract *Marsdenia tenacissima* HEMT @ 366 nm.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	-0.049	0.0000	0.018	0.6697	17.94	0.032	0.5343	0.02135	12.05
2	0.032	0.5343	0.047	0.5795	15.52	0.087	0.1669	0.02238	12.63
3	0.114	0.1597	0.144	0.2008	5.38	0.164	0.1871	0.00931	5.26
4	0.333	0.2998	0.358	0.3273	8.77	0.367	0.3132	0.01039	5.87
5	0.414	0.4448	0.424	0.4560	12.21	0.479	0.1392	0.02131	12.03
6	0.517	0.1483	0.544	0.1731	4.64	0.593	0.1316	0.01179	6.66
7	0.614	0.1340	0.651	0.2135	5.72	0.685	0.1570	0.01259	7.11
8	0.726	0.2310	0.761	0.3459	9.27	0.799	0.1756	0.01952	11.02
9	0.799	0.1756	0.819	0.1905	5.10	0.843	0.1618	0.00796	4.49
10	0.843	0.1618	0.915	0.5767	15.45	0.993	0.0000	0.04053	22.88

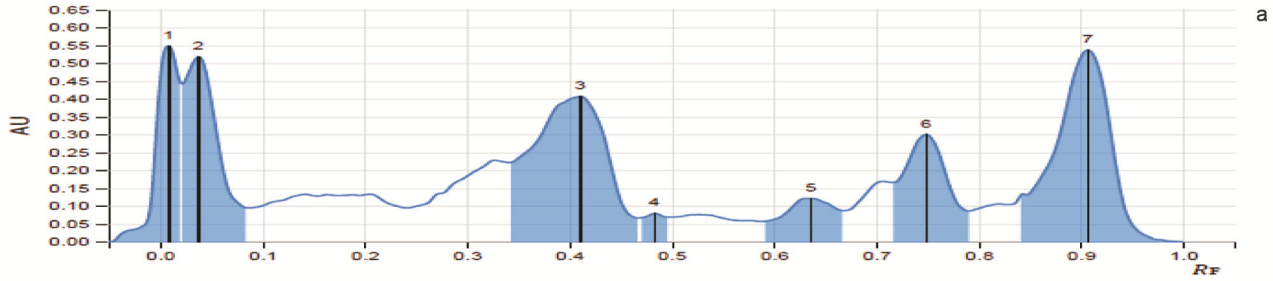


Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	-0.049	0.0000	0.021	0.4871	27.30	0.037	0.3530	0.01362	18.26
2	0.039	0.3529	0.047	0.3642	20.41	0.086	0.0944	0.01071	14.35
3	0.086	0.0944	0.111	0.1488	8.34	0.122	0.1383	0.00459	6.16
4	0.126	0.1412	0.151	0.1631	9.14	0.179	0.1404	0.00792	10.61
5	0.314	0.2076	0.333	0.2290	12.84	0.351	0.2030	0.00810	10.86
6	0.351	0.2030	0.403	0.2736	15.33	0.483	0.0629	0.02442	32.73
7	0.889	0.0383	0.929	0.1184	6.63	0.986	0.0023	0.00526	7.04

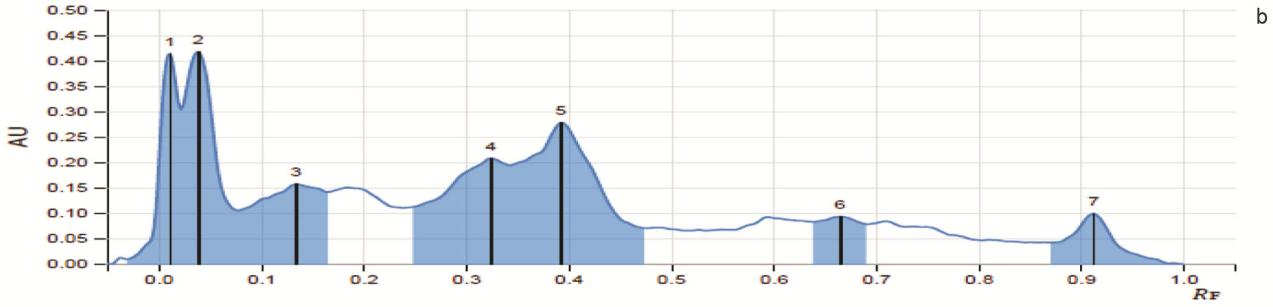


Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	-0.046	0.0000	0.018	0.5980	25.92	0.033	0.4218	0.01720	14.16
2	0.033	0.4218	0.050	0.5058	21.93	0.094	0.1236	0.02094	17.23
3	0.133	0.1486	0.147	0.1701	7.37	0.167	0.1515	0.00538	4.43
4	0.188	0.1499	0.217	0.1687	7.31	0.244	0.1402	0.00894	7.36
5	0.358	0.2733	0.422	0.4849	21.02	0.494	0.1030	0.04469	36.78
6	0.618	0.0718	0.661	0.1264	5.48	0.696	0.0696	0.00785	6.46
7	0.697	0.0695	0.767	0.2530	10.97	0.838	0.0409	0.01651	13.59

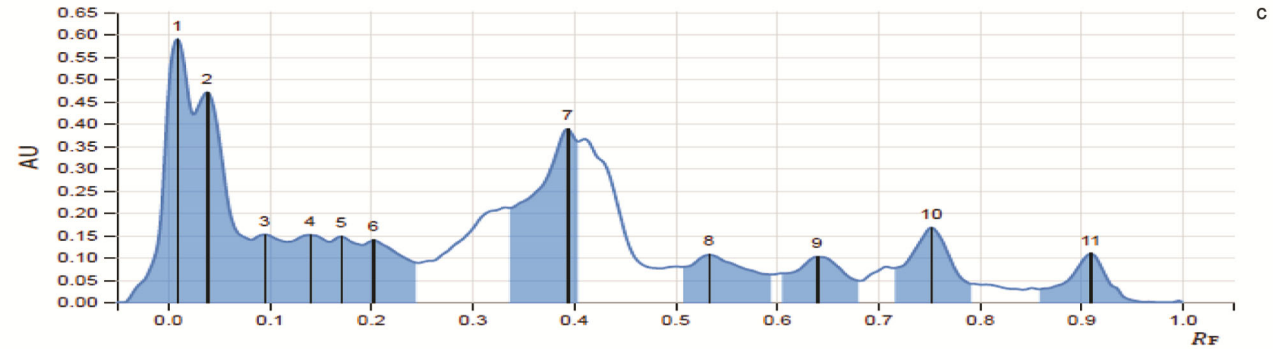
Fig. 11 — Observed R_F bands in the a) Alcoholic extract of *Chonemorpha fragrans*-root: CF, b) Aqueous extract of *Chonemorpha fragrans*: AECF, and c) Hydroalcoholic extract of *Chonemorpha fragrans* HECF @ 545 nm.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	-0.047	0.0000	0.008	0.5475	21.84	0.021	0.4424	0.01427	11.19
2	0.022	0.4419	0.037	0.5170	20.62	0.086	0.0944	0.01986	15.57
3	0.342	0.2214	0.411	0.4065	16.21	0.467	0.0661	0.03515	27.55
4	0.468	0.0660	0.483	0.0791	3.15	0.497	0.0687	0.00211	1.65
5	0.589	0.0564	0.636	0.1211	4.83	0.668	0.0870	0.00746	5.85
6	0.715	0.1659	0.749	0.3002	11.97	0.790	0.0857	0.01533	12.02
7	0.840	0.1259	0.907	0.5360	21.38	1.000	0.0000	0.03338	26.17

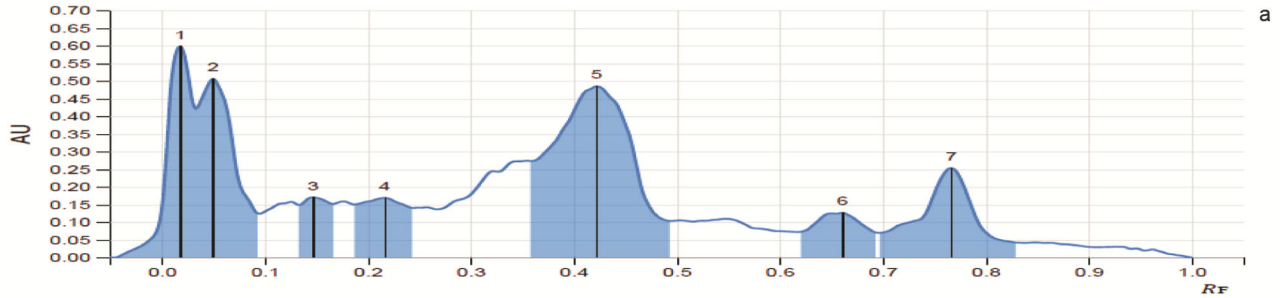


Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	-0.031	0.0091	0.011	0.4127	24.84	0.022	0.3049	0.00896	10.42
2	0.022	0.3049	0.039	0.4163	25.06	0.078	0.1051	0.01499	17.42
3	0.078	0.1051	0.135	0.1563	9.41	0.165	0.1412	0.01196	13.90
4	0.239	0.1099	0.325	0.2073	12.48	0.343	0.1937	0.01664	19.35
5	0.343	0.1937	0.393	0.2774	16.70	0.475	0.0699	0.02377	27.64
6	0.639	0.0826	0.665	0.0929	5.59	0.692	0.0778	0.00457	5.32
7	0.869	0.0420	0.912	0.0984	5.93	0.986	0.0010	0.00512	5.95

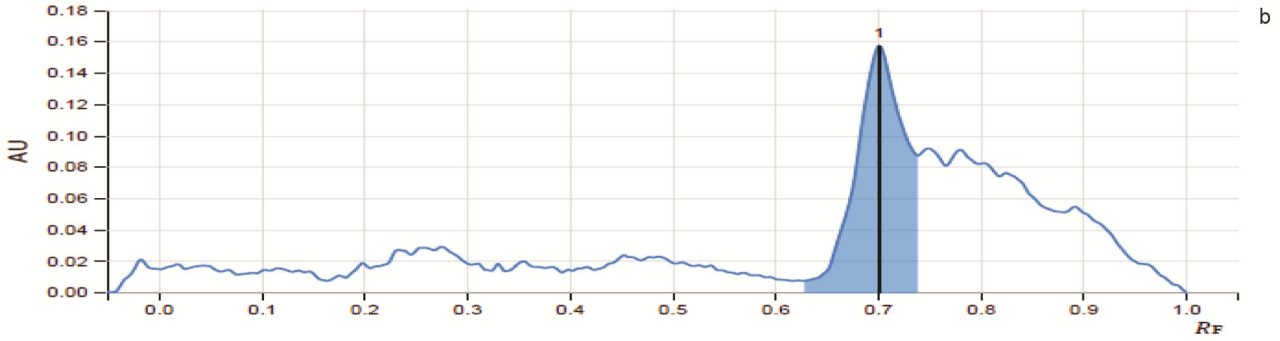


Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	-0.043	0.0000	0.010	0.5884	23.40	0.025	0.4206	0.01719	16.81
2	0.025	0.4206	0.039	0.4698	18.68	0.082	0.1395	0.01753	17.15
3	0.082	0.1395	0.096	0.1513	6.02	0.118	0.1353	0.00520	5.09
4	0.118	0.1353	0.140	0.1504	5.98	0.160	0.1350	0.00598	5.85
5	0.160	0.1350	0.171	0.1470	5.84	0.192	0.1283	0.00441	4.31
6	0.192	0.1283	0.203	0.1384	5.50	0.246	0.0880	0.00633	6.19
7	0.338	0.2109	0.394	0.3869	15.39	0.404	0.3598	0.01919	18.77
8	0.507	0.0790	0.533	0.1059	4.21	0.596	0.0619	0.00743	7.27
9	0.604	0.0647	0.640	0.1014	4.03	0.682	0.0477	0.00602	5.89
10	0.715	0.0764	0.753	0.1667	6.63	0.793	0.0411	0.00812	7.94
11	0.858	0.0291	0.910	0.1085	4.31	0.963	0.0006	0.00485	4.75

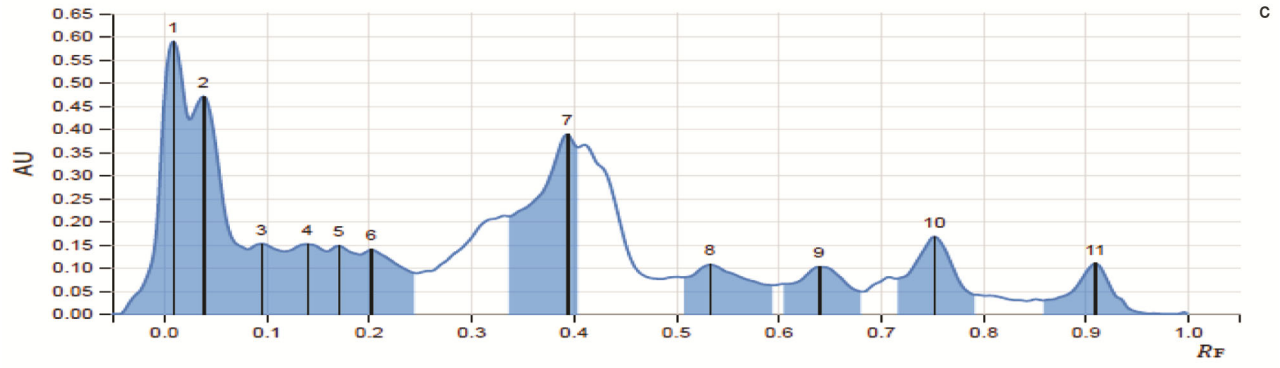
Fig. 12 — Observed R_F bands in the a) Alcoholic extract of *Marsdenia tenacissima*- -root: MT, b) Aqueous extract of *Marsdenia tenacissima*: AEMT, and c) Hydroalcoholic extract *Marsdenia tenacissima*: HEMT @ 545 nm.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	-0.046	0.0000	0.018	0.5980	25.92	0.033	0.4218	0.01720	14.16
2	0.033	0.4218	0.050	0.5058	21.93	0.094	0.1236	0.02094	17.23
3	0.133	0.1486	0.147	0.1701	7.37	0.167	0.1515	0.00538	4.43
4	0.188	0.1499	0.217	0.1687	7.31	0.244	0.1402	0.00894	7.36
5	0.358	0.2733	0.422	0.4849	21.02	0.494	0.1030	0.04469	36.78
6	0.618	0.0718	0.661	0.1264	5.48	0.696	0.0696	0.00785	6.46
7	0.697	0.0695	0.767	0.2530	10.97	0.838	0.0409	0.01651	13.59



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.628	0.0071	0.701	0.1570	100.00	0.739	0.0870	0.00863	100.00



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	-0.043	0.0000	0.010	0.5884	23.40	0.025	0.4206	0.01719	16.81
2	0.025	0.4206	0.039	0.4698	18.68	0.082	0.1395	0.01753	17.15
3	0.082	0.1395	0.096	0.1513	6.02	0.118	0.1353	0.00520	5.09
4	0.118	0.1353	0.140	0.1504	5.98	0.160	0.1350	0.00598	5.85
5	0.160	0.1350	0.171	0.1470	5.84	0.192	0.1283	0.00441	4.31
6	0.192	0.1283	0.203	0.1384	5.50	0.246	0.0880	0.00633	6.19
7	0.338	0.2109	0.394	0.3869	15.39	0.404	0.3598	0.01919	18.77
8	0.507	0.0790	0.533	0.1059	4.21	0.596	0.0619	0.00743	7.27
9	0.604	0.0647	0.640	0.1014	4.03	0.682	0.0477	0.00602	5.89
10	0.715	0.0764	0.753	0.1667	6.63	0.793	0.0411	0.00812	7.94
11	0.858	0.0291	0.910	0.1085	4.31	0.963	0.0006	0.00485	4.75

Fig. 13 — Observed R_F bands in the a) Hydroalcoholic extract of *Chonemorpha fragrans*- -root: HECF, b) Beta-Sitosterol-BS, and c) Hydroalcoholic extract of *Marsdenia tenacissima*: HEMT @ 545 nm.

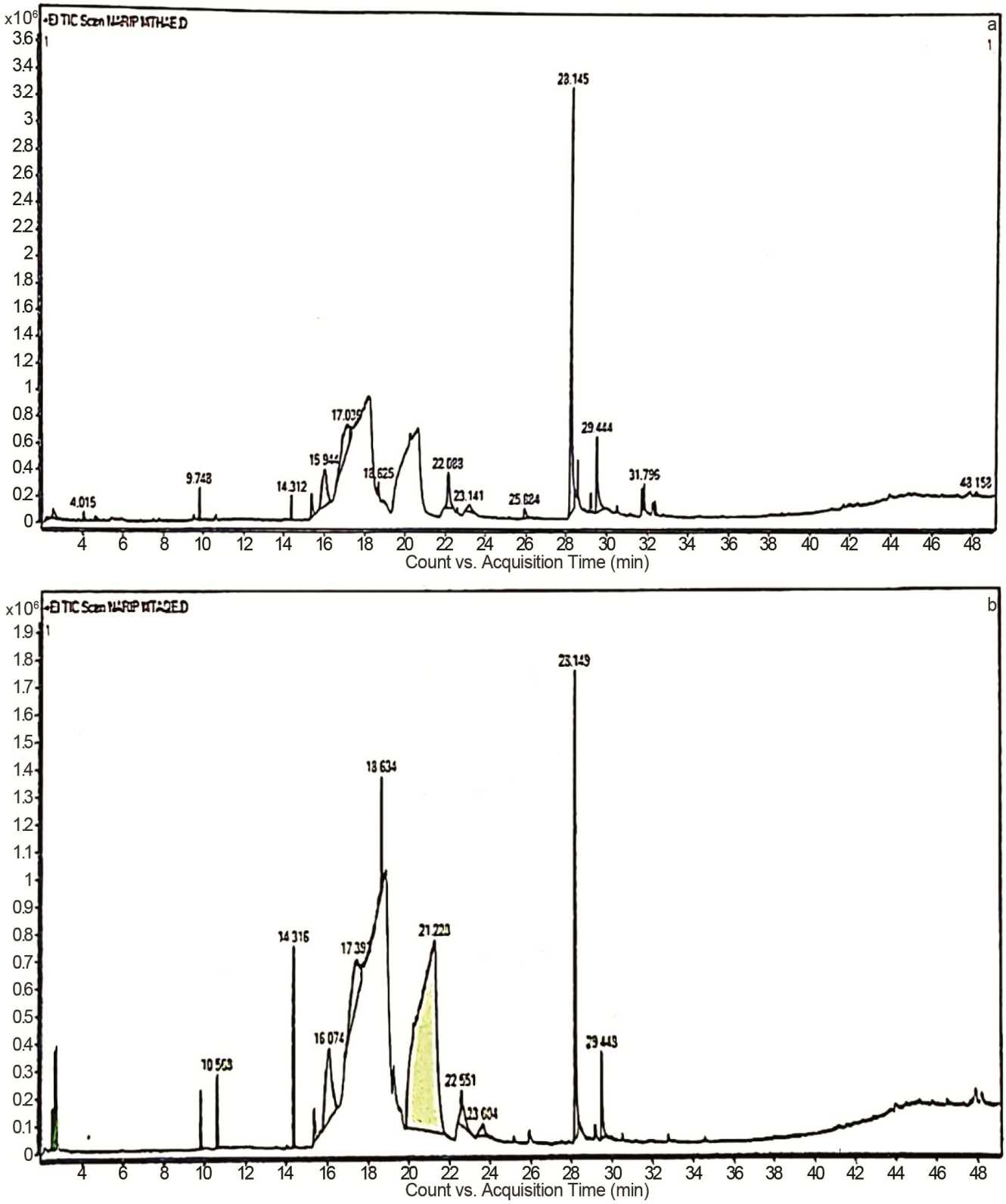


Fig.14 — GC-MS/MS Chromatogram, of a) HEMT, b) AEMT, c) HECF, and d) AECF. (*Contd.*)

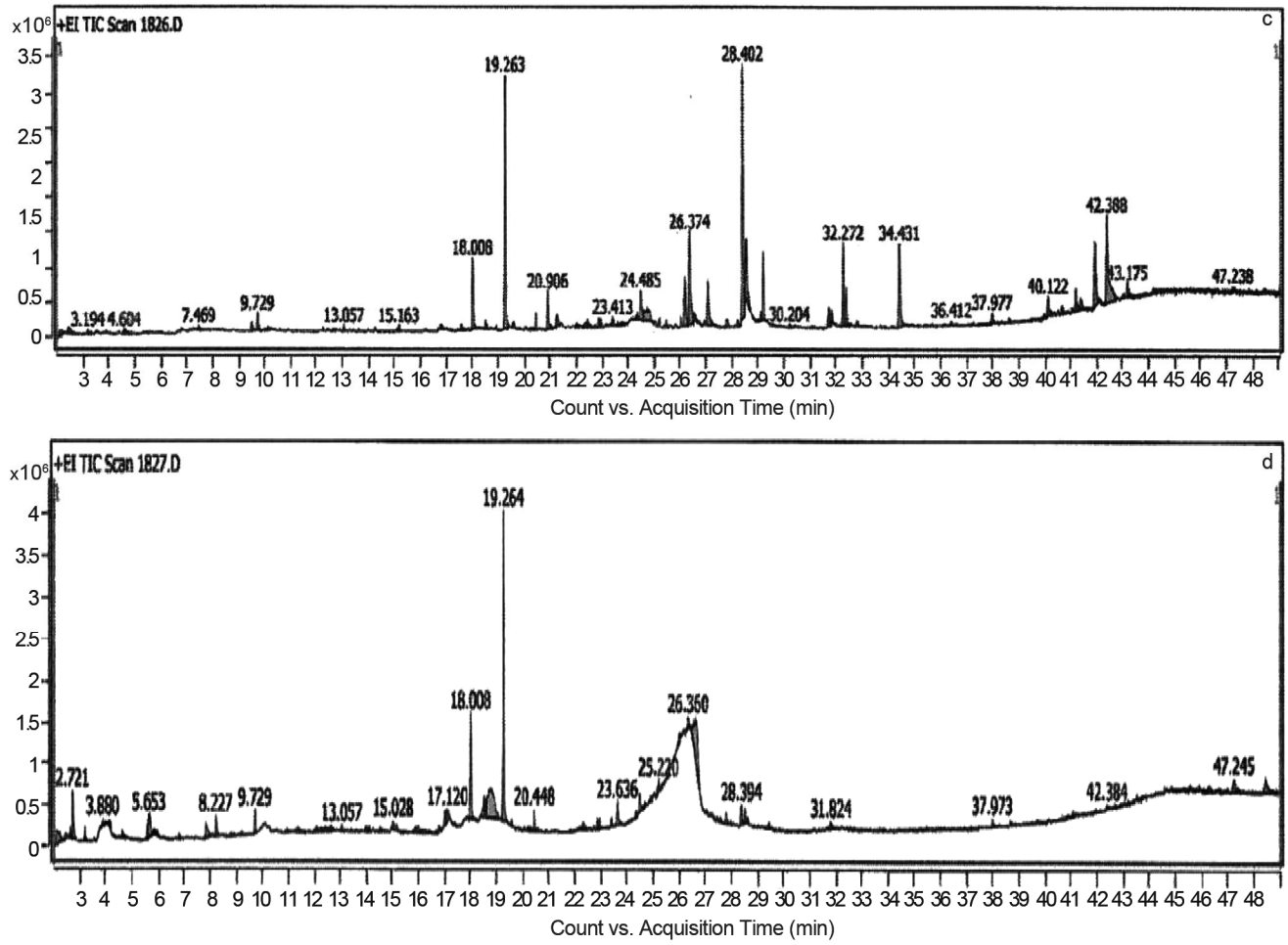


Fig. 14 — GC-MS/MS Chromatogram, of a) HEMT, b) AEMT, c) HECF, and d) AECF.

retention times (Rt), with the most intense peak occurring at Rt: 28.145 and the least intense at Rt: 48.158. Among these, eight major phytochemicals were identified. In contrast, the AEMT displayed 14 chromatographic peaks, with maximum intensity at Rt: 28.149 and minimum at Rt: 23.604, identifying 11 major phytochemicals. Notably, the AEMT extract contained less phytochemicals than the HEMT extract (Table 5).

Further GC-MS/MS analysis was conducted on the HECF and AECF using an in-house method developed at Central Ayurveda Research Institute-Jhansi. The analysis of the HECF sample identified 50 chromatographic peaks at different retention times, with significant peaks occurring at Rt: 19.263, 28.402, 18.008, 26.374, 32.272, 34.431, and 42.388. The area sum percentages of the peaks varied, with the least intense peaks observed between Rt: 3-15, 20-24, and 36-40 minutes. These findings are summarized in Table 6 and Fig.14c, which detail the best-matched library spectra of chemical compounds and their sources.

Similarly, the AECF sample exhibited 49 chromatographic peaks, with prominent peaks at Rt: 19.264, 18.008, and 26.350, indicating these chemicals as more abundant in the extract. Less abundant chemicals were detected within the Rt: range of 30-47 minutes. The chemical composition of AECF, along with the best-matched library spectra, is presented in Table 6 and Fig. 14d; this analysis underscores the complexity of the chemical profiles of both HECF and AECF, with slight differences in the abundance and diversity of the identified compounds, reflecting the influence of solvent choice on extraction efficiency. Comparison of Similar Rt of Probably chemicals observed by the GC-MS/MS of plant extracts are shown in Table 7.

Table 5 — List of the probable Phytochemicals observed in two different extracts of *Marsdenia tenicissima* in GC-MS/MS

S.No.	*Marsdenia tenicissima* Hydro alcoholic extract (HEMT)		*Marsdenia tenicissima* Aqueous extract (AEMT)	
	Retention Time (Rt.)	Probable chemical name	Retention Time (Rt.)	Probable chemical name
1	2.185	Arsenous Acid, tris (trimethyl silyl ester	2.503	*Bezyl (1,2,3-thiadiazol-4-y) carbamate*
2	2.503	*Benzyl (1,2,3-thiadiazol-4-y) carbamate*	2.639	2-(Benzylmethylamino)ethanol N-oxide
3	4.015	Methoxy phenyl oxime	2.701	Petanoic acid
4	4.585	1,2: 3,4-di-O-ethyl boranediylcyclobutane	9.757	*Decamethylcyclopentasiloxane*
5	9.748	*Decamethylcylcopentasiloxane*	14.316	Dodecamethylcyclohexasiloxane
6	14.312	Dodecamethyl	15.326	*4-Amino-3-hydroxy tetrahydrothiophene 1,1-dioxide, TMS derivative*
7	15.312	*4-Amino-3-hydroxy tetrahydrothiophene 1,1-dioxide, TMS Derivative*	16.074	*1,2,3,4-Cylohexanetetrol*
8	15.944	*1,2,3,4-Cyclohexanetetrol*	17.393	*Cyclohexyl S-2-diethyaminoethylethylphosphothiolate*
9	17.036	*Cycolhexyl S-2-diethyaminoethylethylphosphonothiolate*	18.634	*Heptyl S-2-diethyaminoethylamethylphosphonothilate*
10	17.239	O-(iso-Butyl)S-(2-diethyl aminoethyl) ethylphosphonothiolate	21.228	Isobutyl S-2-diethyl amino ethyl propyl phophonothiolate
11	18.625	*Heptyl S-2-Ditheyaminoethyl methyl phosphonothiolate*	22.551	Methyl 2,6-anhydro-alpha-d-altroside
12	22.088	1-deoxy inositol	23.604	1,3,6-trideoxy-3,5-epithio-D-Fructose
13	23.141	Beta. D-Glucosyloxyazoxymethane	28.149	*3,3-Dimethyl-hepta-4,5-dien-2-ol*
14	25.884	*Borinic acid, diethyl, 5 hexynyl ester*	29.448	5,5-Dimethyl-1,3-dioxane-2-ethanol, TBDMS derivative
15	28.145	*3,3 Dimethyl-hepta-4,5-dien-2-ol*		
16	28.502	Methyl 9-methyltetrdecanoate		
17	29.149	Trimethyl-1,2propadienyl-silane		
18	29.444	2,4,6,8-Tetrathiactricyclo [3.3.1.1 (3,7)] decan-1-ol, 3,5,7-trimethyl-monoester with boric acid (H3BO3)		
17	30.463	1-propyl-germacyclopentane		
18	31.690	*Methyl 10,11-tetradecadienoate*		
19	31.796	*Methyl 12,13-tetradecadienoate*		
20	48.158	Tris (terbutyldimethylsilyloxyl)arsane		

Table 6 — List of the probable Phytochemicals observed in two different extracts of *Chonemorpha fragrans* in GC-MS/MS

S.No.	*Chonemorpha fragrans* Hydroalcoholic extract (HECF)		*Chonemorpha fragrans* Aqueous extract (AECF)	
	Retention Time (Rt.)	Probable compound	Retention Time (Rt.)	Probable compound
1	2.180	1H-1,2,3,4-Tetrazole-methoxy phenyl methyl	2.107	*Methyl 12,13-tetradecadienoate*
2	2.470	*Benzyl 1,2,3thiadiazol*	2.445	*Benzyl 1,2,3-thiadiazol*
3	3.194	Carboxymethyl-e	2.639	n-Dodecylpyridinium
4	4.604	*Trans-2,4-dioxide*	2.721	Trimethylalumium
5	7.469	2H-Tetrazole, 2(1,3-methyl)	3.199	Dimethyl mercapto
6	9.512	3-n-Butylthilane	3.880	*Trans-2,4-dioxide*
7	9.729	3-Hydroxy-2-methyl-3-pentane	4.155	*Cyclohexylmethyl S-2-Proylphosphonothiolate*
8	10.135	Methyl phenyl silane	4.624	Pyrrolidinium, 2-carboxy-4-hydroxy-1,1-dimethyl-hydroxide, Inner salt, (2S-trans)
9	12.627	2H-Tetrazole, 2(1,3-dimethyl)	5.653	Ethanethiol, 2-(diethylboryloxy)-
10	13.057	2-Aziod-2,4,4,6,6,8,8-hexamethylheptane	5.856	Boronic acid, ethyl-bis 92-mercaptoethyl ester)
11	15.163	6,6-Dimethyl-2-eptane	5.962	Boronic acid, ethyl-bis (2-mercaptoethyl ester0

(*Contd*.)

Table 6 — List of the probable Phytochemicals observed in two different extracts of *Chonemorpha fragrans* in GC-MS/MS

S.No.	*Chonemorpha fragrans* Hydroalcoholic extract (HECF)		*Chonemorpha fragrans* Aqueous extract (AECF)	
	Retention Time (Rt.)	Probable compound	Retention Time (Rt.)	Probable compound
12	16.805	Pyridine, 2-chloro-3-fluoro-1-oxide	6.817	Cyclohexane, ethenlidene
13	17.573	1,4-Methanophthalizine, 1,4,41,5,6,7,8,8a-octahydro-1,4,9,9-tetramethyl (1alpha, 4alphs, 4a-alpha, 8a-alpha)-	7.841	Propane, 2 isocyanato 2 methyl
14	18.008	*Phosphinic acid, diisopropyl-methyl ester*	8.227	Borane, 2,3-dimethyl-2-butyl (dimer)
15	18.510	2-Azido-2,4,4,6,6-pentamthylheptane	8.831	(4-Methyl-1,2,3-thiadiazol-5yl)-methanol
16	18.573	Cyclohexane, 4-hydroxy-4-phenyl-	9.242	(4-Methyl-1,2,3-thiadiazol-5-yl) methanol
17	19.263	*Benzoic acid, 4-ethoxy, ethyl ester*	9.729	Borinic acid, diethyl, 1-ethynycyclohexyl ester
18	19.573	2-[1-Hexyl-1H-1,2,3-triazol-4yl)methyl]-4-methyl (propyl) amino]isothizolidine,11-dioxide	10.976	(4-Methyl-1,2,3-thiadiazol-5yl)methanol
19	20.287	1H-Thiepine, 2,3,6,7-tertrahydro-4,5-didehydro-3,3,6,6-tetramethyl-1-oxide	11.357	(4-Methyl 1,2,3-thiadiazol-5-yl) methanol
20	20.447	2,4-Dimethoxyphenylisocyante	12.096	Silane, dimethyl
21	20.906	Diethyl Phthalate	12.236	Silane dimethyl
22	21.268	Beta.D-Glucosyloxyazoxymethane	12.429	Silane dimethyl
23	22.017	Methyl 2,2-dimethyl-3,6,9-trioxa-2-silaundecan-11-oate	12.627	Silane dimethyl
24	22.374	(3-Glycidyloxy) propyldiethoxymethylsilane	13.057	(4-Methyl-1,2,3-thiadiazol-5-yl)methanol
25	22.442	Cyclohexane, 1-ethenyl-3-methylene-5-(1-propenylidene)-	13.994	1,2 Benzisothiazol-3(2H)-one, 4,6-diazido-2-methyl, 1-oxide
26	22.867	1-Methyl-4-[4,5-dihydroxyphenyl] pyridinium bromide	14.096	1,2 Benithiazol-3(2H)-one, 4,6-diazido-2-methyl, 1-oxide
27	22.959	(8R, 8aS)-8, 8a-Dimethyl-2-(propan-2-ylidene)-1,2,3,7,8,81-hexahydronapthalene	14.516	Silane, methylene bis [Methyl]-
28	23.157	Borinic acid (2-cyclohexylidene-1,1-diethylpropyl)ethyl-trimethylsilyl ester	15.028	1-(2,6-Dimethyl-phenyl)-1H-tetrazole
29	23.413	Beta-D-Glucosyloxyazoxymethane	15.163	Silane (methylsilyl) methyl
30	23.601	3-Hexene, 3-dimethylboryl-4-trimethylsilyl-	15.975	(4-Methyl-1,2,3-thiadizol-5-yl)methanol
31	23.770	8-Oxononanoic acid, trimethylsilys ester	16.168	(4-Methyl-1,2,3-thiadizol-5-yl)methanol
32	24.355	d-Arabinose	16.395	(4-Methyl-1,2,3-thiadizol-5-yl)methanol
33	24.485	Undccanoic acid	16.694	(4-Methyl-1,2,3-thiadizol-5-yl)methanol
34	24.731	Beta-D-Glucosyloxyazomethane	16.806	1,2-Benzisothiazol-3(2H)-one, 4,6-diazido-2-methyl-1-oxide
35	25.210	Diazoprogesterone	17.042	1,2,4,5-Tetra-O-acetyl-1-deuterio-3-O-Methyl-D-arabinitol
36	25.461	3,6-Methano-1,2,3,4,41,5,6,8a octahydronapthalene,2-(methylimino), N-oxide	17.120	Oct-1-en-3ol, O-TMS
37	26.031	*Borinic acid, diethyl-5-hexynyl ester*	17.395	Diethylborinic acid, TMS Derivative
38	26.195	4-Ethylcyclohexylethylphosphonofluridate (isomer 2)	18.008	*Phosphinic acid, diisopropyl-methyl ester*
39	26.374	4-Methylcyclohexylethylphosphonofluoride	18.506	Silane, diemthyl (tetrahydrofurfuryloxy)ethoxy
40	26.562	Cyclopentlyethylphosophonofluoridate	18.569	2-Butoxy-4-methyl-[1,3,2]dioxaborinane
41	27.069	Dimethyl 2-methoxy hexane-1,6-dioate	18.786	2-Ketohexanoic acid, trimethylsiyl ester
42	27.813	Diethylene glycol 2-ethylhexyl ether, acetate	19.264	*Benzoic acid, 4-ethoxy-ethyl ester*
43	28.200	3,5,5-Trimethylhexylethylphosphonofluroidate	19.578	(4-Methyl-1,2,3-thiadiazol-5-yl)methanol
44	28.402	Difluorophosophoric acid	20.032	(4-Methyl-1,2,3-thiadiazol-5-yl)methanol
45	28.543	Methyl 9-methyltetradeccanoate	20.216	1,2-Dimethyl-3-nitro-4-nitro-benzene

(*Contd.*)

Table 6 — List of the probable Phytochemicals observed in two different extracts of *Chonemorpha fragrans* in GC-MS/MS

S.No.	*Chonemorpha fragrans* Hydroalcoholic extract (HECF)		*Chonemorpha fragrans* Aqueous extract (AECF)	
	Retention Time (Rt.)	Probable compound	Retention Time (Rt.)	Probable compound
46	29.108	7-Oxo-2-oxa-7-thiatricyclo [4,4,0,0 (3,8)] decan-4-ol	20.293	(4-Methyl-1,2,3-thiadiazol-5-yl)methanol
47	29.190	Methyl 2,4-dimethyltetradecanoate	20.448	Nicotinamide N-oxide, TBDMS Dervative
48	30.204	Methyl 13,14-octadecadienoate	20.573	Heptanoic acid, 2,2-dimethyl-6-oxo, methylester
49	31.721	*Methyl 10,11-tetradecadenoate*	21.834	1,2-Benzisothiazol-3(2H)-one, 4,6-diazido-2-methyl-1-oxide
50	31.827	*Methyl 12,13-tetradecadienoate*		

Table 7 — Comparison of similar Rt of probably chemicals observed by the GC-MS/MS of plant extracts

S. No.	Retention time (Rt) in minutes	Name of the Chemo moiety	Name of the plant extract
		M. tenacissima	
1	2.503	Benzyl (1,2,3-thiadiazol-4-y) carbamate	AEMT and HEMT
2	9.748	Decamethylcylcopentasiloxane	
3	15.312	4-Amino-3-hydroxy tetrahydrothiophene 1,1-dioxide, TMS Derivative	
4	15.944	1,2,3,4-Cyclohexanetetrol	
5	17.036	CycolhexylS-2-diethyaminoethylethylphosphonothiolate	
6	18.625	Heptyl, S-2-Ditheyaminoethyl methyl phosphonothiolate	
7	28.145	3,3 Dimethyl-hepta-4,5-dien-2-ol	
		C. fragrans	
1	2.470	Benzyl 1,2,3 thiadiazol	AECF and HECF
2	4.604	Trans-2,4-dioxide	
3	18.008	Phosphinic acid, diisopropyl-methyl ester	
4	19.263	Benzoic acid, 4-ethoxy, ethyl ester	
		M. tenacissima & C. fragrans	
1	26.031	*Borinic acid, diethyl-5-hexynyl ester*	AEMT, HEMT & AECF, HECF
2	31.721	*Methyl 10,11-tetradecadenoate*	
3	31.827	*Methyl 12,13-tetradecadienoate*	

Discussion

Many plants are being used in Ayurveda as substitutes for one another, often without proper scientific validation. This lack of evidence-based standardization raises concerns about the consistency, safety, and efficacy of such substitutions in traditional formulations. This study aimed to scientifically validate the use of *CF* as a substitute/alternative of *Murva* in Ayurvedic formulations. The rationale behind this stems from the discrepancies in botanical descriptions, regional naming conventions, and the diverse interpretations of *Murva* in classical texts[12]. *CF* has a long history of use as the plant associated with *Murva* in southern states like Kerala, where it has been recognized for its medicinal properties and traditional applications[25]. By establishing *CF* as a scientifically sound alternative, this study provides essential scientific backing that can enhance the credibility of Ayurvedic practices and facilitate the adoption of sustainable sourcing methods.

Both *plant* extracts used in the study met stringent quality standards for pesticide residues, aflatoxins, heavy metals, and microbial contamination, confirming their safety for use in medicinal preparations. The physiochemical analysis further revealed that the properties of *MT* extracts were nearly comparable to those of *CF* extracts (Table 3).

This close similarity in physiochemical profiles supports the potential of *CF* as a viable substitute for *MT* in Ayurvedic formulations, ensuring that the substitution does not compromise the quality of the resulting medicinal products.

In the HPTLC Fingerprint analysis of AECF, HECF, and HEMT, AEMT had many more similar bands, but very few were different. Further, similar bands were compared with the Gallic acid (GA), Quercetin (QE) and Beta-Sitosterol (BS). We observed GA in AECF and HEMT, whereas QE in the AECF, HECF, AEMT, and HEMT; however, the BS was observed only in the HECF and HEMT. This shows that both plants have different concentrations of phenols, flavonoids, and sterol chemicals in their extract.

The GC-MS/MS analysis of *both plant* extracts underscores the significant impact of solvent type on phytochemical extraction efficiency. HEMT exhibited a more complex chromatographic profile with 20 peaks, indicating a broader spectrum of phytochemicals than the aqueous extract (AEMT), which had only 14 peaks (Table 5). This suggests that hydroalcoholic solvents may be more effective at extracting diverse compounds. This is consistent with previous findings that such mixtures can extract polar and non-polar compounds, unlike aqueous extracts, which are often limited to more polar substances[26-28]. Similarly, HECF displayed 50 peaks, slightly more than the 49 peaks in the AECF, but HECF showed greater intensity and diversity, indicating a richer phytochemical profile (Table 6).

The GC-MS/MS analysis revealed that AEMT and HEMT shared seven similar compounds at retention times (Rt): 2.03, 9.748, 15.312, 15.944, 17.036, 18.625, and 28.145. Meanwhile, the AECF and HECF shared four similar compounds at Rt: 2.470, 4.604, 18.008, and 19.263. Both plant extracts (MT and CF) had three common compounds at Rt: 26.031, 31.721, and 31.827, as summarized in Table 7. The extracts of the same species (MT or CF) exhibited a more significant chemical composition similarity than extracts from different species. These observations also highlight the superior extraction capabilities of hydroalcoholic solvents, making them preferable for obtaining a comprehensive profile of bioactive compounds. This type of result aligns with findings previously reported by Subra-Paternaultn *et al.*, suggesting that hydroalcoholic extracts had shown more bioactive compounds[29]. It is particularly important in developing herbal medicines and phytochemical research where a full spectrum of compounds is desired. The findings also emphasize the need for tailored extraction methods depending on the specific goals, whether concentrating specific compounds or achieving broad phytochemical extraction[30]. Overall, hydroalcoholic extraction appears more effective in capturing a wide range of bioactive compounds, potentially enhancing the therapeutic efficacy of herbal formulations prepared using these plants.

Future studies could explore these plants pharmacological activities and therapeutic equivalency in clinical settings to validate their efficacy in Ayurvedic treatments. The findings suggest that CF could be a viable substitute for MT in specific Ayurvedic formulations. Continued research and evidence-based substitution are crucial for maintaining the integrity and effectiveness of Ayurvedic medicine amidst environmental and economic challenges. Available comparative analytical studies may not be limited to single-drug pharmacology; they may also encompass comparative studies of the pharmacological activities of polyherbal formulations involving the controversial substitution.

Conclusion

The current study provides a preliminary scientific foundation for utilizing CF as a viable source of *Murva* in Ayurvedic formulations, supported by evidence of its comparable physicochemical properties, HPTLC fingerprints, and phytochemical profiles with MT. The observed chemical similarities and overlapping band patterns between CF and MT strongly indicate the potential for both plants to exhibit similar pharmacological activities.

Future research should prioritize preclinical and clinical validation to confirm the therapeutic efficacy and safety of CF-based formula. Additionally, detailed studies on the pharmacodynamics and pharmacokinetics of these extracts are necessary to understand their modes of action and establish therapeutic equivalency.

To facilitate the integration of CF into Ayurvedic practices as a sustainable and reliable source of *Murva*, it is imperative to develop robust quality control standards and regulatory guidelines for CF-based formulations. These steps will ensure the consistency and safety of the formulations and bridge

traditional Ayurvedic knowledge with modern, evidence-based practices, promoting sustainability while preserving therapeutic authenticity.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

1 Keshari P, Controversy, adulteration and substitution: Burning problems in ayurveda practices, In *Natural Medicinal Plants*, (Intech Open), 2022, doi: 10.5772/intechopen.98220.

2 Dixit V K, Controversial ayurvedic herbs, *J Adv Pharm Technol Res*, 2011, **2**(2), 78-80, doi: 10.4103/2231-4040.82948.

3 Joshi V K, Joshi A and Dhiman K S, The ayurvedic pharmacopoeia of India, development and perspectives, *J Ethnopharmacol*, 2017, **2**(197), 32-38, doi: 10.1016/ j.jep.2016.07.030.

4 Yao R, Heinrich M, Zhang B, Wei X, Qi Y, *et al.*, Single botanical drugs in the Ayurvedic Pharmacopoeia of India—A quantitative ethnobotanical analysis, *Frontiers Pharmacol*, 2023, **14**, 1136446, doi: 10.3389/ fphar.2023.1136446.

5 Thomas V, Nair S V, Ved D K and Shankar D, Controversial identities of medicinal plants in classical literature of Ayurveda, *J Ayu Intger Med*, 2020, **11**(4), 565-572, doi: 10.1016/j.jaim.2019.09.003.

6 Harilal M S, Commercialising traditional medicine: Ayurvedic manufacturing in Kerala, *Econ Political Weekly*, **44**(16), 2009, 44-51.

7 Joshi P R, Patel B R and Shukla V J, An overview of the causes of current practices in Pratinidhi Dravyas (substitution of drugs) in Ayurveda including newer techniques for their evaluation, *Ayu*, 2012, **33**(4), 481-485, doi: 10.4103/0974-8520.1105.

8 Nair P G, Nair P P, Dixit A K, Ekka R and Hazra J, Source plant identification for ayurvedic polyherbal formulation: A hypothetical model-based critical review, *J Drug Res Ayu Sci*, 2020, **5**(4), 249-258, doi: 10.5005/jdras-10059-0104.

9 Joshi P R, Harisha C R and Patel B R, Regionally accepted popular source of Ayurvedic medicinal plants in Southern India, *Int J Pharm Life Sci*, 2011, **2**(10), 1123-1132.

10 Sivarajan V V and Balachandran I, *Ayurvedic drugs and their plant sources*, (Oxford and IBH publishing), 1994.

11 Nair R V, *Controversial drug plants*, (Universities Press India Private Ltd., Hyderabad), 2004.

12 Vaidya B, *Some Controversial Drugs in Indian Medicine*, (ChaukhambhaOrientalia, Varanasi), 1982, 8-15.

13 Mathew A A, Asirvatham R and Tomy D V, Cardioprotective effect of *Marsdenia tenacissima* and *Sansevieria roxburghiana* in doxorubicin-induced cardiotoxicity in rats in vivo: The role of dresgenin and lupeol, *Turk J Pharm Sci*, 2021, **18**(3), 271-281, doi: 10.4274/tjps.galenos.2020.27880.

14 Sasidharan N and Muraleedharan P K, *The Raw Drugs Requirement of Ayurvedic Medicine Manufacturing Industry in Kerala* (KFRI Research Report No. 322, Kerala Forest Research Institute, Peechi, Thrissur), 2009.

15 Jiang S, Qiu L M, Li Y Q, Li L, Wang X Y, *et al*., Effects of *Marsdenia tenacissima* polysaccharide on the immune regulation and tumour growth in H22 tumour-bearing mice, *Carbohydr Polym*, 2016, **137**, 52–58.

16 Mathew A A, Asirvatham R, Gowtham A and Daisy P A, Study of *in-vitro* anti-inflammatory and immunomodulatory effect of Ayurvedic plants – Murva, *Istanbul J Pharm*, 2021, **51**(3), 326-332, doi: 10.26650/ Istanbul J Pharm.2021.877929.

17 Hatapakki B C and Hukkeri V I, Antipyretic activity of root of *Marsdenia tenacissima* in rats, *J Nat Remedies*, 2011, **11**, 98–102.

18 Dong L, Li C P, Song Y Z, Min Z, Sun X M, *et al*., *Marsdenia tenacssima* extract and its functional components inhibits proliferation and induces apoptosis of human Burkitt leukemia/lymphoma cells *in vitro* and *in vivo*, *Leuk. Lymphoma*, 2015, **57**, 419–428.

19 Pang X, Kang L P, Fang X M, Zhao Y, Yu H S, *et al*., Polyoxypregnane glycosides from the roots of *Marsdenia tenacissima* and their anti-HIV activities, *Planta Med*, 2016, **83**, 126–134.

20 Duraisankar M, Devi M and Shanmugasundram P, Hepatoprotective activity of alcoholic extract of Chonemorpha fragrans root in against Paracetamol and Isoniazid-induced liver damage in rats, *Int J Pharmtech Res*, 2015, **8**(7), 232-242.

21 Kedari P and Malpathak N, Exploring antioxidant activity and DNA protection potential of *Chonemorpha fragrans*, *Int J Pharm Sci Res*, 2014, **5**(12), 5452, doi: 10.13040/IJPSR.0975-8232.5(12).5452-62.

22 Dhanya S R, Nair M S, Dileep K B and Sankar V, Chemical constituents from *Chonemorpha fragrans* roots and antibacterial activity studies of sarcorucinine D, *Nat Prod Res*, 2022, **36**(10), 2599-2603, doi: 10.1080/ 14786419.2021.1910263.

23 The Ayurvedic Pharmacopoeia of India, 1st edition, Part I, Vol III, Delhi (IN): Government of India, Ministry of Health and Family Welfare, (Department of Indian Systems of Medicine & Homoeopathy), 2011.

24 The Ayurvedic Pharmacopoeia of India, 1st edition, Part I, Vol III. Delhi (IN): Government of India, Ministry of Health and Family Welfare, (Department of Indian Systems of Medicine & Homoeopathy), 2016.

25 Tiwari V, Singh A and Tiwari A, Phytopharmacological overview on controversial drug: Murva, In *Traditional and Folk Herbal Medicine: Recent Researches*, Vol. II, edited by V K Gupta, (Daya Publsihing house, New Delhi), 2014.

26 Thulasi R, Misro L, Maurya R and Thirupataiah B, Comparative pharmacognostical, phytochemical, high-performance thin-layer chromatography, Fourier-transform

infrared spectroscopy, and ultraviolet spectral studies on leaf and rhizome of *Drynaria quercifolia* (L.) J. Smith, *J Drug Res Ayu Sci*, 2023, **8**(4), 360-369, doi: 10.4103/jdras.jdras_168_22.

27 Borges A, José H, Homem V and Simões M, comparison of techniques and solvents on the antimicrobial and antioxidant potential of extracts from *Acacia dealbata* and *Olea europaea*, *Antibiotics*, 2020, **9**(2), 48, doi: 10.3390/antibiotics9020048.

28 Lefebvre T, Destandau E and Lesellier E, Selective extraction of bioactive compounds from plants using recent extraction techniques: A review, *J Chromatograph A*, 2021, **1635**, 461770, doi: 10.1016/j.chroma.2020.461770.

29 Subra-Paternault P, Garcia-Mendoza M D P, Savoire R and Harscoat-Schiavo C, Impact of hydro-alcoholic solvents on the oil and phenolics extraction from walnut (*Juglans regia* l.) press-cake and the self-emulsification of extracts, *Foods*, 2022, **11**(2), 186, doi: 10.3390/foods11020186.

30 Jha A K and Sit N, Extraction of bioactive compounds from plant materials using combination of various novel methods: A review, *Trends Food Sci Technol*, **119**, 579-591, doi: 10.1016/j.tifs.2021.11.019