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GC-MS analysis of ethanolic extract of *Cabomba furcata* Schult. & Schult.F: An aquatic plant

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Abstract
The present study was designed to determine the bioactive component in the ethanolic extract of the aquatic plant *Cabomba furcata Schult and Schult.* F by using GC-MS analysis.
Methods: The whole plant of *Cabomba furcata* was shade dried and powdered, extracted with ethanol for 4 hours. The phytochemical constituents were analyzed by GC-MS.
Result: Totally 25 components were identified from ethanolic extract, a wide range of fatty acids esters, alcohol, hydrocarbons were reported to possess antioxidant, anticancer, anti-inflammatory properties. Bioactive molecules include hexa decanoic acid (19.47), γ-sitosterol (11.70), α-linoleinic acid (8.89), 1,2-benzene dicarboxylic acid (4.82), dibutyl phthalate(3.19), campesterol (3.04%), phytol (2.41%), Vitamin-E (1.26%), 1,2,3-benezene triol (1.06%), do decanoic acid(0.92%),+-)-lavandulol acetate (0.89%), squalene (0.88), pinostrobin chalcone(0.85%), tetra decanal(0.75%), azelaic acid(0.68%) and various other significant components.
Conclusion: Various constituents were identified as biologically active and pharmaceutically significant for the treatment of various ailments and can have the potential to be commercialized in the pharmaceutical field.

Keywords: Vitamin-E, forked fanwort, anticancer, antioxidant

Introduction
Aquatic plants are one of the most productive ecosystems of the world and essential for life supporting. They provide a wide array of benefits to human kind and is an alternative, aquatic plant can become the potent source for the allelopathic studies. Seaweeds have been traditionally used in human and animal nutrition. Seaweed have been reported for its inhibitory activity and mainly used as antibiotics, antibacterial, antiviral, anti fungal and cytotoxic and larvicidal [1].

Fig 1: *Cabomba furcata*

Cabomba furcata is a water plant, belonging to the family Cabombaceae. This plant can be easily identified because it has divided submerged leaves in the shape of a fan and it submerges completely under water. This plant is much favored by aquarists as an ornamental and oxygenating plant for fish tanks. Its use in the aquarium trade has led to some species being introduced to parts of the world [2].

Cabomba furcata is a species of aquatic plant in the water shield family known by the common names Red Cabomba and Forked Fanwort. It is native to South America and as far north as Cuba and the tip of Florida. It reaches a maximum height between 30 and 80 cm and is up to 8 cm wide. It bears purple flowers [3].

It is naturalized in Peninsular Malaysia because it is so well-established in *Tasik Chini* which is the second largest natural fresh water lake in the peninsular Malaysia. It has seriously impacted the tourist value of the lake and has been given a local name, *ekor kuching* (Cat's tail). Stems: olive-green to reddish brown; Submerged filiform leaves: dark purple, in whorls of three, at the apex often opposite; petiole: 1–2 cm, lamina with linear divisions in one plane, 4 cm long; floating peltate leaves:olive-green, sometimes with a dark purple margin, lamina narrowly rhombic or linear to lanceolate, occasionally sagittate, 20–40 mm × 3 mm; pedicels :2–5.5 cm long; Flowers: purplish, 6–12 mm diameter, 5–10 mm long; sepals obovate-elliptic, 5–9 × 2–4 mm, apex obcordate, basal third yellow, distal two thirds purplish red to bluish violet with darker veins, with a darker rim at margin; petals: ovate-oblong, 5–9 × 2–5 mm, base slightly auriculate, the lobes with two yellow, confluent patches, the claw whitish; stamens: greenish yellow, 5 mm, anthers yellow; carpels: 2, divergent at maturity. Seeds: globose, 1–2 mm diameter, echinate, surface verrucate. In its native habitat, it grows in tropical climates with a brief dry season, in shallow (upto 3m deep), warm (18–30ºC), acidic to slightly acidic (pH 4–7), more-or-less stagnant water in lakes, streams and floodplains. It is a light-demanding plant. It thrives on organic nutrients when it becomes fast growing (e.g., its stem can grow 12 cm a month). It propagates from plant fragments as well as from seed.

It is therefore pre-adapted to conditions in Peninsular Malaysia [4]. *Cabomba furcata* exhibited pronounced seasonal population variations related to high-flow monsoonal rain events. In tropical southern Brazil, *Cabomba furcata* populations persist throughout the year, but even though there are no marked high-flow events, there are seasonal fluctuations in productivity that are related to light intensity and availability of organic carbon [5].

Cabomba furcata has shown the presence of active phytoconsituents such as saponins, flavanoids, tannins (catechin, gallic acid, ellagic acid and syringic acid) [6] and alkaloids like nicotine, tomatine, thebaine and quinine [7]. The further exploration in this plant include the analysis of heavy metal in the lake. Introduction of heavy metals in to the lake is a major concern now and it affects several communities in the lake like fishes, planktons, aquatic plants etc. The plants have the ability to absorb the metals. The accumulation of heavy metals in the plants could be an indicator of water contamination thus can be an excellent biomarker for metal pollution in aquatic system [8]. Thus this plant contributes to a check on water quality. Various other benefits of the plant include economic benefits, social benefits, environmental services etc.

The exotic fresh water weed *Cabomba furcata* is found to have a fast spread in the inland water bodies thus the plant need to be explored further for the analysis of their bioactive molecule. It is important that most of the traditional uses of aquatic plants are novel and they need both popularization and preservation. Hence this study is crucial in screening unknown compounds that is of parmaceutical importance.

Materials and methods

Plant material collection and authentication

The Whole plant of *Cabomba furcata* used or the present research work was collected during the month of October–November from a pond in Guruvayur, Thrissur District. The plant was identified using standard flora and authenticated by Dr. Harinarayanan M.K, Assistant Professor, Sreekrishna College, Guruvayur and herbarium specimen was procured for further reference (Voucher number 101). After the collection the plant was transported to the college laboratory where it is rinsed thoroughly in tap water to remove the attached debris and epiphytes.

Extraction

The fresh plant of *Cabomba furcata* was collected from the pond, shade dried and powdered (100g). Placed in a soxhlet apparatus and extraction was performed with 500ml of ethanol for 4 hours at a temperature not exceeding the boiling point of the solvent. The extract was filtered through Whatman no:1 filter paper and the resulting solution was concentrated *in vaccum* to dryness to give ethanolic extract (10g). The extract obtained was subjected to GC-MS analysis.

GC –MS analysis

Gas chromatography Mass spectroscopy analysis of ethanolic extract was performed using Shimadzu GC-MS Model number: QP2010S equipped with Column - ELITE-5MS (30 meter length, 0.25 mm ID, and 0.25 µm thicknesses). Electron ionization system was used; details of GC programme are given in Table I. The oven temperature was programmed from 70.000C which is given in Table II. Helium gas was used as the carrier gas. Details of GC-MS programme was given in Table III. Programme specifications regarding Mass Spectra are depicted in Table IV. GCMS Software: GCMS Solutions, Libraries used: NIST 11& WILEY 8.

Table 1: GC programme (GC 2010)

GC-Parameters	Programme
Column temperature	70.000C
Injection temperature	260.000C
Injection mode	Split less
Sampling time	2.00 min
Flow control mode	Linear velocity
Pressure	61.5 pka
Total flow	54.1mL/min
Column flow	1.00mL/min
Linear velocity	36.7 cm/sec
Purge velocity	3.0mL/min
Split ratio	50.0

Table 2: Oven temperature program

Rate	Temperature(0c)	Hold time(min)
-	70.0	2.00
10.00	200.0	5.00
-		
5.00	280.0	15.00

Table 3: GC-MS programme (GCMS QP2010)

GC-MS Parameters	Programme
Ion source temperature	200.000c
Interface temperature	280.000c
Solvent cut time	6.50 min
Detector gain mode	Relative
Detector gain	1.01 kV+0.00 kV
Threshold	1000

Table 4: MS table

Mass spectroscopy parameters	Programme
Start time	6-7 min
End time	51.00 min
ACQ time	Scan
Event time	0.50 sec
Scan speed	1000
Start m/z	50.00
End m/z	500.00
Sample inlet unit	GC

Identification of compounds

The constituents in the extract were identified by comparing their relative retention time and confirmation was done by comparing the mass spectra with database from the Library of NIST 11 and Wiley8. GC-MS Chromatogram obtained was given in figure.2.

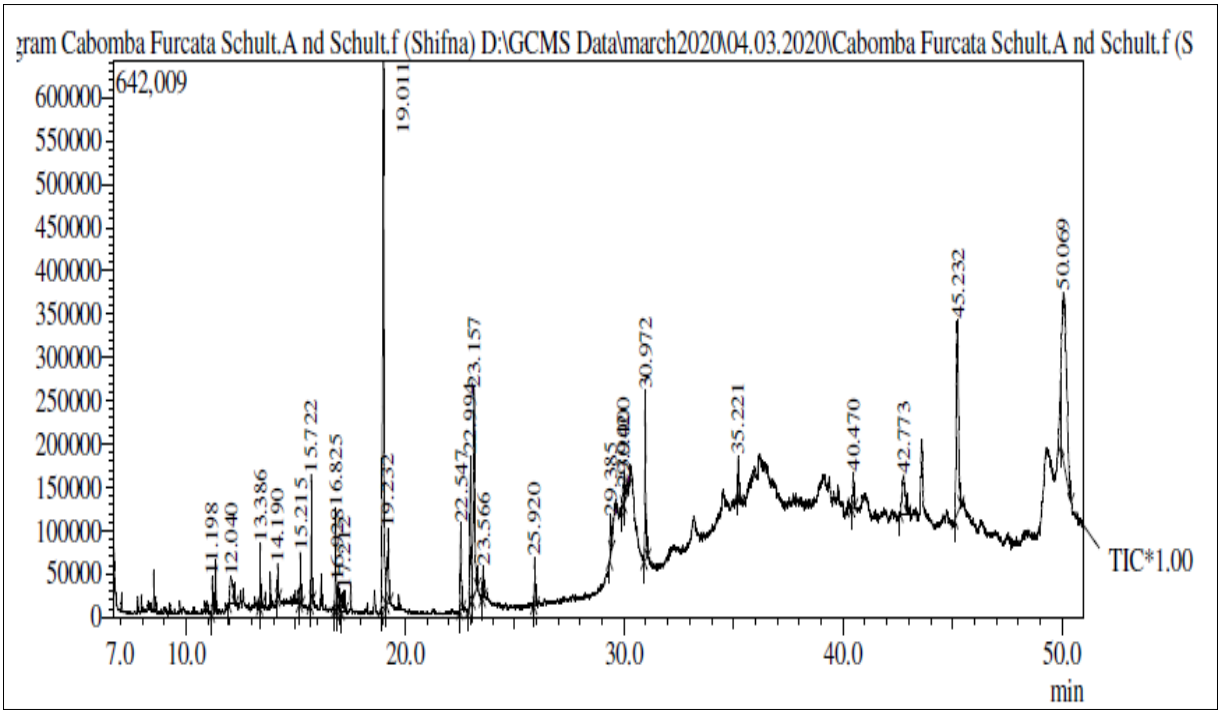

Fig 2: GC–MS chromatogram of ethanolic exract of *Cabomba furcata* Schult. and Schult. F.

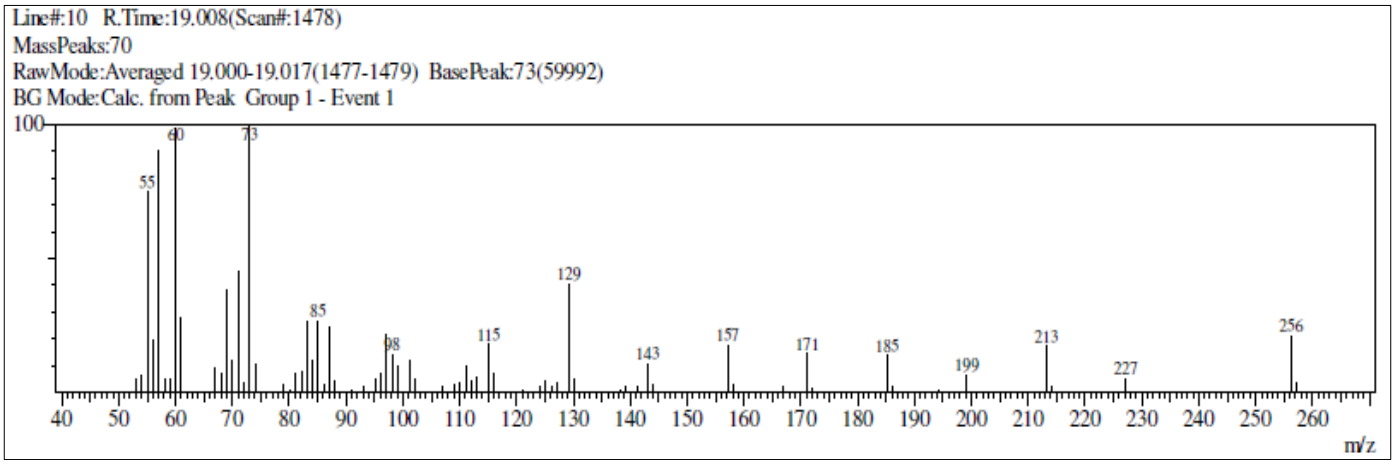

Fig 3: Mass spectroscopic chromatogram of hexadecadienoic acid

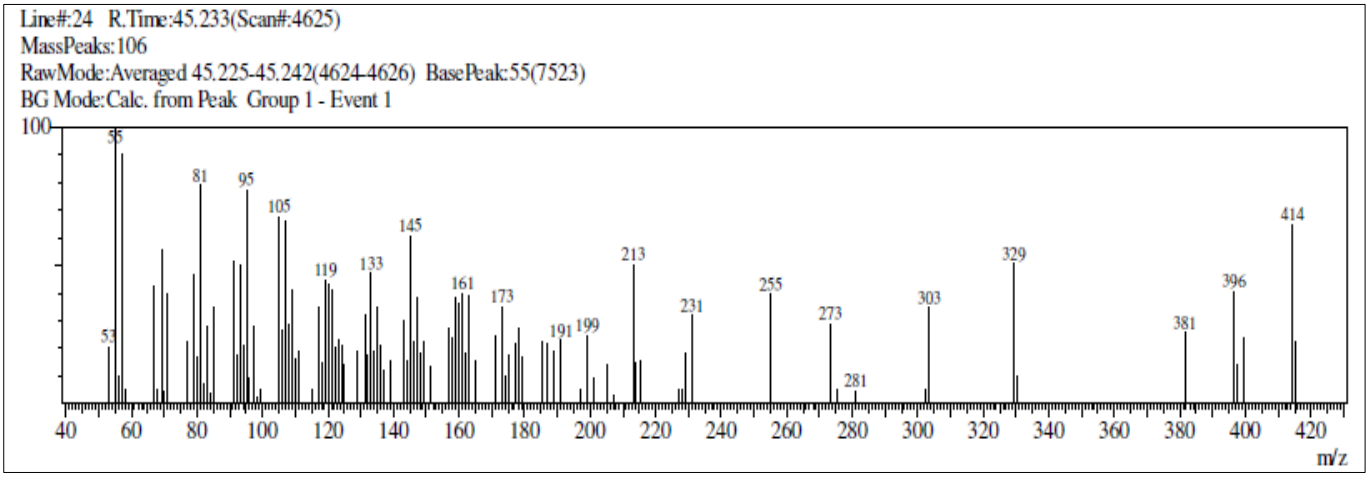

Fig 4: Mass spectroscopic chromatogram of gamma sitosterol

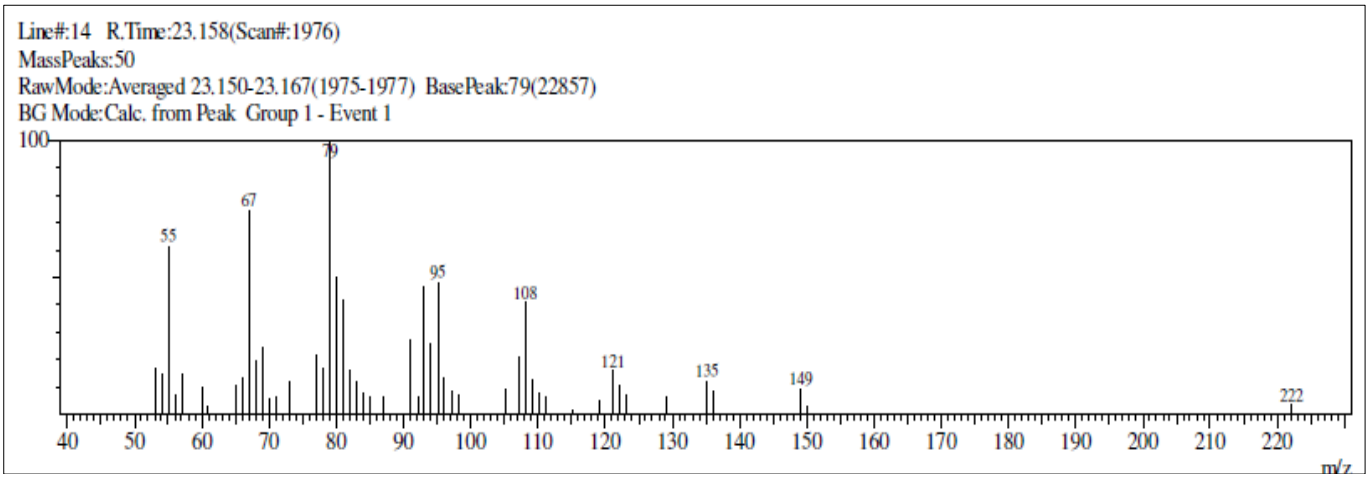

Fig 5: Mass spectroscopic chromatogram of alpha linolenic acid

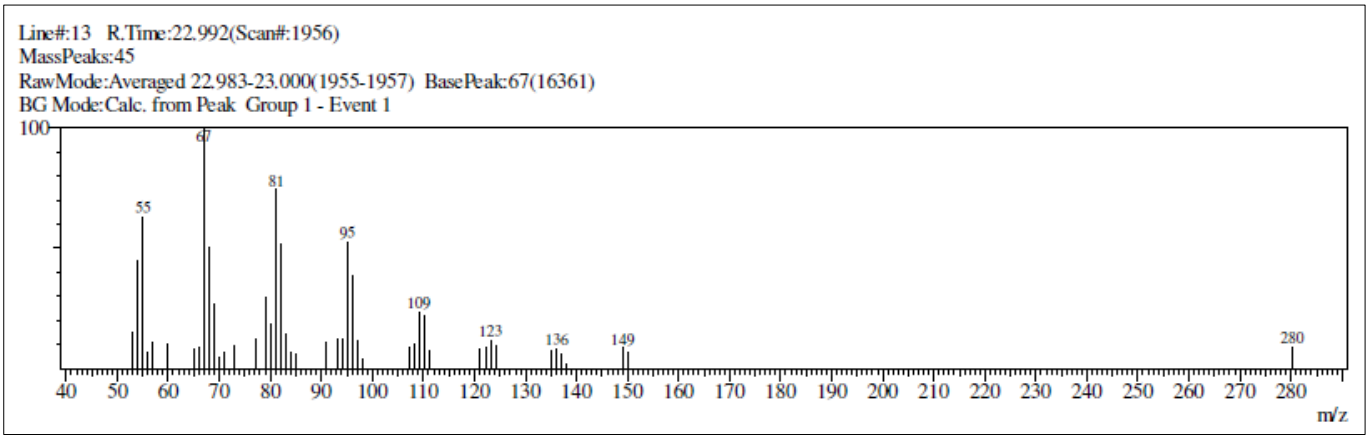

Fig 6: Mass spectroscopic chromatogram of 9,12 octadecadienoic acid

Result and Discussion

GC-MS analysis of ethanolic extract of *Cabomba Furcata* was carried out and a group of 25 compounds were identified as depicted in table 5. The compounds are glycerol trilaurate (22.12%), hexadecanoic acid (19.47%), γ-sitosterol (11.70%), α-linoleinic acid (8.89%), 9,12-octadecadienoic acid(5.15%), 1,2-benzenedicarboxylic acid (4.8%), 1,2,3- benzenetriol (1.06%), nitro iso butyl glycerol (2.37%), do decanoic (0.92%), azelaic acid(0.68%), tetra decanal(0.75%), tetra decanoic acid (2.06%), phytol acetate(2.41%), lauryl glyceryl ether (0.76%),+-)-lavandulol acetate(0.89%), di butyl phthalate (3.19%), phytol (2.68%), docosanoic acid (0.83%), 1-(phenethylthio)-2-phenylethylene(1.11%), pinostrobin chalcone (0.85%), glycerol tri laurate(0.76%), 2-methyltetralosane (1.35%), squalene(0.8%), vitamin-E(1.26%), γ-sitosterol, have been reported as antioxidant, antibacterial antidiabetic and prophylactic activities [9]. Azelaic acid is non toxic and exerts therapeutic effects like antimicrobial, bacteriostatic, antiacne, comedolytic [10]. Pyrogallol have antiproliferative effect [11]. Phytol is used as a precursor for the manufacture of synthetic form of vitamin-E and vitamin-K and have antibacterial effect [12]. Squalene is a precursor of various hormones in humans and sterols in plants, antitumor, anti cancer effect against ovarian, breast lung and colon cancer, lowers cholesterol in blood, protects skin from UV damage [13]. Vitamin-E is an important anti

oxidant, it has been proven that vitamin e can be used to prevent liver fibrosis and cirrhosis in rabbits [14] and vitamin is an adjuvant for cancer treatment [15], α-linoleic acid in the treatment of prostate cancer [16] and also have anti-inflammatory activity, hexa decanoic acid is a good antioxidant, apart from which it have anti inflammatory, antifibrinolytic, haemolytic, lubricant nematicide, antialopecic and used as pesticide and flavor adding, 5-α-reductase inhibitor [17], 1,2-benzenedicarboxylic acid is used in the preparation of perfumes and cosmetics [18], 9,12- octa deca dienoic acid possess anti-inflammatory and anticancer activity [19]. Campestrol exert antioxidant activity [20]. Pinostrobin chalchone exhibit a strong antioxidant property. The mass spectrum of hexadecadienoic acid, gama sitosterol, alpha linolenic acid, 9, 12 octadecadienoic acid are shown in figure (3-6).

Table 5: GC-MS analysis of ethanolic extract of *Cabomba Furcata* Schult and Schult F

Peak#	R.time	Area	Area %	Height	Height %	Name	Base m/z
1	11.198	142347	1.06	41911	1.53	1,2,3 benezene triol	126.10
2	12.040	318979	2.37	32694	1.19	Nitro isobutyl gycerol	57.05
3	13.386	123804	0.92	74195	2.71	Do decanoic acid	60.00
4	14.10	91074	0.68	40249	1.47	Azelaic acid	55.05
5	15.215	100944	0.75	59984	2.19	Tetr decanal	57.05
6	15.722	276785	2.06	148282	5.42	Tetra decanoicacid	60.00
7	16.825	323660	2.41	117396	4.29	Phytol, acetate,	68.05
8	16.928	101961	0.76	31630	1.16	Lauryl glyceryl ether	70.05
9	17.212	119959	0.89	24075	0.88	(+)-Lavandulol acetate	81.10
10	19.011	2617209	19.47	630049	23.02	Hexa decanoic acid	73.05
11	19.232	428277	3.19	8485906	3.14	Dibutyl pthalate	149.05
12	22.547	360543	2.68	100245	3.66	Phytol	71.05
13	22.994	692768	5.15	171391	6.26	9,12-octa decanoic acid	67.05
14	23.157	1194283	8.89	237787	8.69	α- linolenic acid	79.05
15	23.566	110901	0.683	36802	1.34	Docosanoic acid	57.05
16	25.920	149520	1.11	54993	2.01	1-(phenethyl thiol)-2-phenylethylene	240.15
17	29.385	113903	0.85	46447	1.70	Pinostrobin chalcone	166.05
18	29.942	102181	0.76	23108	0.84	Glycerol tri laurate	183.20
19	30.000	181044	1.35	35304	1.29	2-methyl tetracosane	57.05
20	30.972	647875	4.82	195072	7.13	1,2-benzene dicarboxylic acid	149.05
21	35.221	118508	0.88	47231	1.72	Squalene	69.10
22	40.470	169149	1.26	40510	1.48	Vitamin-E	165.10
23	42.773	408195	3.04	44269	1.62	Campesterol	57.05
24	45.232	1572441	11.70	222177	8.12	γ-sitosterol	55.05
25	50.069	2972991	22.12	196033	7.16	Glycerol tri laurate	57.05
		13439301	100.00	2737540	100.00		

Conclusion

From the present study 25 components have been identified from the ethanolic extract of *Cabomba furcata*. The investigation through the present study revealed that the species *Cabomba furcata* is a reliable source of bioactive compounds like fatty acid esters alcohol and hydrocarbons. As GC MS is the first step towards exploration of biomolecules this study can open the way out for further researches in the aquatic flora.

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Conflict of Interest

The authors have no conflict of interest.

Abbreviations used

GC-MS: Gas Chromatography- Mass Spectroscopy; **Rt:** Retention time; **NIST 11**; National Institute Of Standard and Technology 11.

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