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Phytochemical characterization of root and rhizome of critically endangered medicinal plant *Crinum malabaricum* Lekhak and Yadav

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Abstract
Crinum malabaricum Lekhak & Yadav is a critically endangered plant endemic to India (IUCN, 2017). It is an aquatic plant found in streams of Kannur and Kasargod districts of Kerala, India. The underground bulb of *C.malabaricum* is important source of alkaloid galanthamine. It is a selective, reversible, competitive inhibitor of acetylcholine esterase (Olin and Schneider,2002). In this study methanolic, ethyl acetate, chloroform extract of root and rhizome of the plant was studied phytochemically using chromatographic technique like High Performance Thin Layer Chromatography (HPTLC) and Gas Chromatography- Mass Spectrometry (GC-MS). GC-MS result of methanol extract of root showed presence of Hexadecanoic acid, Tetradecanoic acid,12-Methyl-Methyl ester, Ethyl aceate extract showed 1-Hexadecene, Tetratetracontane, Chloroform extract of root showed Heneicosane, Dotriacontane, Methanolic extract of rhizome showed presence of Octadecanoic acid, E-14-hexadecenal, Ethyl acetate extract of rhizome showed Eicosane, Chloroform extract of rhizome showed presence of Hexadecanal, Heptadecanoic acid.

Keywords: *Crinum malabaricum*, HPTLC, GC-MS, hexadecanoic acid, heneicosane, cyclotetracosane

Introduction
Medicinal plants contain various phytochemicals that have different therapeutic activities which can be used to treat various diseases. The family Amaryllidaceae occupies great role in botanical hierarchy because of it's horticultural and ornamental importance as well as it is widely used in traditional medicinal practices. *C.malabaricum* possess tremendous potential of medicinal value and it is comes under the family Amaryllidaceae (Lekhak and Yadav, 2012) [1]. It is an aquatic submerged and its leaves grow 4-5 cm in day length. It's found in streams of Kannur and Kasargod districts of Kerala, India. The Crinum species have commercial, ornamental, economic and medicinal importance. In India it is represented by 14 species (Lekhak *et al.*, 2012) [1]. The natural population of the Amaryllidaceae members are by offsets (Paredes *et al.*, 2014) [18]. Indian crinum species have wide range of therapeutic, anti-aging, anti-oxidant, anti-inflammatory, anti-tumor, anti-microbial activities etc. (Ghane *et al.*, 2018) [17]. Bulbs of *C.malabaricum* sprout during monsoon and dormant during summer season. This species is well known for the source of galanthamine which is used in the treatment of Alzheimer's disease. Galanthamine an alkaloid derived from the underground bulb of the plant. The action of galanthamine is which is competitive inhibitor of acetylcholine esterase. Now a days the natural population of galanthamine producing plants are decreasing due to over exploitation. Plant tissue culture techniques are widely used to conserve the threatened species. The bulbs of *C.malabaricum* contain highest quantity of galanthamine as compared with other species (Jagtap *et al.*, 2014) [14]. Over exploitation of the plant bulbs lead to the depletion of the population. Urbanization and industrial activities also affecting plant habitat. A moth caterpillar feed flowers fruits and tubers of *C.malabaricum*, this is also another threat to the plant species (Punekar *et al.*, 2004).

2. Materials and Methods
2.1 Collection and extraction of plant materials: The fresh samples of *C. malabaricum* were collected from Aravanchal, Kannur district in Kerala, India. The rhizome and root of the sample were washed under running tap water to remove soil particles and adhered debris. The samples were chopped in to pieces, dried under shade at room temperature. The dried samples were grind in to powder and the powdered material is weighed and used for Soxhlet extraction using methanol, ethyl acetate and chloroform. After Soxhlet it was filtered and the filtrate concentrated using the rotary evaporator.

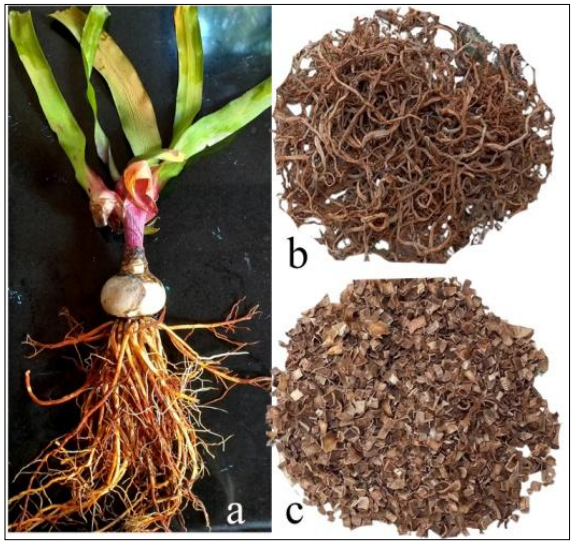

Fig 1: a) habit, b) dried root, c) rhizome

2.2 HPTLC fingerprint profile: HPTLC analyses were performed on aluminium backed pre-coated silica gel 60 F254 TLC plates. Samples were applied to the plates by means of CAMAG Automatic sampler. HPTLC fingerprint profile was carried out by using methanol, ethyl acetate and chloroform extracts of the root and rhizome of *C. malabaricum*.

2.3 GC-MS analysis: Chemical composition was determined by GC-MS (Shimadzu QP-2010 plus with Thermal Desorption System TD 20, fitted with a 60 m x 2025 mm x 0.25 m WCOT column coated with diethylene glycol (AB-Innowax 7031428, Japan). Helium was used as a carrier gas at a flow rate of 1.21 mL/min at a column pressure of 77.6 kPa. Both injector and detector temperatures were maintained at 260 ^{0}C. Samples (6 µL) were injected in to the column with a split ratio of 10:0. Component separation was achieved following a linear temperature program of 70- 260 ^{0}C at 3^{0}C/min and then held at 260^{0}C for 6 min, with a total run time of 44.98 min. The MS parameters used were: electron ionization (EI) voltage 70 eV, peak width 2 s, mass range 40-850 *m/z* and detector voltage 1.5 V. The constituents were identified by comparison of their linear retention indices. The MS fragmentation pattern was checked with National Institute of Standards and Technology (NIST) mass spectra libraries and with those in the literature (Adams, 2001) [4]. The phytochemical compounds were identified and confirmed based on their peak area, molecular formula and retention time.

3. Results

3.1 HPTLC fingerprint profile of Methanol extract of root of *C. malabaricum* at different wave length: The results showing number of peaks, R_f values, and area percentage were presented in table 1. In the HPTLC densitometric the finger print profile of root in methanol extract under 254 nm revealed 12 peaks, major peak at R_f 1.81 with area percentage of 12.97 and minor peak at 0.05 with area percentage of 0.19. In 366nm revealed 7 peaks major peak at R_f 1.95 with area percentage 19.18 followed by peak at 1.81 with area percentage 55.63, minor peak at 0.05 with area percentage 2.47. In 550 nm revealed 12 peaks major peak at 1.96 with area percentage 0.37 followed by R_f 1.72 with area percentage 14.78, minor peak R_f 0.08 with area percentage 3.58.

Table 1: HPTLC fingerprint profile of methanol extract of root of *C.malabaricum* at different wave length.

254 nm			366 nm			550 nm		
No of peaks	**R_f value**	**Area %**	**No of peaks**	**R_f value**	**Area %**	**No of peaks**	**R_f value**	**Area %**
1	0.05	0.19	1	0.05	2.47	1	0.08	3.58
2	0.16	2.18	2	0.14	5.26	2	0.29	5.42
3	0.28	7.33	3	0.77	6.75	3	0.46	14.35
4	0.45	40.50	4	0.93	5.07	4	0.66	5.80
5	0.65	23.24	5	1.39	5.64	5	0.84	2.00
6	0.83	5.03	6	1.81	55.63	6	0.90	0.26
7	0.94	1.07	7	1.95	19.18	7	1.21	23.35
8	1.10	0.44				8	1.41	20.57
9	1.22	4.81				9	1.53	3.19
10	1.54	1.61				10	1.61	6.32
11	1.72	0.63				11	1.72	14.78
12	1.81	12.97				12	1.96	0.37

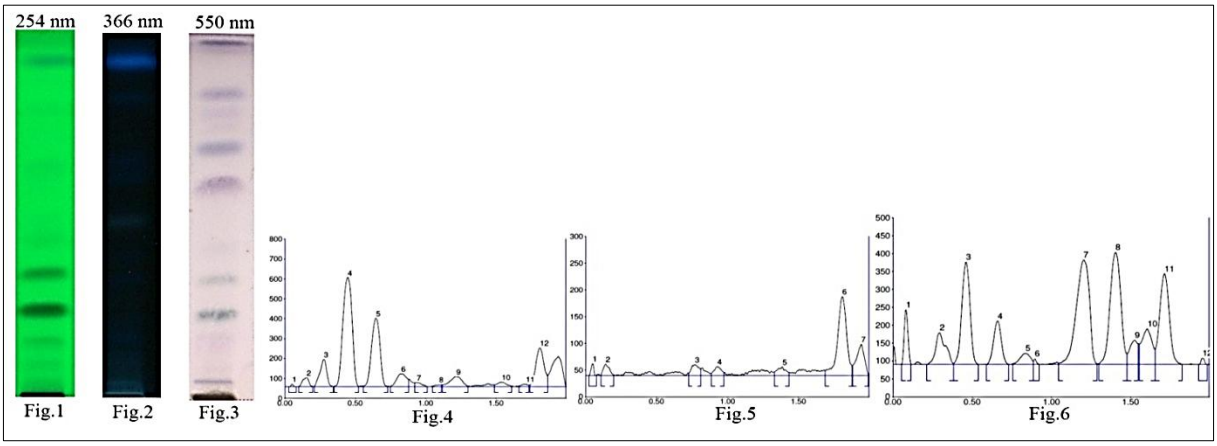

Fig 2: Densitogram of methanol extract of root of *C.malabaricum*. Fig. 4-6 Chromatogram of methanol extract of root of *C.malabaricum*

3.2 HPTLC fingerprint profile of Ethyl acetate extract of root of *C.malabaricum* at different wave length: The results showing number of peaks, R_f values, and area percentage were presented in table 2. In the HPTLC densitometric the finger print profile of root in ethyl acetate extract under 254nm revealed 4 peaks, major peak at R_f 1.97 with area percentage of 6.63 followed by R_f 1.92 with area percentage 69.97 and R_f 1.02 with area percentage 13.35, minor peak at 0.42 with area percentage of 10.04. In 366nm revealed 6 peaks major peak at R_f 1.88 with area percentage 30.19 followed by peak at 1.81 with area percentage 6.58, minor peak at 0.42 with area percentage 6.45. In 550nm revealed 3 peaks major peak at 1.94 with area percentage 56.04 followed by R_f 1.02 with area percentage 25.09, minor peak R_f 0.44 with area percentage 18.88.

Table 2: HPTLC fingerprint profile of ethyl acetate extract of root of *C. malabaricum* at different wave length.

254 nm			366 nm			550 nm		
No of peaks	R_f value	Area %	No of peaks	R_f value	Area %	No of peaks	R_f value	Area%
1	0.42	10.04	1	0.42	6.45	1	0.44	18.88
2	1.02	13.35	2	1.02	10.51	2	1.02	25.09
3	1.92	69.97	3	1.36	25.40	3	1.94	56.04
4	1.97	6.63	4	1.77	20.87			
			5	1.81	6.58			
			6	1.88	30.19			

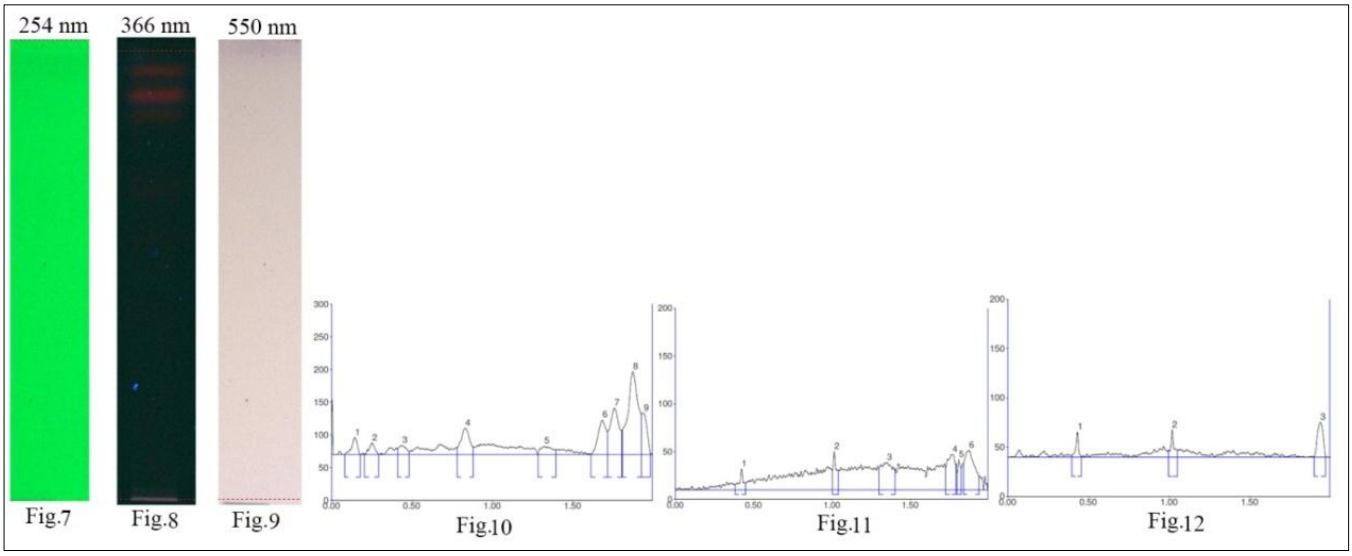

Fig 3: Densitogram of ethyl acetate extract of root of *C.malabaricum*. Fig. 10-12 Chromatogram of ethyl acetate extract of root of *C.malabaricum*

3.3 HPTLC fingerprint profile of Chloroform extract of root of *C.malabaricum* at different wave length: The results showing number of peaks, R_f values, and area percentage were presented in table 3. In the HPTLC densitometric the finger print profile of root in chloroform extract under 254nm revealed 5 peaks, major peak at R_f 1.78 with area percentage of 75.81 and minor peak at 0.20 with area percentage of 5.53. In 366nm revealed 3 peaks major peak at R_f 1.94 with area percentage 27.33 followed by peak at 1.79 with area percentage 70.99, minor peak at 1.02 with area percentage 4.68. In 550nm revealed 6 peaks major peak at 1.69 with area percentage 19.75 followed by R_f 1.59 with area percentage 13.84, minor peak R_f 0.09 with area percentage 8.26.

Table 3: HPTLC fingerprint profile of chloroform extract of root of *C.malabaricum* at different wave length.

254 nm			366 nm			550 nm		
No of peaks	**R_f value**	**Area %**	**No of peaks**	**R_f value**	**Area %**	**No of peaks**	**R_f value**	**Area%**
1	0.20	5.53	1	1.02	4.68	1	0.09	8.26
2	0.36	6.97	2	1.79	70.99	2	1.02	0.42
3	1.02	3.79	3	1.94	24.33	3	1.23	29.70
4	1.20	7.90				4	1.41	28.03
5	1.78	75.81				5	1.59	13.84
						6	1.69	19.75

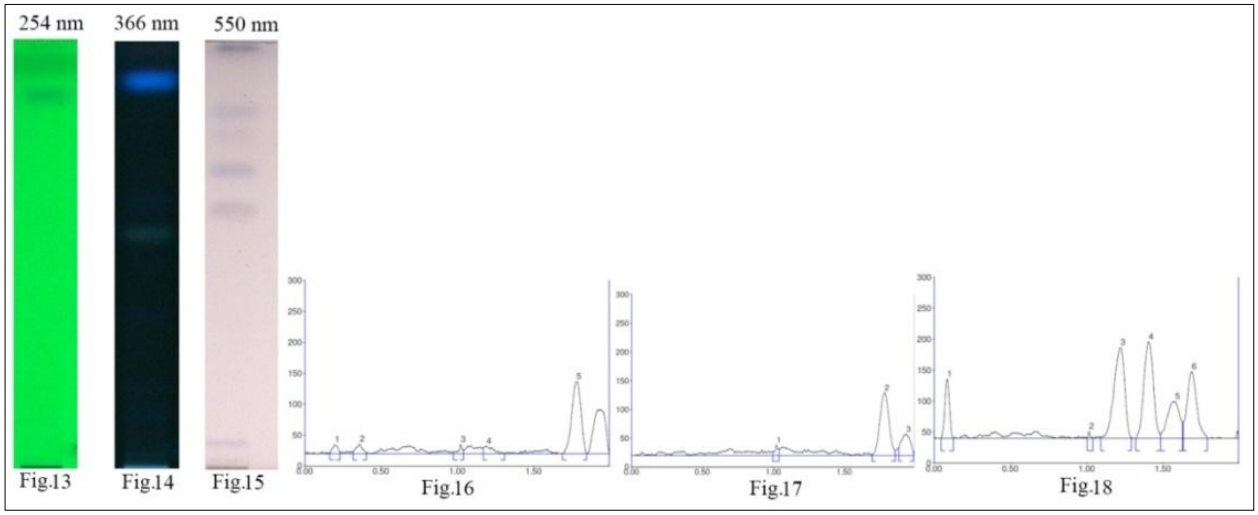

Fig 4: Densitogram of chloroform extract of root of *C.malabaricum*. Fig. 16-18 Chromatogram of chloroform extract of root of *C.malabaricum*

3.4 HPTLC fingerprint profile of Methanol extract of rhizome of *C.malabaricum* at different wave length

Table 4: HPTLC fingerprint profile of methanol extract of rhizome of *C.malabaricum* at different wave length.

254 nm			366 nm			550 nm		
No of peaks	**R_f value**	**Area %**	**No of peaks**	**R_f value**	**Area %**	**No of peaks**	**R_f value**	**Area%**
1	0.26	6.74	1	0.03	1.82	1	0.02	5.63
2	0.90	4.00	2	0.90	7.43	2	0.09	2.35
3	1.00	3.54	3	1.34	7.66	3	0.34	0.67
4	1.35	4.28	4	1.59	7.07	4	0.47	0.78
5	1.81	81.44	5	1.81	76.02	5	0.57	1.26
						6	1.01	1.75
						7	1.17	28.47
						8	1.39	16.81
						9	1.60	18.79
						10	1.70	23.49

The results showing number of peaks, R_f values, and area percentage were presented in table 4. In the HPTLC densitometric the finger print profile of rhizome in methanol extract under 254nm revealed 5 peaks, major peak at R_f 1.81 with area percentage of 81.44 followed by R_f 1.35 with area percentage 4.28 and minor peak at 0.26 with area percentage of 6.74. In 366nm revealed 5 peaks major peak at R_f 1.81 with area percentage 76.02 followed by peak at 1.59 with area percentage 7.07, minor peak at 0.03 with area percentage 1.82. In 550nm revealed 10 peaks major peak at 1.70 with area percentage 23.49 followed by R_f 1.60 with area percentage 18.79, R_f 1.39 with area percentage 16.81, R_f 1.17 with area percentage 28.47, R_f 1.01 with area percentage 1.75, R_f 0.57 with area percentage 1.26, R_f 0.47 with area percentage 0.78, R_f 0.34 with area percentage 0.67, R_f 0.09 with area percentage 2.35 minor peak R_f 0.02 with area percentage 5.63.

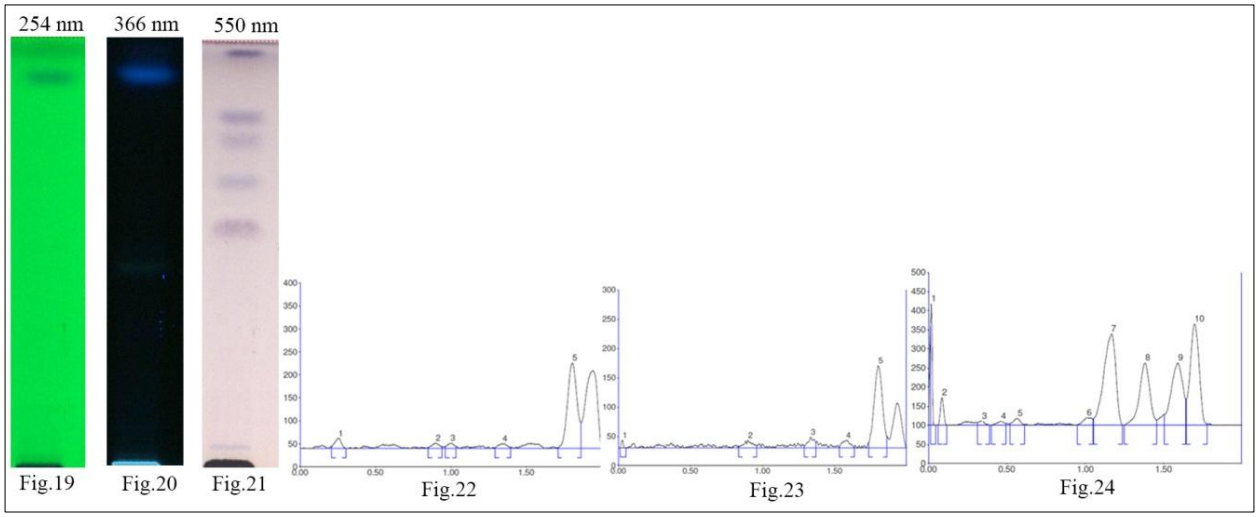

Fig 5: Densitogram of methanol extract of rhizome of *C.malabaricum*. Fig. 22-24 Chromatogram of methanol extract of rhizome of *C.malabaricum*

3.5 HPTLC fingerprint profile of ethyl acetate extract of rhizome of *Crinum malabaricum* at different wave length.

Table 5: HPTLC fingerprint profile of ethyl acetate extract of rhizome of *C.malabaricum* at different wave length.

254 nm			366 nm			550 nm		
No of peaks	**R_f value**	**Area %**	**No of peaks**	**R_f value**	**Area %**	**No of peaks**	**R_f value**	**Area%**
1	1.74	63.89	1	1.74	70.80	1	0.07	3.34
2	1.91	36.11	2	1.87	29.20	2	0.55	3.78
						3	1.11	21.37
						4	1.36	14.00
						5	1.54	18.57
						6	1.65	24.56
						7	1.96	14.38

The results showing number of peaks, R_f values, and area percentage were presented in table 5. In the HPTLC densitometric the finger print profile of rhizome in ethyl acetate extract under 254nm revealed 2 peaks, major peak at R_f 1.91 with area percentage of 36.11 and minor peak at 1.74 with area percentage of 63.89. In 366nm revealed 2 peaks major peak at R_f 1.87 with area percentage 29.20 minor peak at 1.74 with area percentage 70.80. In 550nm revealed 7 peaks major peak at 1.96 with area percentage 14.38 followed by R_f 1.65 with area percentage 24.56, R_f 1.54 with area percentage 18.57, R_f 1.36 with area percentage 14.00, R_f 1.11 with area percentage 21.37, R_f 0.55 with area percentage 3.58 minor peak R_f 0.07 with area percentage 3.34.

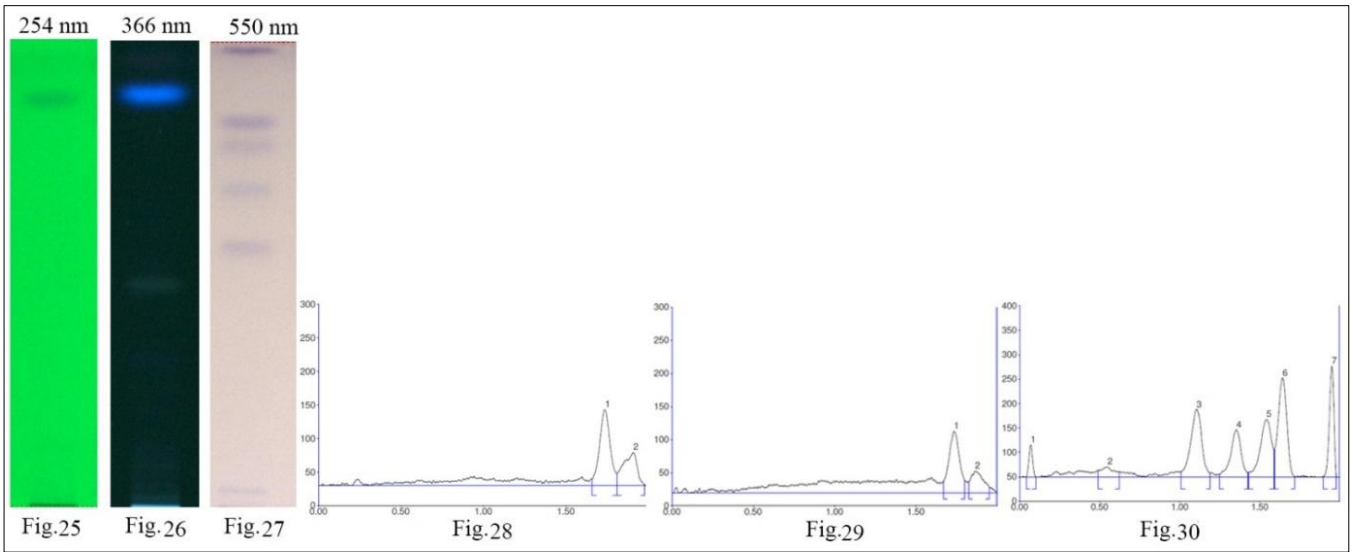

Fig 6: Densitogram of ethyl acetate extract of rhizome of *C.malabaricum*. Fig. 28-30 Chromatogram of ethyl acetate extract of rhizome of *C.malabaricum*

3.6 HPTLC fingerprint profile of chloroform extract of rhizome of *Crinum malabaricum* at different wave length.

Table 6: HPTLC fingerprint profile of chloroform extract of rhizome of *C.malabaricum* at different wave length.

254 nm			366 nm			550 nm		
No of peaks	**R_f value**	**Area %**	**No of peaks**	**R_f value**	**Area %**	**No of peaks**	**R_f value**	**Area%**
1	0.76	1.93	1	1.06	6.79	1	0.09	4.08
2	1.07	7.51	2	1.63	6.55	2	0.66	1.86
3	1.63	4.65	3	1.78	70.79	3	1.23	25.59
4	1.78	85.91	4	1.93	15.86	4	1.40	17.63
						5	1.58	20.93
						6	1.69	29.91

The results showing number of peaks, R_f values, and area percentage were presented in table 6. In the HPTLC densitometric the finger print profile of rhizome in chloroform extract under 254nm revealed 4 peaks, major peak at R_f 1.78 with area percentage of 85.91, followed by R_f 1.63 with area percentage 4.65, R_f 1.07 with area percentage 7.51 and minor peak at 0.76 with area percentage of 1.93. In 366nm revealed 4 peaks major peak at R_f 1.93 with area percentage 15.86 followed by peak at 1.78 with area percentage 70.79, R_f 1.63 with area percentage 6.55, minor peak at 1.06 with area percentage 6.79. In 550nm revealed 6 peaks major peak at 1.69 with area percentage 29.91 followed by R_f 1.58 with area percentage 20.93, R_f 1.40 with area percentage 17.63, R_f 1.23 with area percentage 25.59, R_f 0.66 with area percentage 1.86 minor peak R_f 0.09 with area percentage 4.08.

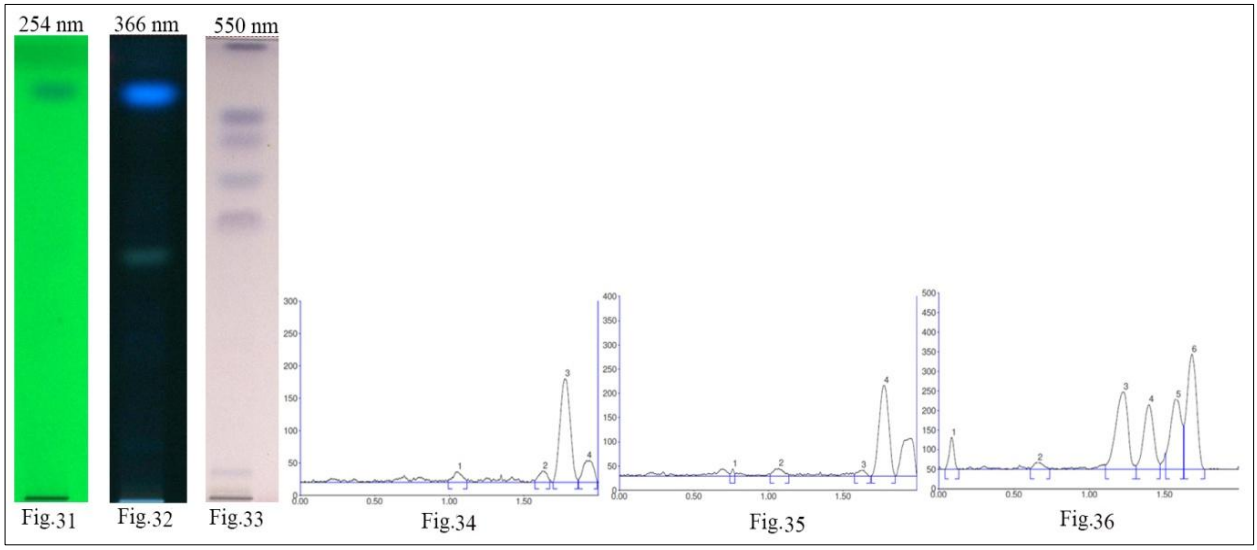

Fig 7: Densitogram of chloroform extract of rhizome of *C.malabaricum*. Fig. 34-36 Chromatogram of chloroform extract of rhizome of *C.malabaricum*

3.7 Metabolite profiling by GC-MS Methanol root

7 bioactive compounds have been identified in the methanol root extract of *Crinum malabaricum* (table 7). The major bioactive compounds include Hexadecanoic acid, Methyl ester has anti-inflammatory effects, Hexadecanoic acid has antioxidant, anti-fibrinolytic, anti-microbial effects, 9,12-Octadecadienoic acid, methyl ester has anti-microbial effects, Tetradecanoic acid,12-Methyl-,Methyl ester has anti-fungal effects.

Table 7: Metabolite profile of methanol extract of root of *C.malabaricum*

Peaks	RT	Area %	Name of compound	Biological activity
1	14.725	27.00	5-(Hydroxymethyl)-2-(dimethoxymethyl) furan	-
2	15.073	0.98	2-Methoxy-4-vinylphenol	-
3	22.406	4.31	Hexadecanoic acid, Methyl ester	Anti-inflammatory (Silva *et al.*,2021) [32]
4	22.744	7.26	Hexadecanoic acid	antioxidant, anti-fibrinolytic, anti-microbial (Gonzalez *et al.*,2023) [33]
5	24.050	8.87	9,12-Octadecadienoic acid, methyl ester	Anti-microbial (Rukachaisirikul *et al.*, 2004) [34]
6	24.109	4.37	9-Octadecenoic acid (Z) -, Methyl ester	-
7	24.341	2.00	Tetradecanoic acid,12-Methyl -, Methyl ester	Anti-fungal (Zalbayu *et al.*, 2021)

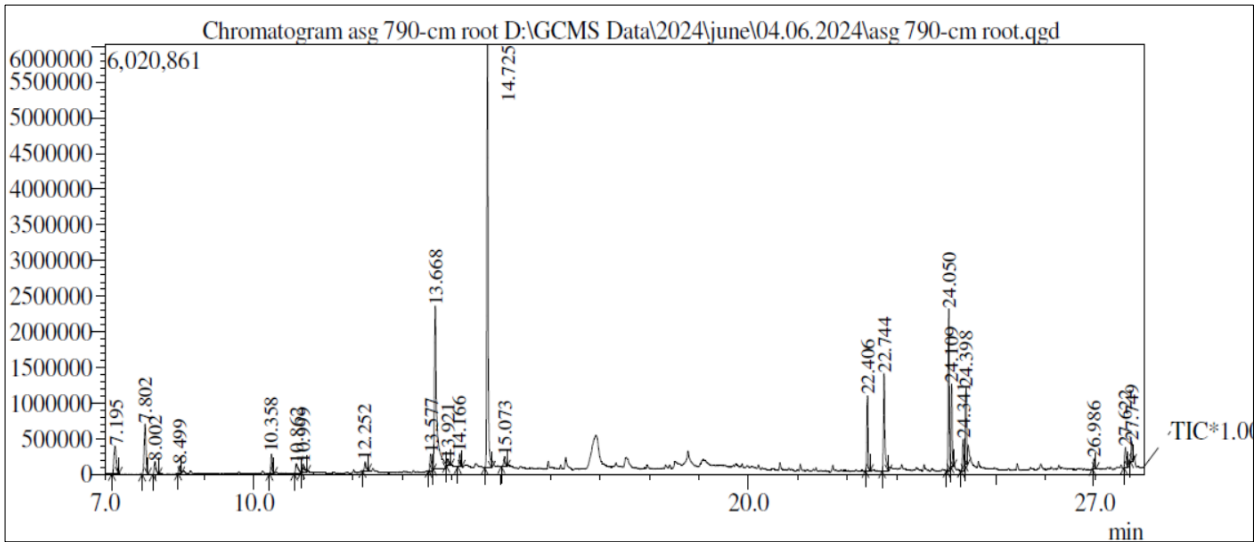

Fig 8: GC-MS chromatogram of methanol extract of root of *C. malabaricum*

Ethyl acetate root

18 bioactive compounds have been identified in the ethyl acetate root extract of *Crinum malabaricum* (table 8), the major bio active compounds include, 1-Hexadecene which has Antimicrobial, antifungal, antioxidants activities, Cyclotetracosane has anti-fungal activities, Squalene is a natural anti-oxidant, Tetratetracontane is an anti-oxidant.

Table 8: Metabolite profile of ethyl acetate extract of root of *C. malabaricum*

Peaks	RT	Area %	Name of compound	Biological activity
1	10.932	7.74	1-Dodecene	-
2	13.327	2.02	Undecane,47-Dimethyl-	-
3	14.636	1.34	Hexane,2,3,4-Trimethyl-	-
4	16.549	10.86	1-Tetradecene	-
5	16.762	1.93	Nonane,3,7-dimethyl	-
6	19.019	1.49	Sulfurous acid, 2-ethylhexyl isohexyl ester	-
7	19.535	5.21	Phenol,2,4-Bis[1,1-Dimethylethyl]	-
8	19.877	4.38	Benzoic acid,Ethoxy-,Ethyl ester	-
9	21.561	10.89	1-Hexadecene	Antimicrobial, antifungal, antioxidants (Belakhdar *et al.*, 2015)
10	26.044	9.31	1-Tetradecanol	-
11	30.096	6.75	(Trans)-2-Nonadecene	-
12	33.794	3.39	Cyclotetracosane	Anti-fungal (Mongalo *et al.*, 2019) [7]
13	37.190	2.04	Phosphonic acid,Dioctadecyl Ester	-
14	39.307	9.44	1,2-Benzenedicarboxylic Acid	-
15	42.446	8.26	1,4-Benzenedicarboxylic acid,bis (2-ethylhexyl) ester	-
16	43.459	4.03	Squalene	Natural anti-oxidant (Smith *et al.*, 2000)
17	44.713	3.41	2-Bromotetradecane	-
18	48.240	7.52	Tetratetracontane	Anti-oxidant (Asnaashari *et al.*, 2019) [8]

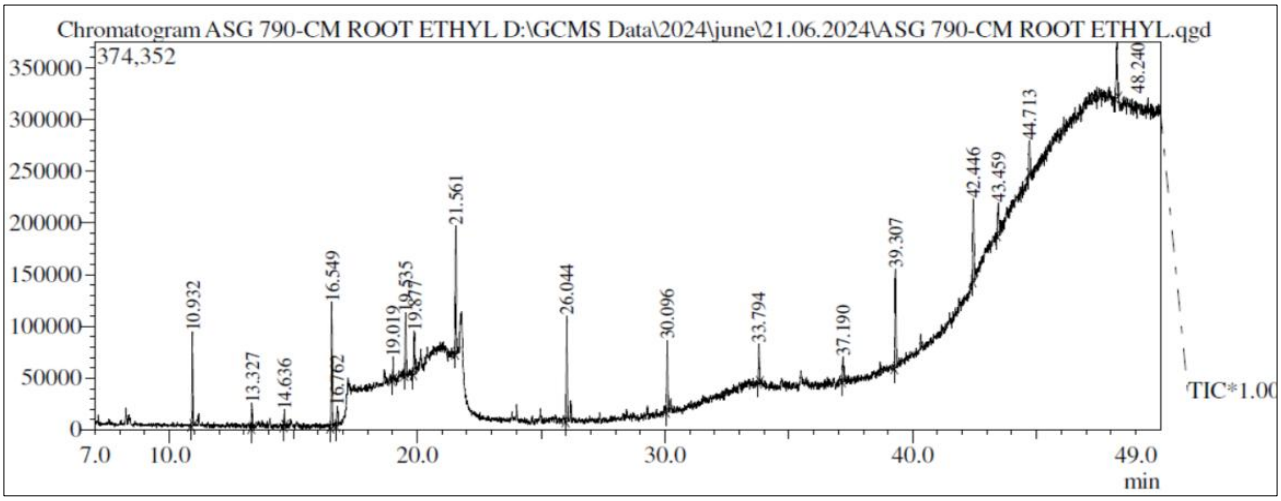

Fig 9: GC-MS chromatogram of ethyl acetate extract of root of *C. malabaricum*

Chloroform root

16 bioactive compounds have been identified in the chloroform root extract of *Crinum malabaricum* (table 9), the major bio active compounds include 1-Nonadecene it has Anti-microbial, anti-cancer, anti-oxidant activities, Heneicosane it has anti-microbial activities, n-Hexadecanoic acid has anti- oxidant activities, Behenic alcohol has anti-oxidant activities, 9,12-Octadecadienoic acid (Z,Z)- has hapato protective activities, 1-Heptacosanol has anti-bacterial activities, 1-Hexacosanol has larvicidal activities, Dotriacontane used as anti-convulsant, 1-Hexacosanol has acetylcholinesterase inhibitory effect.

Table 9: Metabolite profile of chloroform extract of root of *C.malabaricum*

Peaks	RT	Area %	Name of compound	Biological activity
1	15.325	0.48	Heptadecane	-
2	18.418	4.38	1-Nonadecene	Anti-microbial,anti-cancer,anti-oxidant (Rukachaisirikul *et al.*, 2004) [34]
3	18.522	0.92	Heneicosane	Antimicrobial (Vanitha *et al.*, 2020) [19]
4	20.871	5.83	n-Hexadecanoic acid	Anti-oxidant (Subavathy., 2016) [39]
5	21.337	4.65	Behenic alcohol	Anti-oxidant (Ertas *et al.*, 2015) [9]
6	23.199	1.76	9,12-Octadecadienoic acid (Z, Z)-	Hepatoprotective (Darmstad *et al.*, 2002)
7	23.304	1.17	Oxazole,4,5-dihydro-2-pentadecyl-	-
8	24.003	3.93	1-Heptacosanol	Anti-bacterial (Gade *et al.*, 2017) [11]
9	26.467	2.82	1-Hexacosanol	Larvicidal activity
10	26.903	1.99	Nonacosanal	-
11	27.755	0.75	Dotriacontane	Anticonvulsant (Signe *et al.*, 2007)
12	28.141	8.38	Bis (2-ethylhexyl) phthalate	-
13	28.936	2.49	1-Hexacosanol	-
14	32.806	5.89	Dotriacontane	-
15	34.309	1.32	1-Hexacosanol	acetylcholinesterase inhibitory effect (Gade *et al.*, 2017) [12]
16	36.248	6.03	Tetrapentacontane	-

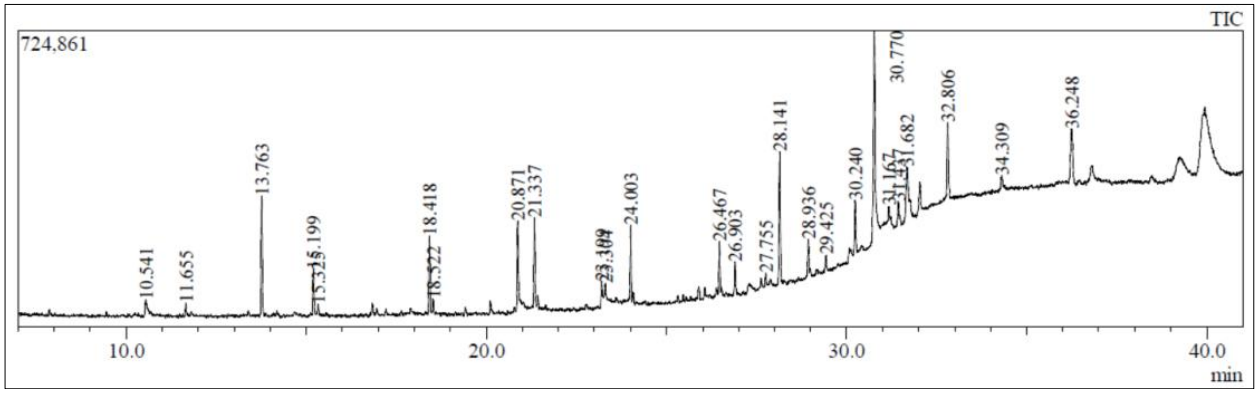

Fig 10: GC-MS chromatogram of chloroform extract of root of *C.malabaricum*

Methanol rhizome: 15 bioactive compounds have been identified in the methanol rhizome extract of *Crinum malabaricum* (table 10). E-14-hexadecenal has anti-bacterial and anti-oxidant property. Hexadecanoic acid, methyl ester has Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic, flavor, Hemolytic, 5-Alpha reductase inhibitor, 9,12-Octadecadienoic acid (Z, Z)-, Methyl ester has anti-inflammatory, nematicide actions, Octadecanoic acid has anti-inflammatory, antiarthritic activity, antibacterial activity role. Octadecanoic acid has anti-inflammatory, antiarthritic activity, antibacterial activity, Fenbufen used in non-steroidal anti-inflammatory drug synthesis. Hexadecanoic acid,2-Hydroxy-1- (Hydroxy methyl) ethyl ester used as anti-oxidant. Naphthalene has Antihypertensive, antidiabetic activity.

Table 10: Metabolite profile of methanol extract of rhizome of *C.malabaricum* at different wave length.

Peaks	RT	Area %	Name of compound	Biological activity
3	12.014	1.14	Ethanamine,N-Ethyl-N-Nitroso	-
4	12.253	5.27	4H-pyran-4-One,2,3-Dihydro-3,5-Dihydroxy-6-Methyl	-
5	13.084	4.26	Naphthalene	Antihypertensive, antidiabetic (Gayathri *et al.*, 2023) [20]
6	20.639	1.36	Tetradecanoic acid	-
7	21.050	0.39	E-14-hexadecenal	Antibacterial, antioxidant
8	22.405	1.72	Hexadecanoic acid,methyl ester	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Antiandrogenic, flavor, Hemolytic, 5-Alpha reductase Inhibitor (Arpana *et al.*, 2007) [38].
9	22.742	8.39	Hexadecanoic acid	-
10	24.048	5.76	9,12-Octadecadienoic acid(Z,Z)-,Methyl ester	Anti-inflammatory, nematicide (Dukes data base)
11	24.394	4.89	9,12-Octadecadienoic acid	-
12	24.445	1.02	7-Tetradecenal, (Z)	-
13	24.650	0.76	Octadecanoic acid	anti-inflammatory, antiarthritic activity, antibacterial activity
14	24.435	0.68	Fenbufen	non-steroidal anti-inflammatory drug (NCBI,2024) [21]
15	26.987	0.59	Octadecanal	-

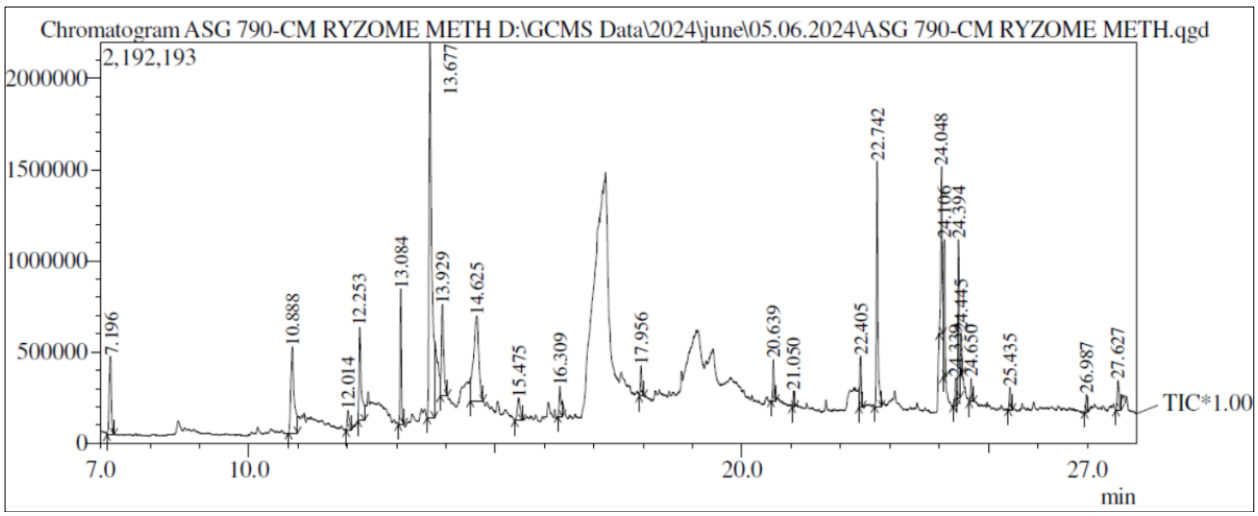

Fig 11: GC-MS chromatogram of methanol extract of rhizome of *C. malabaricum*

Ethyl acetate rhizome

16 bioactive compounds have been identified in the rhizome ethyl acetate extract of *Crinum malabaricum* (table 11). The major compounds include 1-Decene used as flavors, perfumes, pharmaceuticals, dyes, oils. Undecane, 4,7-Dimethyl- has Anti-allergic, anti-inflammatory, Heptadecane

has anti-fungal property, Nonadecane has anti-microbial property, 2,4-Ditert-Butylphenol has a Antifungal activity, antioxidant activity. Nonadecane has anti-microbial property. Hexadecane has Antioxidant, antibacterial and antifungal activity. Eicosane has anti-tumor activity. Hexadecanoic acid has anti-inflammatory activity.

Table 11: Metabolite profile of ethyl acetate extract of rhizome of *C. malabaricum* at different wave length.

Peaks	RT	Area %	Name of compound	Biological activity
1	6.199	0.52	1-Decene	Flavors, perfumes, pharmaceuticals, dyes, oils
2	7.726	0.88	Undecane,4,7-Dimethyl-	Anti-allergic, anti-inflammatory (Choi *et al.*, 2024) [22]
3	7.868	0.32	Octadecane	-
4	11.352	1.59	Cyclopropane,nonyl-	Anethetic agent
5	13.611	1.61	Heptadecane	Anti-fungal (Abubacker *et al.*, 2015) [36]
8	19.134	1.59	Nonadecane	Anti-microbial (Lakshmi *et al.*, 2017) [23]
9	19.657	2.14	2,4-Ditert-Butylphenol	Antifungal activity, antioxidant activity
11	20.243	1.31	Nonadecane	Anti-microbial ((Lakshmi *et al.*, 2017) [23]
12	21.648	2.13	1-Hexadecene	-
13	21.823	5.09	Hexadecane	Antioxidant, antibacterial and antifungal (Arora *et al.*, 2017) [24].
14	24.081	1.20	Eicosane	Anti-tumor activity (Sivasubramanian *et al.*, 2013) [25]
15	29.534	1.11	Hexadecanoic acid	Anti-inflammatory (Kolar *et al.*, 2019) [26]
16	30.161	1.17	Cyclooctacosane	-

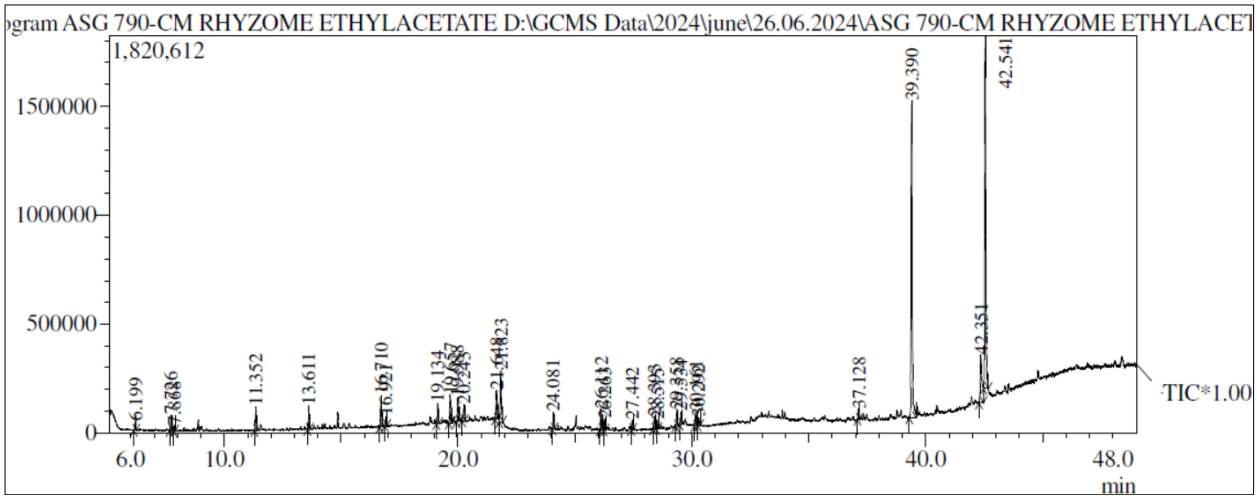

Fig 12: GC-MS chromatogram of ethyl acetate extract of rhizome of *C.malabaricum*

Chloroform rhizome

18 bioactive compounds have been identified in the rhizome chloroform extract of *Crinum malabaricum* (table 12). The major compounds include 1-Tetradecene it has anti-microbial action, E-15-Heptadecenal has anti-bacterial role, Heptadecanoic acid it has anti-microbial role, 1-Tricosene has anti-tumor effects, 9-Octadecenamide used for the treatment of the sleep disorders, Hexadecanal has antioxidant role, 1,2-Benzenedicarboxylic acid has anti-bacteriak role.

Table 12: Metabolite profile of chloroform extract of rhizome of *C.malabaricum* at different wave length.

Peaks	RT	Area %	Name of compound	Biological activity
1	14.705	1.63	1-Tetradecene	Anti-microbial (Naragani *et al.*, 2016) [27]
2	17.633	13.75	2,4-Ditert-Butylphenol	-
3	19.617	3.77	Cetene	-
4	21.871	1.43	1- Hexadecanol, Acrylate	-
5	24.078	6.46	E-15-Heptadecenal	Anti-bacterial (Kumar *et al.*, 2011) [28]
6	24.230	0.89	Heptadecane	-
7	27.734	4.09	Heptadecanoic acid	Anti-microbial (Jubie *et al.*, 2012) [29]
8	28.514	6.73	E-15-Heptadecenal	-
9	33.177	2.20	Octanamide,N-(2-hydroxyethyl)	-
10	34.969	5.18	1-Tricosene	Anti-tumor (Lee *et al.*, 2008) [30]
11	37.084	0.94	1-Eicosene	-
12	39.019	1.40	9-Octadecenamide	Used as treatment for sleep disorders
13	39.919	3.89	Heptadecyl trifluoroacetate	-
14	40.615	2.41	Hexadecanal	Anti-oxidant (Arora *et al.*, 2017) [24].
15	42.515	14.44	1,2-Benzenedicarboxylic acid	Antibacterial (Amudha *et al.*, 2018) [31]
16	43.535	1.83	Phenanthro[1,2-b] furan-10,11-dione,1,6-dimethyl	-
17	43.755	2.46	Tricosyl trifluoroacetate	-
18	45.905	26.50	Liriodendromine	-

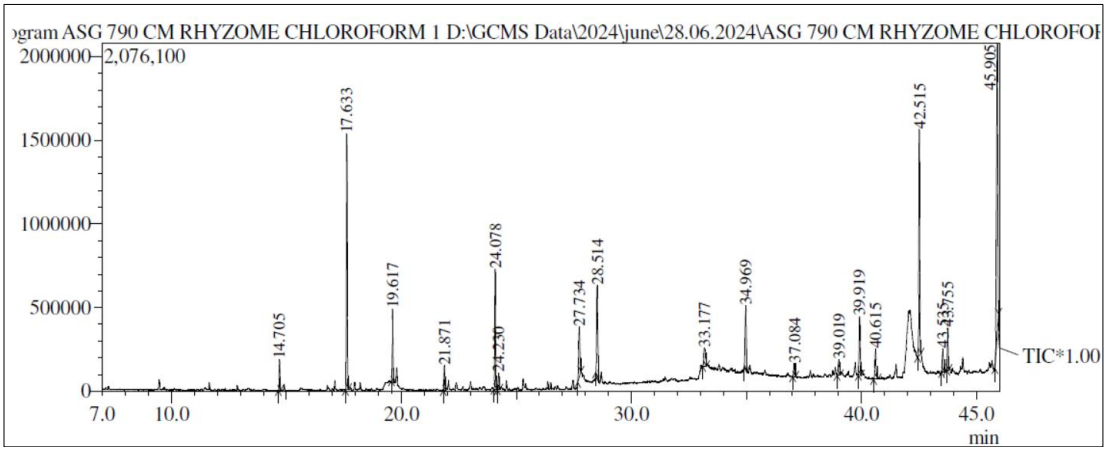

Fig 13: GC-MS chromatogram of chloroform extract of rhizome of *C.malabaricum*

4. Conclusion

The present study focuses on the HPTLC, GC-MS analysis of root, rhizome extract of *C.malabaricum*. GC-MS proflile revealed the presence of various primary and secondary metabolites with valuable biological and pharmacological activity. GC-MS fingerprint analysis has become the most important tool because of it''s simplicity and reliability. Each of these compounds has documented therapeutic potentials. The presence of these bioactive compounds helpful in the treatment of various ailments. The present study helps to identify the various bio active compounds. Methanolic extract of root showed Hexadecanoic acid,Methyl ester it has anti-inflammatory effect, 9,12-Octadecadienoic acid, methyl ester shows anti-microbial effects, ethyl acetate extract of root showed 1-Hexadecene which has Antimicrobial, antifungal, antioxidants activities, Cyclotetracosane has anti-fungal properties. Chloroform extract of root showed 1-Nonadecene it has Anti-microbial, anti-cancer,anti-oxidant activities, Heneicosane it has anti-microbial activities, n-Hexadecanoic acid has anti-oxidant activities, Behenic alcohol has anti-oxidant activities, 9,12-Octadecadienoic acid (Z,Z)- has hapato protective activities.

Methanolic extract of rhizome showed E-14-hexadecenal has anti-bacterial and anti-oxidant property. Hexadecanoic acid, methyl ester has Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic, flavor, Hemolytic, 5-Alpha reductase inhibitor, 9,12-Octadecadienoic acid (Z, Z)-, Methyl ester has anti-inflammatory, Ethyl acetate extract of rhizome showed 1-Decene used as flavors, perfumes, pharmaceuticals, dyes, oils. Undecane,4,7-Dimethyl- has Anti-allergic, anti-inflammatory, Heptadecane has anti-fungal property, Nonadecane has anti-microbial property, chloroform extract of rhizome showed 1-Tetradecene it has anti-microbial action, E-15-Heptadecenal has anti-bacterial role, Heptadecanoic acid it has anti-microbial role, 1-Tricosene has anti-tumor effects. Further investigation may leads to the isolation of such bioactive compounds. Medical potentials of these compounds need further research on toxicological aspect to develop safe drugs.

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