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Exploring Neurological Phytochemical Chemical Constituents Using Aqueous Extraction of *Benincasa Hispida* Fruit: Molecular Bombardment Study of Electrospray Ionisation by Liquid Chromatography-Mass Spectroscopy Method

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Abstract

The *Benincasa hispida*, also known as budkumbalikai and belong to the family Cucurbitaceae. Fruit of this used by native people for the treatment central nerve system. The aim of the study was to identify the phenolic compound from the fruit of *Benincasa hispida* by using LC-MS and GC-MS-based targeted and untargeted analysis. LC-MS untargeted chromatogram peak showed flavonoid molecules in graph. In GC-MS untargeted analysis showed the 48 bioactive phytochemical constitutes from different classes were annotated there are 48 bioactive molecules including phenolic acids, glycoside, organic acids, flavonoids and other compounds. The *Benincasa hispida* has excellent potential as edible vegetable fruit-based food due to its phytochemical profile and high phenolic content.

Keywords: Liquide chromatography- mass spectroscopy, gas chromatography-liquid chromatography

INTRODUCTION

The wax gourd, or *Benincasa Hispida*, is a popular vegetable with health benefits. It belongs to the Cucurbitaceae family. The wax gourd's ability to prevent oxidation varies depending on its parts, including the peel, pulp, and core; the seeds exhibit the strongest antioxidant potential [1]. Sanskrit texts claim that it can help with a wide range of illnesses, including epilepsy, dyspepsia, nerve disorders, and insanity. Numerous scientific investigations have been carried out to determine its anticonvulsant, antioxidant, and anti-inflammatory qualities [2]. Triterpenoids, flavonoids, glycosides, saccharides, proteins, carotenes, vitamins, minerals, ß-sitosterol, and uronic acid are among the main substances found in these seeds [3]. Identifying the compounds found in the extracts is the primary goal of these techniques. When quantification is carried out, the calibration curve for only a small number of primary phenolic compounds that are purportedly present in the residues is used with the external standard method. As a result, these techniques are not designed to quantify a large number of phenolics, and even when they are created to test a large number of compounds, they are not proven to be suitable for routine analysis of this kind of analyte that remains in the solid residue matrix following the distillation of essential oils. Target analyte isolation and quantification from various plant matrices is widely acknowledged to necessitate the development of suitable techniques with high selectivity and sensitivity. To quantitatively extract all the analytes from the composite plant matrix, suitable extraction and purification methods should be developed before applying an optimized chromatographic method. From the literature review, the author Doshi GM (2016) described the hyphenated technique of liquid chromatography-mass chromatography for the elucidation of flavonoids from plant extracts of Carissa congesta, Polyalthia longifolia, and Benincasa hispida. The use of spectroscopy Flavonoids like quercetin (m/z 301) and rutin (m/z 610) were found in the extracts as parent ions, and close analogues like quercetin-O-hexoside, vicenin 2, quercetin-3-O-xyloside/arabinoside, and quercetin-3-

O-glucoside were found as fragments [4]. Choi SI in 2024 reported that UPLC-QTOF-MS/MS was used to identify marker compounds that are produced or increased during fermentation in *Benincasa hispida*. HPLC-PDA was used to verify the analysis method and perform content analysis. By comparing their retention times, UV and MS spectra, and molecular formulas with those documented in earlier investigations, the marker compounds obtained or increased in content were determined to be 2-furoic acid, 2,3-dihydroxybenzoic acid, and rubinaphthin A [5]. According to Luo C. et al., the wax gourd (*Benincasa hispida*)'s genome-wide identification of the saur gene family and functional characterization of bhsaur60 during fruit development [6]. Data regarding the *Benincasa hispida* phytochemical composition have not been sufficiently addressed in the literature. Therefore, studies regarding the phenolic profile are required as they can contribute to research aimed at analysis the relation between diet and diseases. In addition, they can facilitate the development of new products and further uses, and promote the sustainable development and contribute to the conservation of its biodiversity. The aim of this study, therefore, was to investigate the profile of the phytochemical compounds, especially the phenolic compounds found in the *Benincasa hispida* fruit.

METHODOLOGY

Collection of plant specimen

The fruit of *Benincasa hispida* was gathered in a section of the Nelamangala market during May. SSCP/No/15/22 is the specimen number that was verified by a Pharmacognosiste at Sree Siddaganga, B.H. Road, Tumkur.

Preparation of plant specimen

A mixer was used to grind the 200g of fresh *Benincasa hispida* fruit. Through a cold maceration process, the collected homogenate paste was combined with an aqueous solvent for 72 hours. A dry extract powder was obtained by lyophilizing the homogenate after it had been filtered through muslin cloth and Whatman No. 1 filter paper. For upcoming research, the lyophilized powder extract was kept in airtight amber-colored bottles [7].

Liquid Chromatography-Mass Spectroscopy analysis of aqueous extract of *Benincasa hispida*

The instrument Waters ELSD 2420 was used to perform the chromatographic separation. A Diode array detector and an Agilent 8453 were used to analyze the separated fractions under UV light. Reversed phase HPLC was used to separate the mixture on an Adsorbosphere column-NH2 (250 x 4.6 mm column) using gradient and isocratic elution with acetonitrile/water, and Waters ELSD 2420 was used for detection. The mobile phase in ELSD is initially evaporated. The sample's solid remnants are subsequently transported as a mist into a cell, where a laser detects them. Using an LCMSD/Trap System with an electro-spray interface, HPLC–MS analysis was carried out. In the positive ion mode, the MS spectra were obtained. 0.10% formic acid in HPLC-grade deionized water (A) (milli-q-water exposed to infrared radiation through 3.5-micron filters) and methanol (B) extracted from the stationary phase of an Atlantis dc 18 column (50 x 4.6mm - 5µm) made up the mobile phase. The different gradient program was as follows: at a flow rate of 1.2 mL min-1, 10% B to 95% B in 4 minutes, 95% B to 95% B in 1 minute, 95% B to 10% B in 0.5 minutes, and 10% B in 1.5 minutes. A 2.0 µL injection volume and a 40°C column oven temperature were maintained. Product mass spectra were captured between 150 and 1000 m/z. The run was preceded by the optimization of the instrumental parameters [8].

RESULTS AND DISCUSSION

The diverse chemical components that make up plants are said to have biological activity and are in charge of displaying a variety of pharmacological effects. Many of the secondary metabolites found in plants which posses natural antioxidants, which have been shown to be safer than synthetic ones. Depending on the solvent's and the target compound's characteristics, the study used earlier analytical methods to identify various compounds chemical compound contained in the corresponding fractions in specific plants [1]. Also from gas chromatography-mass spectroscpgy investigation of aqueous extract reveals the presence of forty-eight bioactive phytochemical compounds respectively, as shown in Table 01. However, out of 48 bioactive compound we found a flavonoid bioactive phytochemical identified as 2,3-Diphenyl-5,8-dimethoxy-6-acetamido quinoxaline,1-(9-Furan-2-ylmethyl-9-azabicyclo-[3,3,1]-non-3-yl)-3-(3-ethoxy-phenyl)thiourea,4-(4-Iodo-phenyl)-3,4-dihydro-1H-benzo-quinolin-2-

one,Glucuronamide,3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-3-(4-methoxyphenyl) was present in the aqueous extract and has been established to possess certain biological functions ranging from antioxidant to anti-inflammatory properties.

Liquid Chromatography-Mass Spectroscopy of aqueous fraction of *Benincasa hispida*

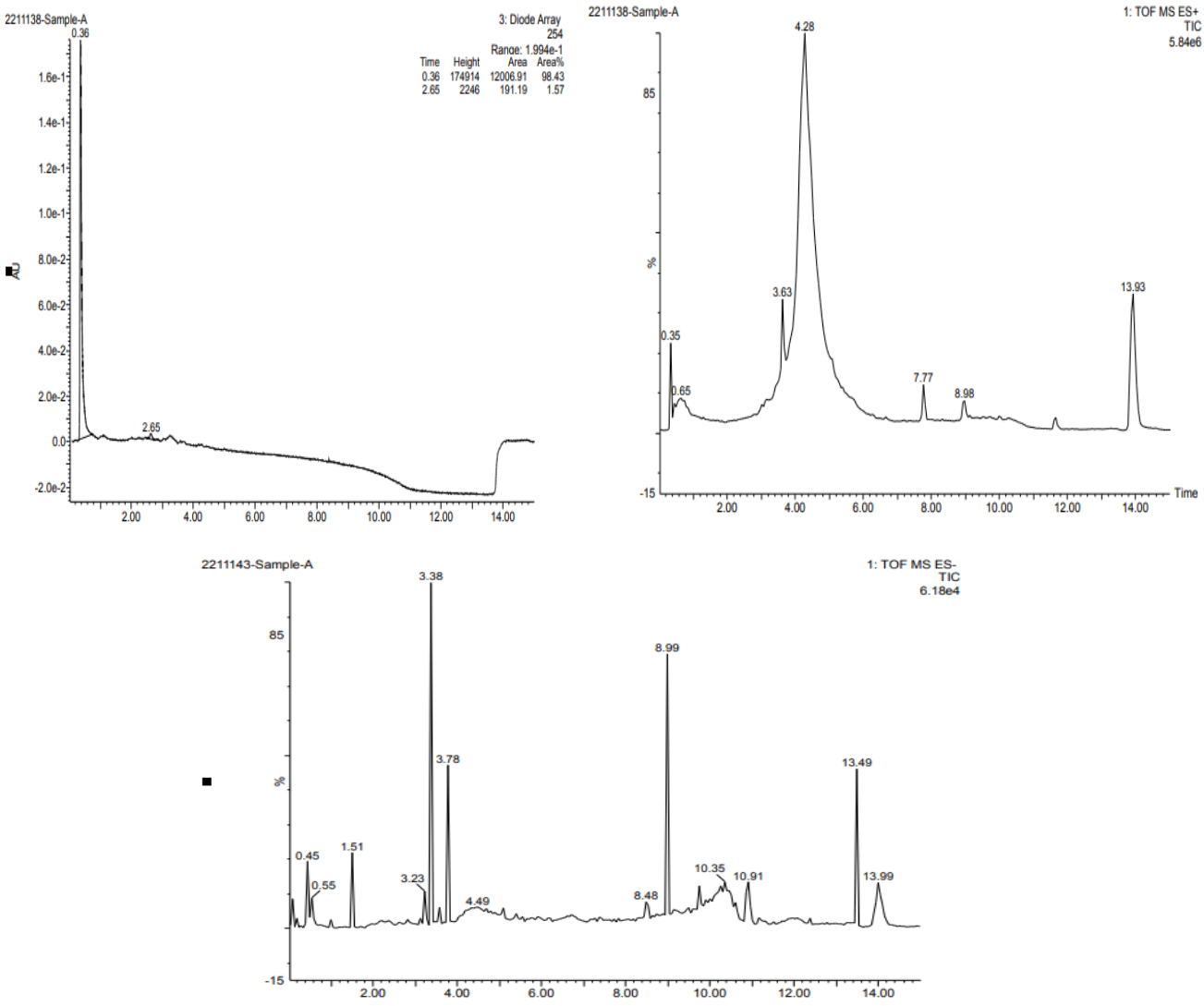
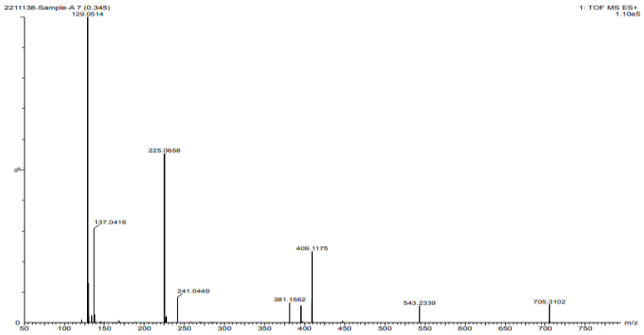


Figure 1. LC-MS chromatogram of aqueous fraction of *Benincasa hispida* extract.

Mass- spectroscopy of aqueous *Benincasa hispida* extract


2211138-Sample-A 7 (0.345)
1: TOF MS ES+
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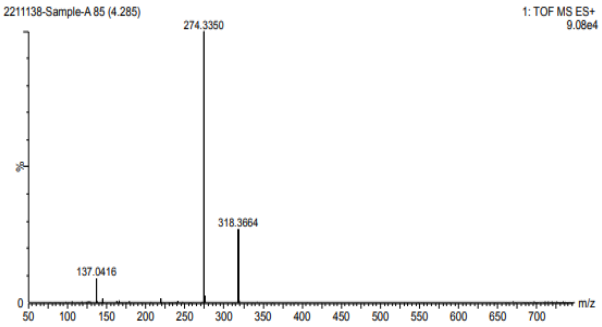
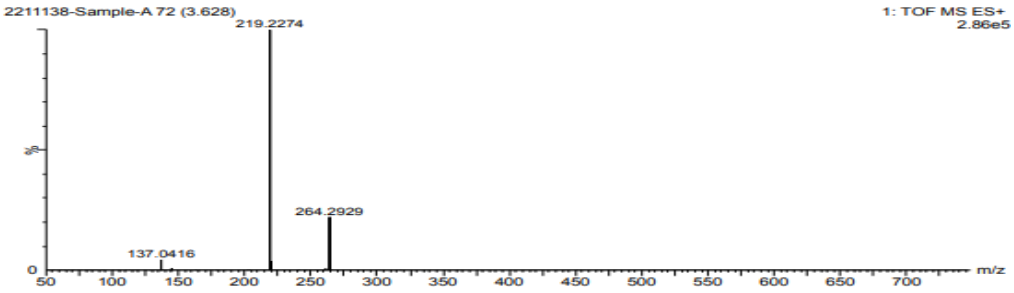
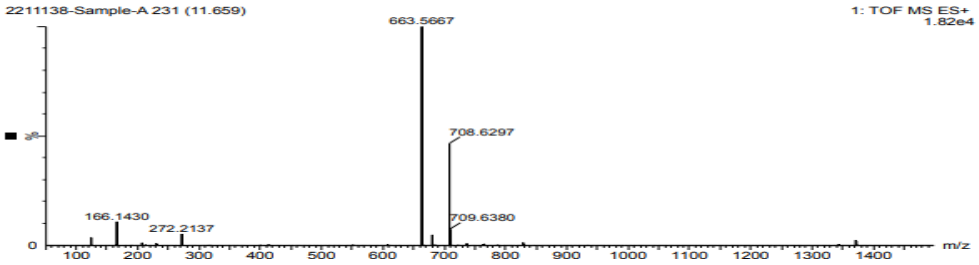
129.0514
225.0658
137.0416
409.1175
241.0449
381.1562
543.2339
705.3102
m/z


2211138-Sample-A 85 (4.285)
1: TOF MS ES+
9.08e4
274.3350
318.3664
137.0416
%
m/z


2211138-Sample-A 72 (3.628)
1: TOF MS ES+
2.86e5
219.2274
264.2929
137.0416
%
m/z


2211138-Sample-A 231 (11.659)
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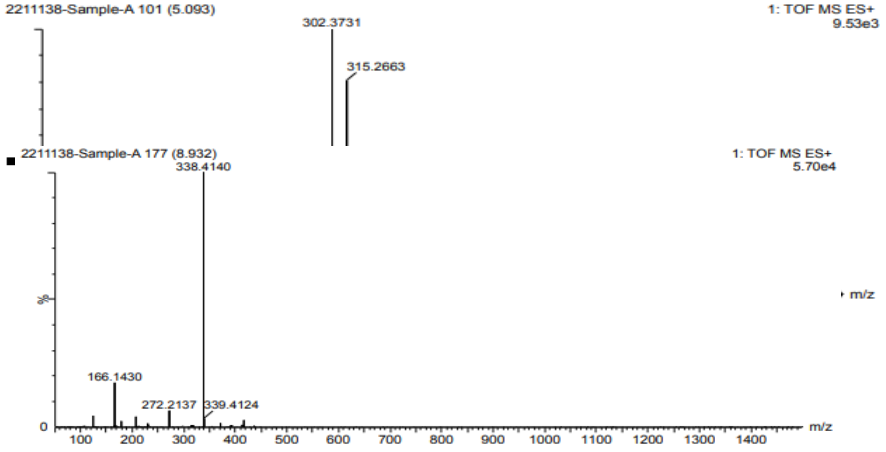
1.82e4
663.5667
708.6297
709.6380
166.1430
272.2137
%
m/z


2211138-Sample-A 101 (5.093)
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302.3731
315.2663
2211138-Sample-A 177 (8.932)
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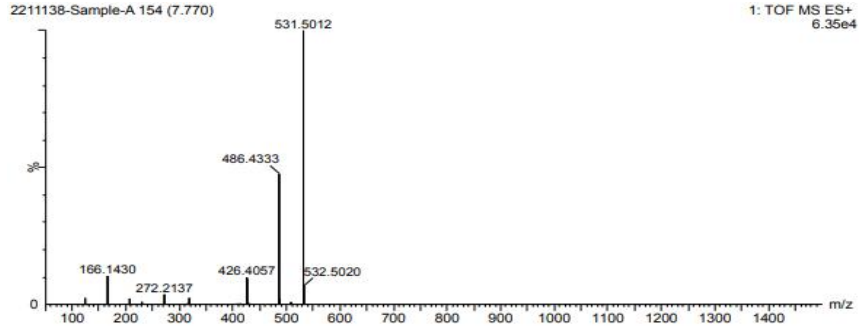
166.1430
272.2137
339.4124
%
m/z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166.1430
272.2137
426.4057
532.5020
%
m/z

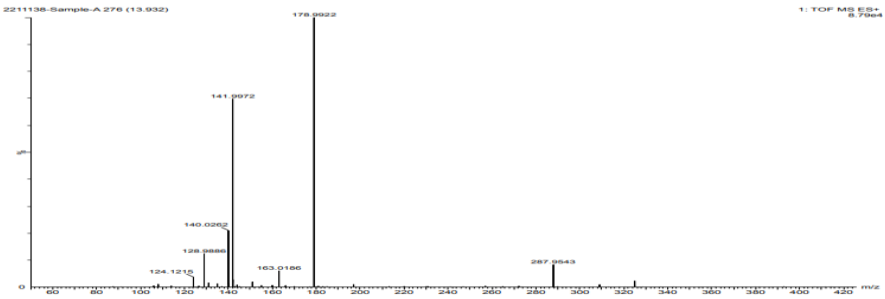

Figure 2. Mass- spectra fraction of aqueous extraction of *Benincasa hispida*

Table 01: Bioactive molecules from aqueous extract of *Benincasa hispida*.

Sl. No.	Name of the Compound	Molecular Formula	Molecular Weight	Peak area (%)
1.	Methyl-2,3,4-tri-O-trifluoracetylβd-xylopyranoside	$C_{12}H_9F_9O_8$	452	17.5
2.	Acridine	$C_{14}H_9Cl_2NO$	277	9.04
3.	1,3-Bis(trichloromethyl)benzene	$C_8H_4Cl_6$	310	7.63
4.	1,2-Cyclohexanedicarboxylic acid	$C_{22}H_{22}Cl_2O_4$	420	7.04
5.	1,2-Cyclohexanedicarboxylic acid	$C_{22}H_{22}Cl_2O_4$	564	6.50
6.	Cyclopent-2-enone,5-benzylideno-3-(4-chlorophenyl).	$C_{18}H_{13}ClO$	554	4.58
7.	4,5-Dibromo-2-nitrobenzaldehyde	$C_7H_3Br_2NO_3$	552	4.23
8.	3-Chloro-2-fluorobenzamide	$C_{14}H_{11}ClFNO_2$	279	3.90
9.	Succinic acid	$C_{16}H_{19}C_{l3}O_4$	380	3.60
10.	Benzamide	$C_{14}H_{11}ClFNO_2$	279	2.90
11.	4-Isoxazolecarboxylic acid	$C_{25}H_{21}NO_4$	399	26.8
12.	Benzamide	$C_{19}H_{24}F_3N_3O_3$	399	25.7
13.	2,3-Diphenyl-5,8-dimethoxy-6-acetamidoquinoxaline	$C_{24}H_{21}N_3O_3$	399	21.7
14.	Ethyl-4-[5-(2,5-dimethyl-4-nitrophenyl)-2-furyl]-1,2,3,4-tetrahydro-6-methyl-2-oxo-5-pyrimidine carboxylate	$C_{20}H_{21}N_3O_6$	399	14.9
15.	Benzamide	$C_{23}H_{36}F_3NO$	399	3.12
16.	1-(9-Furan-2-ylmethyl-9-azabicyclo[3.3.1]non-3-yl)-3-(3-ethoxy phenyl)thiourea	$C_{22}H_{29}N_3O_2S$	399	2.14
17.	Cyclopenta[5,6]naphth[2,1-c]azepin-3(2H)-one,8β-(1,5-dimethylhexyl)-1,5a,5bα,6,7,7a,8,9,10,10aα,10bβ,11,12,12aα-tetradecahydro-5aβ,7aβ-dimethyl	$C_{27}H_{45}NO$	399	1.97
18.	3H-Naphtho[2,3-b]furan-2-one,4-hydroxy-3-[[2-(2-methoxyphenyl) ethylamino]methyl]-4a,5-dimethyl-3a,4,4a, 5,6,7,9,9a-octahydro	$C_{24}H_{33}NO_4$	399	1.59
19.	4-(4-Iodo-phenyl)-3,4-dihydro-1H-benzo[h]quinolin-2-one	$C_{19}H1_4INO$	399	0.40
20.	4-(3-Iodo-phenyl)-3,4-dihydro-1H-benzo[h]quinolin-2-one	$C_{19}H_{14}INO$	399	0.26
21.	2-Dimethylsilyloxytetradecane	$C_{16}H_{36}OSi$	272	8.83

22.	Dimethyl(1-cyclopentyl ethoxy)silane	$C_9H_{20}OSi$	172	8.15
23.	Ethyl propargyl sulfone	$C_5H_8O_2S$	132	7.51
24.	2-Dimethylsilyloxytridecane	$C_{15}H_{34}OSi$	258	6.93
25.	Ethoxy(dimethyl)isopropylsilane	$C_7H_{18}OSi$	146	5.59
26.	(E)-1-Butenyl ethyl sulfone	$C_6H_{12}O_2S$	148	4.72
27.	Glucuronamide	$C_6H_{11}NO_6$	193	3.61
28.	Octanoic acid, 3-hydroxy-, methyl ester	$C_9H_{18}O_3$	174	3.47
29.	Cinnamic acid, (E)-, TMS derivative	$C_{12}H_{16}O_2Si$	220	3.47
30.	2-Butanethiol, 2-methyl Or tert-Amyl mercaptan	$C_5H_{12}S$	104	3.34
31.	benzamide, N-(4,5-dihydro-5-oxo-1-phenyl-1H-pyrazol-3-yl)	$C_{16}H_{13}N_3O_2$	279	8.66
32.	3H-pyrazol-3-one, 4-ethyl-2,4-dihydro-2-phenyl-5-(phenylamino)-	$C_{17}H_{17}N_3O$	279	3.94
33.	Dibenz-a,b-acridine	$C_{21}H_{13}N$	279	3.94
34.	Pyridine-3-carbonitrile, 2-oxo-4-phenyl-6-(thiophen-2-yl)-1,2-dihydro	$C_{16}H_{10}N_2OS$	278	3.20
35.	Fumaric acid, pentafluorobenzyl hept-2-yl ester	$C_{18}H_{19}F_5O_4$	394	2.83
36.	3,6-Diacetyl-9-ethylcarbazole	$C_{18}H_{17}NO_2$	279	2.72
37.	5,8-etheno-1H-cyclobuta[d][1,2,4]triazolo[1,2-a]pyridazine-1,3(2H)-dione, 5,5a,7a,8-tetrahydro-2-phenyl	$C_{16}H_{13}N_3O_2$	279	2.72
38.	Chromium,cyclopentadienyl-(hexamethylbenzene)	$C_{17}H_{23}Cr$	279	2.40
39.	4'-Hydroxy-7-methylcinchophenic aci	$C_{17}H_{13}NO_3$	279	2.12
40.	1,2,3,5-Benzenetetracarboxylic acid, tetramethyl ester	$C_{14}H_{14}O_8$	310	2.04
41.	11-Nitrodibenzo(a,c)phenazine	$C_{20}H_{11}N_3O_2$	325	12.3
42.	1H-Pyrazolo[3,4-b]pyridine-5-carboxylic acid, 1,3-dimethyl-4-(2-phenylhydrazino)-, ethyl ester	$C_{17}H_{19}N_5O_2$	325	9.69
43.	Propionic acid, 3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-3-(4-methoxyphenyl)	$C_{18}H_{15}NO_5$	325	
44.	benzamide, N-(4,5-dihydro-5-oxo-1-phenyl-1H-pyrazol-3-yl)-	$C_{16}H_{13}N_3O_2$	279	5.22
45.	Sebacic acid, decyl 2-fluorophenyl ester	$C_{26}H_{41}FO_4$	436	4.82
46.	Fumaric acid, pentafluorobenzyl hept-2-yl ester	$C_{18}H_{19}F_5O_4$	394	3.49
47.	3H-pyrazol-3-one, 4-ethyl-2,4-dihydro-2-phenyl-5-(phenylamino)	$C_{17}H_{17}N_3O$	279	2.33
48.	Pyridine-3-carbonitrile, 2-oxo-4-phenyl-6-(thiophen-2-yl)-1,2-dihydro	$C_{16}H_{10}N_2OS$	278	1.98

Figure 3. Gas-Chromatography of Aqueous extract of *Benincasa hispida*

CONCLUSION

Benincasa hispida aqueous extracts investigation on Liquid chromatography- mass spectroscopy and gas chromatography predicted new possible bioactive molecules which may have therapeutic benefits, offer encouraging approaches to treating these changes in the central nervous system and call for more mechanistic research.

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Conflict of Interest
Nil

Funding
Nil

Ethics Statement
Not applicable

Abbreviations
BHA: *Benincasa hispida* aqueous; LC-MS- Liquid chromatography and mass spectroscopy; GC-MS: Gas chromatography-mass spectroscopy

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