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Phytochemical Characterization On Leaf Of Critically Endangered Medicinal Plant Crinum Malabaricum Lekhak And Yadav

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Abstract

Crinum malabaricum (malabar river-lily) is endemic plant group and described new to science (Lekhak and Yadav 2012).Which comes under the family Amaryllidaceae and it was assessed as critically endangered using the IUCN criteria(Lansdown, R.V. 2016).The plant is a source of the acetylcholinesterase(AChE) inhibiting alkaloid galanthamine used to treat Alzheimer's and Parkinson's diseases.The bulbs of this plant contain the highest amount of galanthamine among Crinum species (Mani *et al.*, 2023). In this study methanolic, ethyl acetate ,chloroform extract of leaves of the plant was studied phytochemically using various quantitative assays and chromatographic technique like High Performance Thin Layer Chromatography (HPTLC) and Gas Chromatography- Mass Spectrometry (GC-MS). On quantitative assays *C. malabaricum* showed higher flavanoid content and phenolic content. GC-MS result of methanol extract leaves showed presence of Lycorine , Hexadecanal, 9-octadecanoic acid, phytol.Lycorine possesses multiple pharmacological effects. Which has action of inhibition of acetylcholinesterase(AChE), anti tumor, anti bacterial, anti viral, antifungal effects. Ethyl extract leaves showed presence of pentadecane, cyclopropane nonyl, E14 hexadecenal and Chloroform extract of leaves showed presence of loliolide, octadecanal, 2,4-ditert butyl phenol. Loliolide can prevent neurological cell death and better remedy for Parkinsons disease.

Key words: *Crinum malabaricum*, Lycorin, Loliolide, Phytol, Liriodendromine, Hexadecanoic acid, Heptadecanoic acid

1. Introduction

Crinum malabaricum is an aquatic plant under the family Amaryllidaceae. The plant is found in streams of Kannur and Kasargod districts of Kerala, India. The underground bulb of the plant is important source of an alkaloid galanthamine , which is a selective ,reversible competitive inhibitor of Acetylcholinesterase (AchE). This species is considered as critically endangered because it is restricted to a population of approximately 1000 individuals in a single stream. It is threatened by human habitation and fringing the stream results in the death of the plants (Lansdown,2016). Alzheimer's is a neurodegenerative disease, it affects memory, language, problem solving. Alzheimer's disease affects most of the people with dementia and it is correlated with increasing age. Galanthamine, an isoquinoline alkaloid and is used to treat against Alzheimer's disease. The action of Galanthamine is which increase dopaminergic neurotransmission, and decreases neuro degeneration and cognitive decline. Which promoting the process of learning , memory and attention (Tsvetkova *et al* 2016). Lycorine obtained from methanolic extract of leaves has great

significance in drug industry. Lycorine belong's to the same family as galanthamine which is the active component of a drug used for the treatment of Alzheimer's disease. Lycorine shows Acetylcholinesterase (AChE) inhibitory activity. The patients with Alzheimer's disease shows decrease in Acetylcholine(Ach) leads to difficulties with memory and attention. AChE is the enzyme which degrades the acetylcholine, hence suppression of the action of AChE is the better option in treatment of Alzheimer's disease. Now a days the production of galanthamine from plants is not sufficient to meet the demands of pharmaceutical industry. Over exploitation of the plant material leads to the depletion of available plant polulations(Jagtap *et al*.,2014). Lycorine from the methanolic extract of leaves of *C.malabaricum* can be used as alternative source of galanthamine which save the plants, because tuber is not take here , and leaves of the plant is only collect. IUPAC name of lycorine is 2,4,5,7,12b,12c-hexahydro-lH-(1,3)dioxolo(4,5-j)-pyrrolo(3,2,1-de)phenanthridine- 1-diol, and its molecular formula is C16H17NO4 with the relative molecular mass of 287.31, physical state is colourless crystal and melting point is 260-262^{0}C.

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 leaves 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.

2.2 Preliminary phytochemical characterization

Preliminary phytochemical screening of methanolic extract of leaves of *C.malabaricum* has been conducted to find out various secondary metabolites. The methanolic extracts obtained from solvent extraction were then subjected to various qualitative chemical tests for the identification of various plants constituents like alkaloids, glycosides, phenolics and tannins. The preliminary phytochemical screening tests may be useful for the detection of the bioactive principles and subsequently may lead to drug development (Varadarajan *et al*.,2007). The major class of compounds detected in methanolic extract of leaves of *C.malabaricum* were listed in table 1.

Table 1. Preliminary phytochemical screening of leaf of Crinum malabaricum
ME=Methanol extract ,EA=Ethyl Acetate extract, CE=Chloroform extract,+=Presence,-=Absence.

	Crinum *malabaricum*		Leaf		
Sl no	Name of compound	Name of the test	ME	EA	CH
1	Carbohydrate	Fehling's test	+	+	+
2	Proteins and amino acids	Biuret test	+	+	+
3	Phenolic compound and tannin	Ferric chloride test	+	+	+
4	Saponins	Foam test	-	-	-
5	Flavonoids	Aqueous sodium hydroxide	+	+	+

2.3 Quantitative estimation of phytochemicals

Estimation of total phenolics

Total phenolic content was determined using Folin-Ciocalteu (FC) reagent (Oueslati *et al*.,2012). The plant extract (0.5 mL) was mixed with 0.5 mL of FC reagent (1:1 diluted with distilled water) and incubated for 5 min at 22^0C followed by addition of 2 mL of 20% Na_2CO_3 . The mixture was then incubated further at 22^0C for 90 min and the absorbance was measured at 650 nm. The total phenolic content (mg/mL) was calculated using gallic acid equivalents per gram of dry weight (mg GAE/gDW) by using regression equation. Samples were analysed in triplicates, result shown in table 2. Standard curve used for the determination of total phenolic content was prepared by using different concentrations of gallic acid equivalent(GAE) and it's respective optical density were shown in fig.1.

Estimation of total flavonoids

Total flavonoid content was measured by the aluminium chloride colorimetric assay
(Zhishen *et al*) An aliquot (1ml) of extracts or standard solutions of quercetin (20,40,60,80 and 100µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water . To the flask was added 0.30 ml 5% $NaNO_2$, after was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510nm. Total flavonoid content from the methanolic extract of leaves, rhizome and root of *C.malabaricum* shown in table 2. Total flavonoid content was prepared by using different concentration of quercetene equallents(QUE) and it's respective optical density was shown in fig 2.

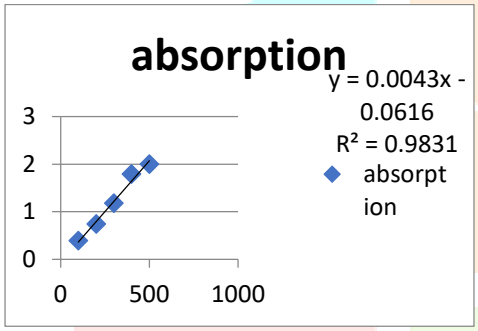

Fig.1. Standard calibration curve for gallic acid

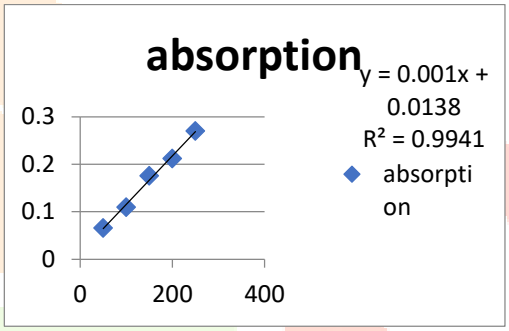

Fig.2. Standard calibration curve for quercetin

2.4 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, chloroform extracts of the leaf of *C.malabaricum*. The better mobile phase for extract was toluene, ethyl acetate and methanol in 7:3:1 ratio.

2.5 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 3^0C/min and then held at 260^0C 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 2s, 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).

3. Results

3.1 Preliminary phytochemical screening

Phytochemical analysis revealed presence of different alkaloids present in the leaf extract. The result of phytochemical screening of methanol extract of the leaves of *C.malabaricum* were presented in table 1. Carbohydrate, protein, phenolic compound and tannin, and flavonoids were the major phytochemicals found in the leaves of *C.malabaricum*.

Quantitative estimation of phytochemicals

The results of the bioactive components of methanolic extract of leaves of *C.malabaricum* revealed presence of carbohydrate, proteins, amino acids, phenolics, tannin, flavonoids, and alkaloids. In the ethyl acetate extract carbohydrate, proteins and amino acids, phenolics, tannin, flavonoids, alkaloids were present. But saponins were absent. Chloroform extract of leaves contains , proteins, amino acids, phenolics, tannin, flavonoids, and alkaloids. These bioactive compounds are important in the pharmaceutical and medicinal field due to their anti oxidant, anti microbial and other biological properties. Flavonoids have been shown to have anti bacterial properties (Ebrahimzadeh.,2019), saponins have been reported to have anti bacterial, anti fungal activities(Shi *et al*., 2019). Tannin possess anti oxidant, anti viral properties(Okwu & Josiah, 2006) Alkaloids have antimalarial, anti bacterial properties (Siddique & Brunton, 2019).

Total phenolic content

Standard curve used for the determination of total phenolic content was prepared by using different concentration of gallic acid equivalent and it's optical density were shown in fig.1.

Table .2 Estimation of total phenolics,flavonoids content in leaves of *C. malabaricum*

Total Flavonoid content in mg QE/100g	Total phenolic content in mg GAE/100g	Ratio of TF/TP
9.89±0.55	9.08±0.09	1.08

Total flavonoid content

Standard curve used for the determination of total flavonoid content was prepared by using different concentration of quercetin equivalent(QUE) and its optical density were shown in fig.2

3.2 HPTLC fingerprint profile of Methanol extract of leaf of *C.malabaricum* at different wave length.

The densitogram for HPTLC fingerprint profile of methanol extract of leaves at different wave length is shown in fig.3-5 and respective chromatogram were shown in fig.6-8. The results showing number of peaks, R_f values and area percentage are presented in table 3. In the HPTLC densitometric scan the finger print profile of leaf in methanol extract under 254nm revealed 10 peaks, major peak at R_f 1.84 with area percentage of 31.48, followed by peak at Rf 1.74 with area percentage of 12.62 and minor peak at R_f 0.05 with area percentage of 3.05.In 366 nm revealed 17 peaks , major peak at R_f 1.80 with area percentage of 33.66, followed by peak at 1.72 with area percentage of 9.20, minor peak at 0.05 with area percentage 6.09. In 550nm revealed 16 peaks major peak at R_f 1.96 with area percentage of 0.65, followed by peak at 1.89 with the area percentage 2.00 and minor peak at 0.02 with area percentage 3.67.

254 nm			366nm			550nm		
No of peaks	$R_f$value	Area %	No of peaks	$R_f$value	Area %	No of peaks	$R_f$value	Area%
1	0.05	3.05	1	0.05	6.09	1	0.02	3.67
2	0.16	16.69	2	0.18	17.61	2	0.10	1.06
3	0.26	3.72	3	0.25	1.81	3	0.17	2.59
4	0.28	5.20	4	0.54	0.95	4	0.35	1.20
5	0.39	2.44	5	0.56	0.68	5	0.47	2.12
6	0.47	11.62	6	0.71	1.74	6	0.51	1.43
7	0.58	3.30	7	0.73	1.09	7	0.59	3.12
8	0.97	9.88	8	0.87	2.85	8	0.71	0.87
9	1.74	12.62	9	0.89	2.37	9	0.75	1.23
10	1.84	31.48	10	0.91	1.93	10	1.28	46.42
			11	0.97	2.01	11	1.47	18.42
			12	1.01	2.66	12	1.56	1.41
			13	1.37	11.59	13	1.65	4.08
			14	1.44	2.01	14	1.79	9.73
			15	1.59	2.03	15	1.89	2.00
			16	1.72	9.20	16	1.96	0.65
			17	1.80	33.36			

Table 3. HPTLC fingerprint profile of methanol extract of leaves of *C.malabaricum* at different wave length.

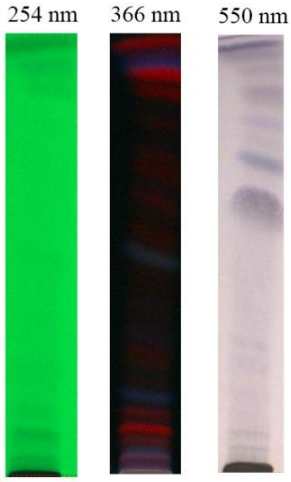

Fig.3 Fig.4 Fig.5

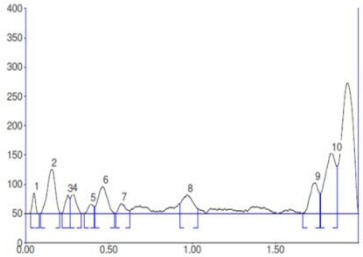

Fig.6

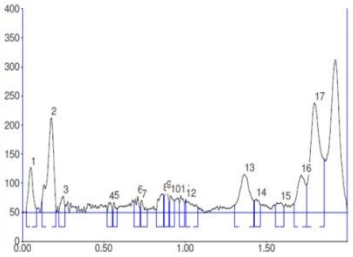

Fig.7

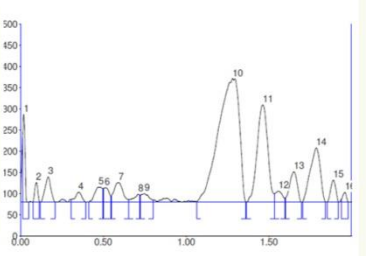

Fig.8

Fig. 3-5 Densitogram of methanol extract of leaves of *C.malabaricum* Fig. 6-8 Chromatogram of methanol extract of leaves of *C.malabaricum*

3.3 Metabolite profiling by GC-MS

In the present study, 23 bioactive compounds have been identified. Each of these compounds documented therapeutic potentials. 23 bioactive compounds have been identified in the methanol leaf extract of *Crinum malabaricum***.** 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- used as anti oxidant. Tetradecanoic acid has anti oxidant property. Tetradecanoic acid Antifungal, antibacterial and hypercholesterolemic activities . 9-Octadecanoic acid (Z)- has anti inflammation properties. Hexadecanoic acid,Methyl ester has anti fungal property. Methyl Octadeca-9,12-Dienoate has anti oxidant and anti cancer property. Cis,cis,cis-7,10,13-Hexadecatrienal has larvicidal effect on mosquitoes. 9-Octadecanoic acid(Z)- has anti microbial and anti bacterial action. Hexadecanal used as anti oxidant. Phenanthro[1,2-

b]furan-10,11-dione,1,6-dimethyl- has anti cancer activities.The major compound obtained from methanolic extract of leaf is lycorine. It has inhibition of Inhibition of acetylcholinesterase activity.

Peaks	RT	Area %	Name of compound	Biological activity
1	7.567	3.43	Thymine	-
2	9.553	4.70	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	Antioxidant (Čechovská *et al*.,2011)
3	11.985	22.50	5-Hydroxymethylfurfural	-
4	12.383	0.73	Ethanol,2,2-Diethoxy-	-
5	13.465	0.71	2,4(3H,5H)-Furandione,3-Propyl	-
6	14.313	0.61	2,4,4-Trimethyl-2-Pentyl-3-Oxa-Zolidinyloxy	-
7	16.032	1.17	L-Proline,5-Oxo-,Methyl Ester	-
8	25.359	0.42	Tetradecanoic acid	Antifungal, antibacterial and hypercholesterolemic activities (Elaiyaraja *et al.*,2016).
9	26.966	0.80	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne	-
10	27.459	0.42	9-Octadecanoic acid (Z)-	Anti inflammation (Kang *et al*.,2011)
11	28.736	0.95	Hexadecanoic acid,Methyl ester	Antifungal(Abubacker *et al*.,2013)
12	29.532	16.46	Hexadecanoic acid	Antioxidant, antifibrinolytic, anti microbial (Gabriel *et al*.,2013)
13	31.392	0.59	Heptadecanoic acid	-
14	31.936	0.60	Methyl Octadeca-9,12-Dienoate	Anti oxidant, anti cancer(Gabriel *et al*.,2013)
15	32.039	0.87	9,12,15-Octadecatrienoic acid,Methyl Ester,(Z,Z,Z)-	-
16	32.278	2.88	Phytol	Cytotoxic, antioxidant, anti-inflammatory, immune-modulating, autophagy- and apoptosis-inducing, and antimicrobial
17	32.832	27.12	Cis,cis,cis-7,10,13-Hexadecatrienal	Larvicidal effect on some species of mosquitoes(Ogunlesi *et al*.,2019)
18	33.248	2.61	9-Octadecanoic acid(Z)-	Anti microbial, anti bacterial (Kang *et al*.,2018)
19	37.765	0.33	Hexadecanal	Anti oxidant (Okwu & Josiah, 2006)
20	38.871	2.21	Dihydro-desmethoxyambelline	-
21	40.389	0.97	Phenanthro[1,2-b]furan-10,11-dione,1,6-dimethyl-	-
22	42.267	0.54	Liriodendromine	-
23	42.516	7.38	Lycorin	Inhibition of acetylcholinesterase(AChE) (Kola *et al*.,2023).

Table.4 Metabolite profile of methanol extract of leaf of *C.malabaricum*

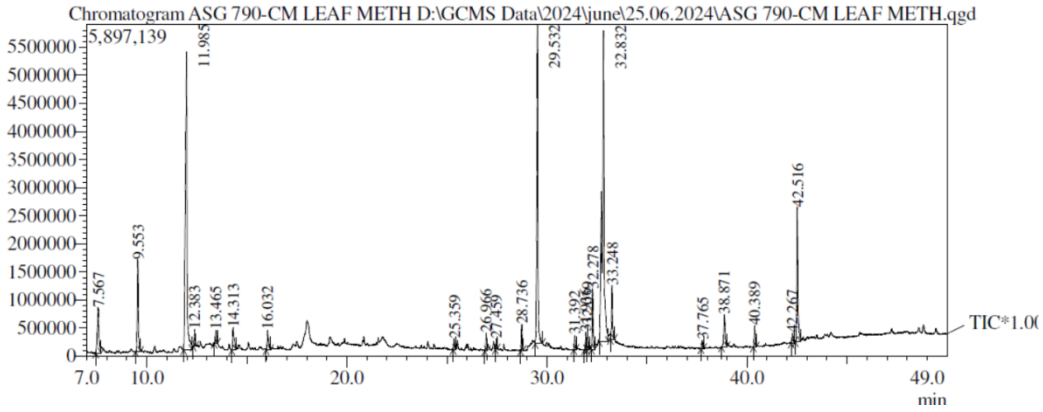

Fig 9.GC-MS chromatogram of methanol extract of leaves of *C.malabaricum*

3.4 HPTLC fingerprint profile of Ethyl Acetate extract of leaf of *C.malabaricum* at different wave length.

The densitogram for HPTLC finger print profile of leaf in ethyl acetate extract at different wave length is shown in fig.10, fig.11, fig.12 and respective chromatogram were shown in fig.13,fig.14,fig15. The results showing number of peaks, R_f values, and area percentage are presented in table 5. In the HPTLC densitometric the finger print profile of leaf in ethyl acetate extract under 254nm revealed 9 peaks, major peak at R_f 1.94 with area percentage of 9.10 and minor peak at 0.08 with area percentage of 4.04. In 366nm revealed 11 peaks major peak at R_f 1.88 with area percentage 30.94 followed by peak at 1.77 with area percentage 29.06, minor peak at 0.05 with area percentage 1.50. In 550nm revealed 13 peaks major peak at 1.94 with area percentage 37.55, minor peak with area percentage 0.66 and R_f at 0.07.

Table.5 HPTLC fingerprint profile of ethyl acetate extract of leaves of *crinum malabaricum* at different wave length.

254 nm			366nm			550nm		
No of peaks	$R_f$value	Area %	No of peaks	$R_f$value	Area %	No of peaks	$R_f$value	Area%
1	0.08	4.04	1	0.05	1.50	1	0.07	0.66
2	0.21	2.84	2	0.10	0.46	2	0.14	0.86
3	0.41	3.01	3	0.14	3.90	3	0.41	0.92
4	0.78	10.00	4	0.20	0.43	4	0.51	0.69
5	1.29	4.07	5	0.59	1.91	5	0.56	0.61
6	1.62	11.76	6	0.68	4.03	6	0.67	0.66
7	1.72	17.94	7	0.83	11.92	7	0.82	1.74
8	1.81	37.24	8	1.34	6.19	8	1.14	19.65
9	1.94	9.10	9	1.69	9.65	9	1.35	8.40
			10	1.77	29.06	10	1.52	5.43
			11	1.88	30.94	11	1.69	11.82
						12	1.79	11.20
						13	1.94	37.35

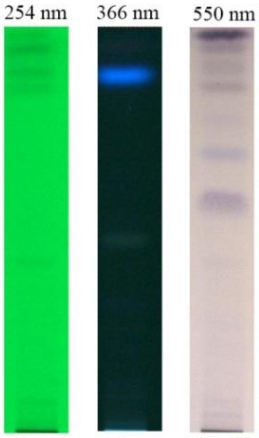

Fig.10 Fig.11 Fig.12

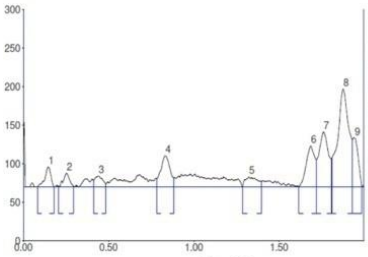

Fig.13

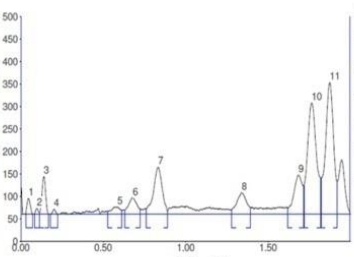

Fig.14

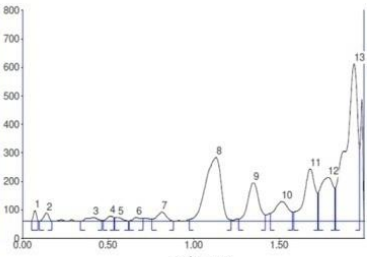

Fig.15

Fig. 10-12 Densitogram of ethyl acetate extract of leaves of *C.malabaricum.* Fig. 13-15 Chromatogram of ethyl acetate extract of leaves of *C.malabaricum*

3.5 Metabolite profiling by GC-MS

In the present study , fifteen bioactive compounds have been identified in the ethyl acetate extract of leaves of *C.malabaricum*. The presence of various phyto chemicals contributes to the medicinal activity of the plant. Each of these compound has documented therapeutic potentials. Undecane,4,7-Dimethyl shows Anti allergic ,anti inflammatory, Cyclopropane,nonyl- used as general anaesthetic, Octadecane has bacterial metabolite activity, 1-Tetradecene shows anti fungal ,anti bacterial activity, E-14-Hexadecenal has anti bacterial and anto oxidanat activity, Tridecanal shows role in natural mosquito control, E-15-Heptadecenal has anti bacterial activity, (E)-Phytol has anti microbial and anti oxidant activity, Hexa decanal has anti oxidant activity.

Table.6 Metabolite profile of ethyl acetate extract of leaf of *C.malabaricum*

Peaks	RT	Area %	Name of compound	Biological activity
1	7.705	0.44	Undecane,4,7-Dimethyl-	Anti allergic ,anti inflammatory (Govindarajan *et al*.,2016)
2	11.327	3.45	Cyclopropane,nonyl-	General anesthetic (Budavari,1996)
3	13.589	0.63	Octadecane	Bacterial metabolite
4	16.682	4.83	1-Tetradecene	Antifungal,antibacterial (Ibrahim *et al*,2017)
6	19.598	2.96	Phenol,2,4-Bis(1,1-Dimethylethyl)-	Antifungal (Amaral *et al*,2014)
7	21.615	6.17	E-14-Hexadecenal	Antibacterial,antioxidant (Kumar *et al*,2011)
10	24.371	1.47	Tridecanal	Natural mosquito control (Tabanca *et al*.,2014)
11	26.076	4.05	E-15-Heptadecenal	Antibacterial activity
12	26.225	1.46	Pentadecane	Antimicrobial (Bruno *et al*,2015)
13	32.312	14.03	(E)-Phytol	Anti microbial, anti oxidant(Rivera *et al*.,2023)
15	37.773	2.07	Hexadecanal	Antioxidant(Amutha *et al*.,2018)

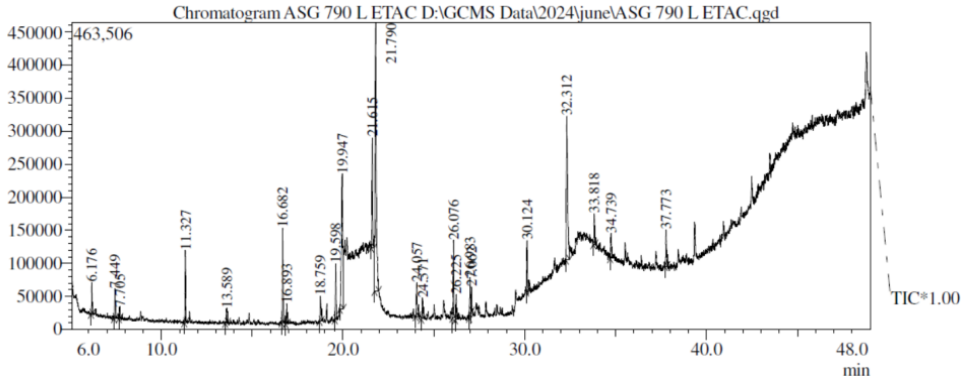

Fig 16 GC-MS chromatogram of ethyl acetate extract of leaves of *C.malabaricum*

3.6 HPTLC fingerprint profile of chloroform extract of leaf of *C. malabaricum* at different wave length.

Table 7. HPTLC fingerprint profile of chloroform extract of leaves of *C.malabaricum* at different wave length.

	254 nm		366nm			550nm		
No of peaks	$R_f$value	Area %	No of peaks	$R_f$value	Area %	No of peaks	$R_f$value	Area%
1	0.20	11.00	1	0.03	1.06	1	0.10	1.62
2	0.35	6.27	2	0.11	1.07	2	0.21	1.80
3	0.47	14.60	3	0.16	8.33	3	0.70	4.77
4	0.58	11.28	4	0.24	1.27	4	0.85	5.19
5	0.83	12.30	5	0.84	4.84	5	1.01	5.45
6	1.01	22.16	6	1.01	18.18	6	1.26	46.79
7	1.08	3.74	7	1.39	5.90	7	1.45	17.47
8	1.12	6.22	8	1.76	5.57	8	1.61	6.66
9	1.75	7.34	9	1.84	26.60	9	1.77	7.71
10	1.84	5.10	10	1.94	27.18	10	1.88	2.55

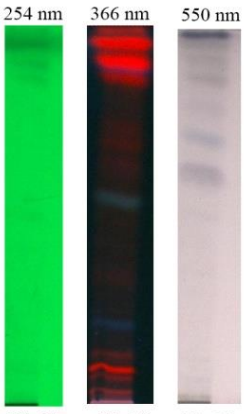

Fig.17 Fig.18 Fig.19

The results showing number of peaks, R_f values, and area percentage are presented in table. 7. In the HPTLC densitometric the finger print profile of leaf in chloroform extract under 254nm revealed 10 peaks, major peak at R_f 1.84 with area percentage of 5.10 and minor peak at 0.20 with area percentage of 11.00. In 366nm revealed 10 peaks major peak at R_f 1.94 with area percentage 27.18 followed by peak at 1.84 with area percentage 26.20, minor peak at 0.03 with area percentage 1.06. In 550nm revealed 10 peaks major peak at 1.88 with area percentage 2.55 followed by R_f 1.77 with area percentage 7.71, minor peak R_f 0.10 with area percentage 1.62.

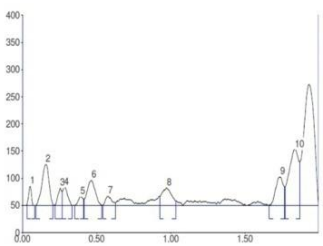
Fig.20

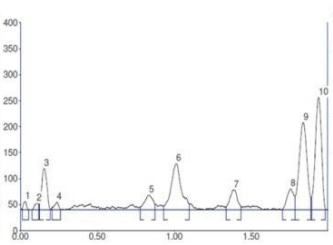
Fig.21

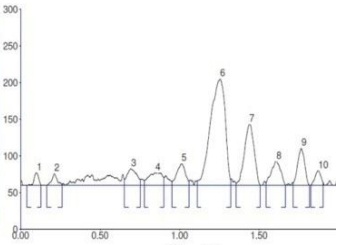
Fig.22

Fig. 17-19 Densitogram of chloroform extract of leaves of *C.malabaricum*. Fig. 20-22 Chromatogram of chloroform extract of leaves of *C.malabaricum*

3.7 Metabolite profiling by GC-MS

9 bioactive compounds have been identified in the chloroform leaf extract of *C.malabaricum*. The major bioactive compounds include 2,4-Ditert-Butylphenol used as anti oxidant. Loliolide has Neuroprotective and Anti-Inflammatory activities. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol has anti microbial and anti oxidant property. Hexadecanoic acid has anti bacterial and anti fungal property. E-15-heptadecenal has anti bacterial property. Phytol has anti bacterial and anti inflammatory actions. Octadecanal has anti bacterial actions.

Table.8 Metabolite profile of chloroform extract of leaf of *C.malabaricum*

Peaks	RT	Area %	Name of compound	Biological activity
1	17.623	10.18	2,4-Ditert-Butylphenol	Antioxidant (Gabriel *et al*.,2013)
2	22.374	1.42	Hexadecanal	
3	23.558	1.46	loliolide	Neuroprotective and Anti-Inflammatory Activities (Silva *et al*.,2021).
4	24.071	13.18	E-15-Heptadecenal	Antimicrobial (Kumar *et al*.,2011)
5	25.860	0.92	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Antioxidant, Antimicrobial (Thiyagarajan *et al*.,2011)
6	27.727	7.42	Hexadecanoic acid	Anti bacterial, anti fungal(Aparna *et al*.,2012)
7	28.504	10.88	E-15-heptadecenal	Anti bacterial(Shi *et al*., 2019)
8	32.194	13.39	Phytol	Anti oxidant(Okwu & Josiah, 2006)
9	40.604	1.40	Octadecanal	Anti bacterial (Kumar *et al*.,2011)

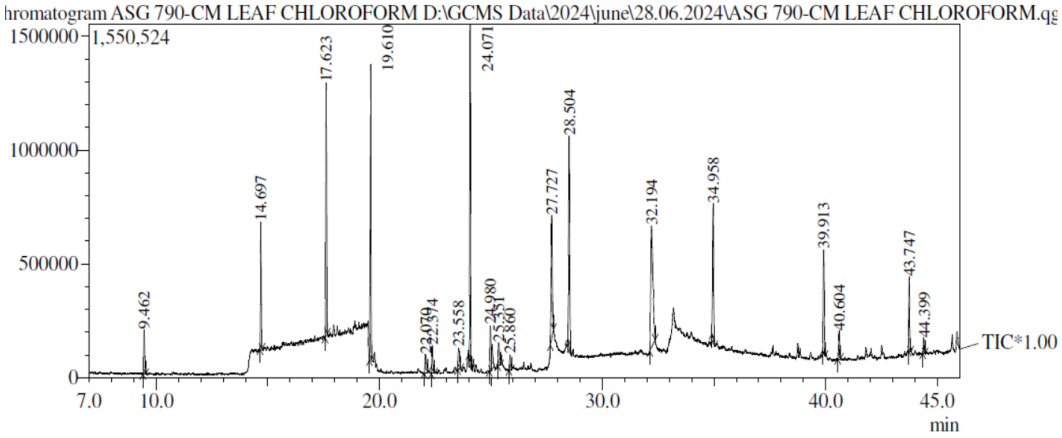

Fig 23 GC-MS chromatogram of chloroform extract of leaves of *C.malabaricum*

4. Discussion

Galanthamine is a natural alkaloid obtained from Amaryllidaceae plants and and ingredient of drug for Alzheimer's disease. Lycorine is a galanthamine type of compound possess anti tumor, anti bacterial, anti viral and cardiovascular effects. It's multiple biological and pharmacological effects especially low toxicity and mild side effects. Alzheimer's disease is a neuro degenerative disease. It affects memory, language, and problem solving ability. Lycorine exhibits broad spectrum of biological functions, especially the synthesis of Alzheimer's drug . Loliolide obtained from chloroform extract of leaf of *C.malabaricum* is a new therapeutic option for neurological disease (Silva *et al*.,2021). Parkinson's disease is another most common neuro degenerative disease worldwide. Its symptoms include expressionless face, cramped hand writing, difficulty swallowing, difficulty retrieving memory, sleep disturbances,depression, anxiety. Destruction of brain cells are the main reason for the disease. Loliolide obtained from chloroform extract of the leaves of *C.malabaricum* can prevent neurological cell death and it's a better remedy for parkinson's disease.

5. Conclusion

Phytochemical screening of methanol, ethyl acetate, chloroform extract of leaf of *C.malabaricum* revealed presence of proteins, phenolics, flavonoids, and glycosides. Alzheimer's is a neurodegenerative disease, leading to memory loss. Lycorine another alkaloid shows resemblance with galanthamine obtained from the methanolic extract of leaves of *C.malabaricum* is useful in drug development. The alkaloid lycorine which present in methanolic extract of leaves has interesting biological properties, it include mainly neuroprotective functions. Lycorine could be use as a alternative choice of galanthamine. Chloroform extract of leaves contain loliolide which possess neuroprotective properties. Loliolide is helpful in the synthesis of neuro protective drugs. *C.malabaricum* is critically endangered plant so it need more conservation activities. The plant also possess greatest role in the synthesis of many drugs. According to IUCN red data book the plant is critically endangered and need more care in its natural habitat. At present the production of galanthamine from natural plant sources is not sufficient to meet the demands of pharmaceutical industry. Over exploitation of the plant material leads to the depletion of available populations.

References

1)Lekhak, M.M. and Yadav, S.R. 2012. *Crinum malabaricum* (Amaryllidaceae), a remarkable new aquatic species from Kerala, India and lectotypification of Crinum thaianum. Kew Bulletin 67(521-526).
2)Lansdown, R.V. 2016. Crinum malabaricum. The IUCN Red List of Threatened Species 2016: e.T69726391A69727184.
3) Mani M, Mathiyazhagan C, Dey A, Faisal M,Alatar AA, Alok A, Shekhawat MS. Micro morpho anatomical transitions at various stages of in vitro development of Crinum malabaricum Lekhak and Yadav: A critically endangered medicinal plant. Plant Biol(Stuttg). 2023 Jan;25(1):142-151.
4) Ebrahimzadeh, M. A., Pourmorad, F., & Hafezi, S. (2008). Antioxidant activities of Iranian corn silk. Turkish Journal of biology, 32(1), 43-49.
5) Okwu, D. E., & Josiah, C. (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. African Journal of Biotechnology, 5(4), 357-361. doi:10.4314/ajb.v5i4.
6) Siddique, M. A. B. & Brunton, N. (2019). Food Glycoalkaloids: Distribution, Structure, Cytotoxicity, Extraction, and Biological Activity. In J. Kurek (Ed.), Alkaloids-Their Importance in Nature and Human Life.
7)Zhishen J, Mengcheng T and Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999; 64: 555-559.
8)Varadarajan, P., Rathinaswamy, G. and Asirvatham, D. 2008. Antimicrobial properties and phytochemica constituents of Rheo discolour. Ethnobot.Leaflet 12:841-84596)
9)Oueslati, S., ksouri, R., Falleh, H., Pichette ,A., Abdelly , C. and Legault, J. 2012. Phenolic content ,antioxidant , anti inflammatory and anti cancer activities of the edible halophyte *Suaeda fruticosa* Forssk. Food Chem.. 132: 943-947.
10) Adams, R. P. (2001). Identification of essential oils by Capillary gas Chromatography/Mass Spectroscopy , Allured , Carol Stream, IL,USA.
11)Zhang,W.; Yang, J.; Chen, Y.; Xue, R.; Mao, Z.; Lu,W.; Jiang, Y. Lycorine Hydrochloride Suppresses Stress-Induced Premature Cellular Senescence by Stabilizing the Genome of Human Cells. Aging Cell **2021**, 20, e13307.
12) Silva J, Alves C, Martins A, Susano P, Simões M, Guedes M, Rehfeldt S, Pinteus S, Gaspar H, Rodrigues A, Goettert MI, Alfonso A, Pedrosa R. Loliolide, a New Therapeutic Option for Neurological Diseases? In Vitro Neuroprotective and Anti-Inflammatory Activities of a Monoterpenoid Lactone Isolated from *Codium tomentosum*. Int J Mol Sci. 2021 Feb 14;22(4):1888.
13) Thiyagarajan B, Ganeshan Janani, Manokaran S,Rajangam U. Phytochemical Analysis of Methanolic and Benzene Extracts of Aerial Part of Raphanus sativus by Gas Chromatography-Mass Spectrometry(GC-MS). Bull. Env.Pharmacol. Life Sci., Vol 12 [8] July2023: 137-148
14). Vasudevan Aparna, Kalarickal V. Dileep, Pradeep K. Mandal, Ponnuraj Karthe, Chittalakkottu Sadasivan, Madathilkovilakathu Haridas, Anti-Inflammatory Property of *n*-Hexadecanoic Acid: Structural Evidence and Kinetic Assessment,Volume80, Issue3September 2012,Pages 434-43.
15) Vinay Kumar, A.K., J. Bhatnagar and J.N. Srivastava, 2011. Antibacterial activity of crude extracts of *Spirulina platensis* and its structural elucidation of bioactive compound. Journal of Medicinal Plants Research, 5(32): 7043-7048.
16) Kola, A.; Lamponi, S.;Currò, F.; Valensin, D. A Comparative Study between Lycorine and Galantamine Abilities to Interact with AMYLOID _ and Reduce In Vitro Neurotoxicity. Int. J. Mol. Sci. **2023**, 24, 2500. https://doi.org/10.3390/ijms24032500.
17) Jagtap, U.B., Lekhak, M.M., Fulzele, D.P., Yadav, S.R., Bapat, V.A., 2014. Analysis of selected *Crinum* species for galanthamine alkaloid: an anti-Alzheimer drug. Current Science 107, 2008–2010.
18) Tabanca, N., Gao, Z., Demirci, B., Techen, N., Wedge, D. E., Ali, A., Sampson, B. J., Werle, C., Bernier, U. R., Khan, I. A., Baser, K. H. C., 2014. Molecular and Phytochemical Investigation of *Angelica dahurica* and *Angelica pubescentis* .
19) Elaiyaraja, A., Chandramohan, G., 2016. Comparative phytochemical profile of *Indoneesiella echioides* (L.) Nees leaves using GC-MS. Journal of Pharmacognosy and Phytochemistry, 5(6), 158-171.
20) Kang MC, Ham YM, Heo SJ, Yoon SA, Cho SH, Kwon SH, Jeong MS, Jeon YJ, Sanjeewa K, Yoon WJ, Kim KN. Anti-inflammation effects of 8-oxo-9-octadecenoic acid isolated from *Undaria peterseniana* in lipopolysaccharide-stimulated macrophage cells. EXCLI J. 2018 Aug 2;17:775-783. doi: 10.17179/excli2018-1422. PMID: 30190667; PMCID: PMC6123615.

21) Gabriel A. Olatunji, Oluwatoyin Adenike Fabiyi, Adekunle J. Adeniji, and Omonike O. Ogbole Phytochemicals from *Kigelia pinnata* Leaves Show Antioxidant and Anticancer Potential on Human Cancer Cell Line Olubunmi Atolani, Journal of Medicinal Food 2013 16:10, 878-885
22) Abubacker MN, Devi PK. *In vitro* antifungal potentials of bioactive compounds heptadecane, 9- hexyl and Ethyl iso-allocholate isolated from *Lepidagathis cristata* Wild. (*Acanthaceae)* leaf. BBB 2015;3:336-43.
23) Dobrina Tsvetkova, Danka Obreshkova,Stefka Ivanova,Bozhidarka Hadjieva,2016.galantamine increase dopaminergic neurotransmission, and decreases neurodegeneration and cognitive decline. Which promoting the process of learning,memory and attention,(July-2016);099-116.
24) Čechovská, L., Cejpek, K., Konečný, M. *et al.* On the role of 2,3-dihydro-3,5-dihydroxy-6-methyl-(4*H*)-pyran-4-one in antioxidant capacity of prunes. *Eur Food Res Technol***233**, 367–376 (2011).26) Budavari. (1996). The Merck Index – AnEncyclopedia of Chemicals, Drugs, and Biologicals.Whitehouse Station, NJ: Merck and Co., Inc, p. 464.
25) Federica Bruno,GermanoCastelli,Antonella Migliazzo, Maria Piazza,Antonella Galante, Vincenza Lo Verde,SimonaCalderone,GiuseppaNucatolo, Fabrizio VitaleCytotoxic Screening and In Vitro Evaluation of Pentadecane Against *Leishmania infantum* Promastigotes and Amastigotes,*J Parasitol* (2015) 101 (6): 701–705.
26) Gonzalez-Rivera, M.L.; Barragan-Galvez, J.C.; Gasca-Martínez, D.; Hidalgo-Figueroa, S.; Isiordia-Espinoza, M.; Alonso-Castro, A.J. In Vivo Neuropharmacological Effects of Neophytadiene. *Molecules*2023, *28*, 3457.
27) Kang MC, Ham YM, Heo SJ, Yoon SA, Cho SH, Kwon SH, Jeong MS, Jeon YJ, Sanjeewa K, Yoon WJ, Kim KN. Anti-inflammation effects of 8-oxo-9-octadecenoic acid isolated from *Undaria peterseniana* in lipopolysaccharide-stimulated macrophage cells. EXCLI J. 2018 Aug 2;17:775-783. doi: 10.17179/excli2018-1422. PMID: 30190667; PMCID: PMC6123615.
28) M. Ogunlesi,W. Okiei, E. Ofor, and A. Eniola, Medicinal and Pesticidal Potentials of the Constituents of the Essential Oil from Adenia cissampeloides Leaves. 68 (1-2) (2019) 7–21.
29) Amudha P, Jayalaksmi M, Pushpabharathi N and Vanitha V 2018. Identification of bioactive components in *Enhalus acoroides* seagrass extract by Gas Chromatography–Mass Spectrometry. Asian J. Pharm.Clinic. Res. **11**: 131-137.