PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS PRESENT IN ETHANOLIC EXTRACTS OF FLOWERS OF *CLITORIA TERNATEA* LINN.

Shammah C J*, Srikanth M, Snega M, Barkavi G K, Santhosh T, Praveen Kumar R, Mahalakshmi G(2)

1. Department of Pharmaceutical Chemistry, SNS College of Pharmacy and Health Sciences, Coimbatore.

2. Department of Pharmaceutical Chemistry, Arulmigu Kalasalingam College of Pharmacy, Srivilliputur.

*Correspondence: shammahcj@gmail.com

KEYWORDS

GC-MS analysis, *Clitoria ternatea*, Bioactive compounds, Ethanol extract, Anti-cancer, Anti-inflammatory.

ABSTRACT

Clitoria ternatea Linn, commonly known as butterfly pea, is a medicinal plant widely recognized for its therapeutic properties. This study aimed to investigate the phytochemical constituents and bioactive compounds present in the ethanolic extract of *Clitoria ternatea* flowers using phytochemical screening and Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds which are known for their diverse pharmacological activities. GC-MS analysis identified several bioactive compounds, including Lidocaine, Campesterol, Squalene, Phytol, Eicosanic acid which contribute to the plant's anti-cancer, antioxidant, anti-inflammatory, antimicrobial, and neuroprotective properties. The findings suggest that the ethanolic extract of *Clitoria ternatea* flowers is a rich source of bioactive compounds with potential therapeutic applications, indicating further investigation into its pharmacological and medicinal uses.

INTRODUCTION:

The oldest type of medicines is made from medicinal plants, which have been used for thousands of years in traditional medicine in many nations worldwide. Over the millennia, human groups passed down empirical evidence of their positive impacts. Medicinal plants have been found in nature for a very long time. These therapeutic plants are a gift from God that may treat countless illnesses in people and other living things. In every medical system and other traditional systems around the world, they have been the primary source of pharmaceuticals. Any plant that has compounds in one or more of its organs that have therapeutic value or that serve as building blocks for the production of beneficial medications is considered medicinal plant [1].

An illness known as cancer occurs when some body cells proliferate out of control and spread to other bodily organs. Most definitions of cancer broadly conform to the current NCI definition: “Cancer is a disease in which some of the body’s cells grow uncontrollably and spread to other parts of the body” [2]. One of the diseases that kill more people globally these days is cancer. A projected 10.3 million people died from cancer worldwide in 2020, while 19.3 million new cases were reported. The anatomy is subjected to several external factors on a daily basis, including pollution, tobacco smoke, and ultraviolet (UV) rays [5]. These factors cause reactive species, especially oxidants and free radicals, to accumulate, which can lead to the development

of various diseases, including cancer. Natural antioxidants with anti-inflammatory and antioxidant properties, such as vitamins, polyphenols, and bioactive chemicals produced from plants, are employed as preventive and therapeutic medications against harmful molecules that harm the body.

The bright blue blossom of *Clitoria ternatea*, also known as the butterfly pea, belongs to the Fabaceae family and is used as a decorative plant, traditional medicine, and a natural food coloring. An abundance of blue-colored anthocyanins is seen in blue pea flowers. Ternatins are polyacylated anthocyanins found in blue pea flowers. The anthocyanins found in blue peas have good thermal and storage durability. Blue pea flower anthocyanins are a viable substitute for genipin and spirulina. Many nations around the world, including Thailand, Malaysia, Kenya, Australia, the United States, Sri Lanka, Brazil, Cuba, Sudan, and others, are home to blue pea plants. In semi-arid tropical Australia as well as humid and sub-humid regions of Asia, America, and Africa, the genus is now scarce [10]. The phytochemical investigation of Clitoria ternatea flowers for the presence of alkaloids, tannins, glycosides, resins, steroids, saponins, flavonoids, and phenols is the focus of this study [12]. The medicinal properties are validated and reported to have antioxidant, antidiabetic, and hepatoprotective properties. The water extract of flowers has shown antiproliferative properties inhibiting cancer cell lines [11].

Fig.1:*Clitoria Ternatea*

MATERIALS AND METHODS:

Collection and Authentication of Plant Materials:

Fresh, healthy flowers of *Clitoria ternatea* used for the investigation were collected from local region, Coimbatore, Tamil Nadu, India. The plant were authenticated from the Botanical Survey of India at the Agricultural University in Coimbatore by Dr. M.U. Sharief, Scientist ‘F’ and Head of office at TNAU.

Preparation of Flower Extracts:

Freshly collected *Clitoria ternatea* flowers were washed with running tap water. Flowers were dried under shade for two weeks. The properly dried flowers were powdered in an electric blender. To prevent the effects of humidity, the coarsely ground components were stored in sealed containers and kept at room temperature until they were needed. About 100 g of dry powdered samples were extracted with 500ml of ethanol (99.9%w/v) by Cold Maceration for 3 days. The extracts were placed on the evaporator to evaporate the ethanol after being individually filtered

through Whatman no. 41 filter paper. After evaporate extract were obtained. The extract were kept in airtight container and stored at room temperature [4].

Preliminary Phytochemical Analysis:

Using conventional techniques, the ethanolic extracts were utilized to qualitatively identify a variety of secondary metabolites.

Gas Chromatography-Mass Spectroscopy (GC-MS):

GC-MS, or gas chromatography-mass spectrometry, is a crucial tool for analysing unknown bioactive plant components. GC-MS analysis was used to determine the chemical composition of ethanolic extracts of *Clitoria ternatea* flowers. GC-MS analysis of these ethanolic extract were carried out using the equipment CH-GCMSMS02, 8890 GC System, 7000 GC/TQ gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing following condition: Equipped with a column Elite- HP5MS, fused silica capillary column (30 m X 250 µ X 0.25 µ), helium gas (99.999) was used as carrier gas at the injection volume was 2µl, and the flow rate was 1 ml/min. The injector temperature is set at 280 °C, and aux temperature is 280 °C. Scan range is 30-900 m/z, Total running time was 38 mins. The average peak areas of each component were compared to determine their relative proportion.

RESULTS:

Preliminary Phytochemical Analysis:

The distribution of different phytochemical constituents in ethanolic extracts of *Clitoria ternatea* were evaluated qualitatively and shown in Table 1, it was clear that a lot of active biochemical compounds like alkaloids, carbohydrates, flavonoids, resins, and tannins were present in the ethanol extracts of *Clitoria ternatea* flowers.

TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACTS OF FLOWERS OF *CLITORIA TERNATEA*

S.NO	Phytochemical constitutions	Results (Flowers)
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Tannins	+
4.	Resins	+
5.	Saponins	-
6.	Flavonoids	+
7.	Phenols	-
8.	Steroids	-

Gas Chromatography-Mass Spectroscopy (GCMS) Analysis:

Different phytochemical/bioactive compounds of the ethanolic extracts of flowers of *Clitoria ternatea* were analysed by using GC-MS. The chromatograms of the extracts were shown in Fig:2 and summarized in Tables 2.

GC-MS chromatogram of *Clitoria ternatea* flower extract showed 17 major peaks which indicated the presence of 17 different bioactive compounds Fig:2**.** The results revealed that the percentage of major bioactive compounds viz., Cyclobutane, 1R,3E-bis(4-methoxy-2-oxo-2H-pyran-6-yl)-2Z,4E-bis(4-ethylphenyl) (98.53%), D-Alanine, N-(2,5-ditrifluoromethylbenzoyl)-, pentyl ester (64.49%), Hexadecenoic acid, 2-hydroxy-1(hydroxymethyl)ethyl ester (17.27%), Octadecanoic acid, 2,3-dihydroxypropyl ester (14.38%), Lidocaine (6.89%), delta. -Tocopherol, O-methyl- (11.06%), Campesterol (23.05%), Acetic acid (2.09%), Eicosanoic acid (8.58%), Phytol (6.03%), 5-Hydroxymethylfurfural (19.29%), Diethyl Phthalate (5.47%), Alanine, N-methyl-n-propoxycarbonyl-, dodecyl ester (3.56%), Benzofuran, 2,3-dihydro (5.49%), Squalene (3.38%), delta. -Tocopherol (2.97%) and Phenol, 5-ethenyl-2-methoxy (5.12%) were found as the major compounds in the ethanolic extract of flower of *Clitoria ternatea* Table 2.

TABLE 2: ACTIVITY OF BIOACTIVE COMPOUND IDENTIFIED IN ETHANOLIC EXTRACT OF *CLITORIA TERNATEA* FLOWERS

S.no	Name of the compounds	Retention time	Peak area %	Molecular formula	Reported biological activity
1.	Cyclobutane, 1R,3E-bis(4-methoxy-2-oxo-2H-pyran-6-yl)-2Z,4E-bis(4-ethylphenyl)	25.4926	98.53%	$C_{32}H_{32}O_6$	Anti-cancer,anti-viral, neuroprotective, anti-oxidant
2.	D-Alanine, N-(2,5-ditrifluoromethylbenzoyl)-, pentyl ester	27.7431	64.49%	$C_{17}H_{19}F_6NO_3$	Anti-bacterial, anti-fungal, immunomodulatory
3.	Octadecanoic acid, 2,3-dihydroxypropyl ester	32.4455	14.38%	$C_{21}H_{42}O_4$	Anti-microbial, anti-inflammatory, emollient
4.	Lidocaine	24.6695	6.89%	$C_{14}H_{22}N_2O$	Local anaesthetic effect, anti-arrhythmic effect
5.	delta. -Tocopherol, O-methyl-	35.2786	11.06%	$C_{28}H_{48}O_2$	Anti-cancer, anti-oxidant, neuroprotective, anti-inflammatory
6.	Campesterol	37.5000	23.05%	$C_{28}H_{48}O$	Anti-cancer, anti-oxidant, lower cholesterol effect
7.	Acetic acid	4.1379	2.09%	$C_2H_4O_2$	Anti-microbial, anti-inflammatory
8.	Eicosanoic acid	29.5054	8.58%	$C_{20}H_{40}O_2$	Anti-microbial, anti-inflammatory, wound healing activity
9.	Phytol	26.8785	6.03%	$C_{20}H_{40}O$	Anti-microbial, anti-inflammatory, anti-cancer, anti-oxidant
10.	5-Hydroxymethylfurfural	14.3011	19.29%	$C_6H_6O_3$	Anti-inflammatory, anti-oxidant
11.	Diethyl Phthalate	20.8892	5.47%	$C_{12}H_{14}O_4$	Endocrine disruption, toxicity effect

12.	Alanine, N-methyl-n-propoxycarbonyl-,dodecyl ester	11.3606	3.56%	$C_{18}H_{35}NO_4$	Anti-microbial, anti-inflammatory, cytotoxicity
13.	Benzofuran, 2,3-dihydro-	12.7890	5.49%	C_8H_8O	Anti-cancer, neuroprotective, anti-oxidant, anti-inflammatory, analgesic effect
14.	Squalene	33.2222	3.38%	$C_{30}H_{50}$	Anti-cancer, neuroprotective, anti-oxidant, anti-inflammatory, CVS, skin wound healing effect
15.	delta. -Tocopherol	34.2319	2.97%	$C_{28}H_{48}O_2$	Anti-cancer, neuroprotective, anti-oxidant, anti-inflammatory, CVS, immune system support
16.	Phenol, 5-ethenyl-2-methoxy	15.1093	5.12%	$C_{10}H_{12}O_2$	Anti-microbial, anti-inflammatory, anti-cancer, analgesic
17.	Hexadecenoic acid, 2-hydroxy-1(hydroxymethyl)ethyl ester	30.7974	17.27%	$C_{19}H_{38}O_4$	Emollient, anti-microbial, skin penetration enhancement

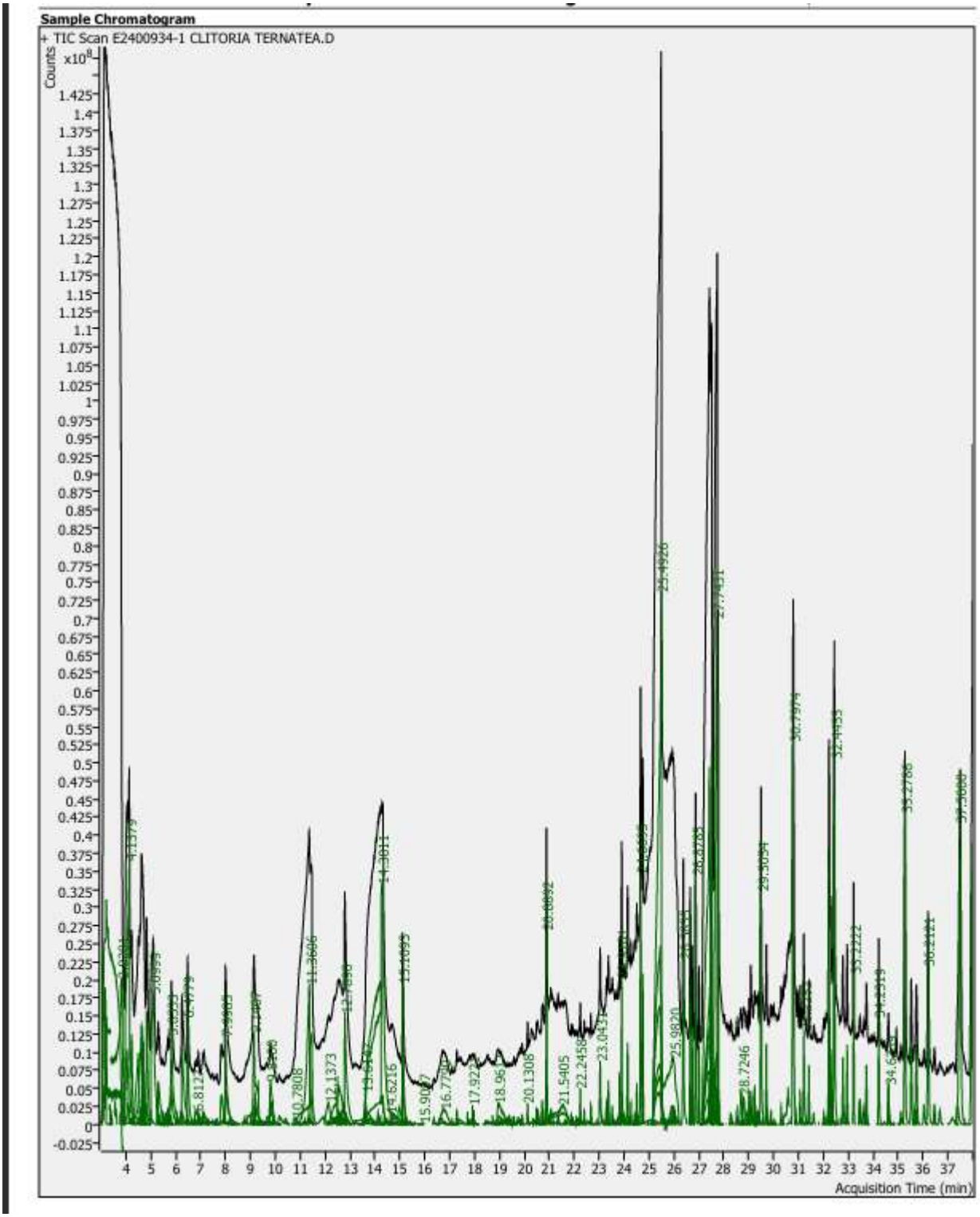
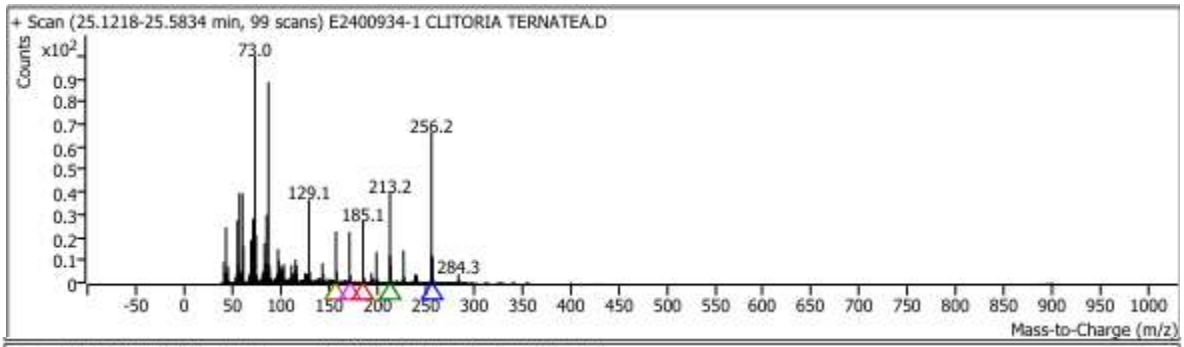


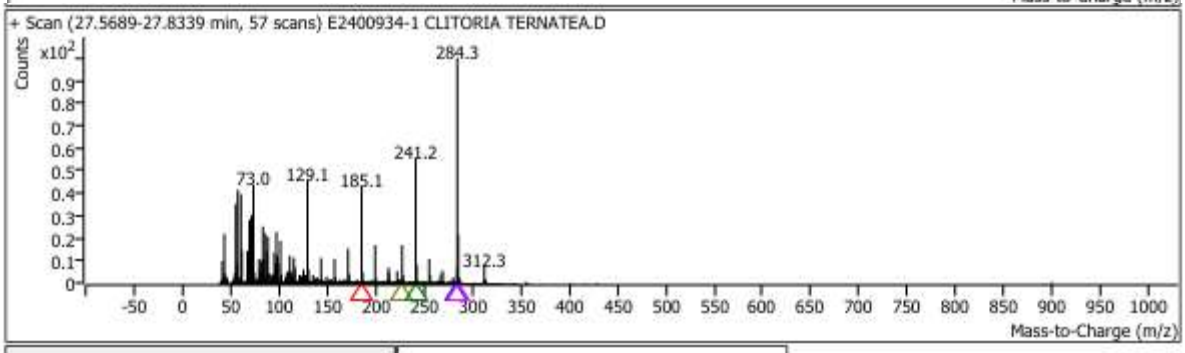
FIG.2: GC-MS CHROMOTOGRAM OF ETHANOLIC EXTRACT OF *CLITORIA TERNATEA* FLOWER

E

A


+ Scan (25.1218-25.5834 min, 99 scans) E2400934-1 CLITORIA TERNATEA.D
Counts
x10^2
73.0
256.2
129.1
213.2
185.1
284.3
Mass-to-Charge (m/z)

B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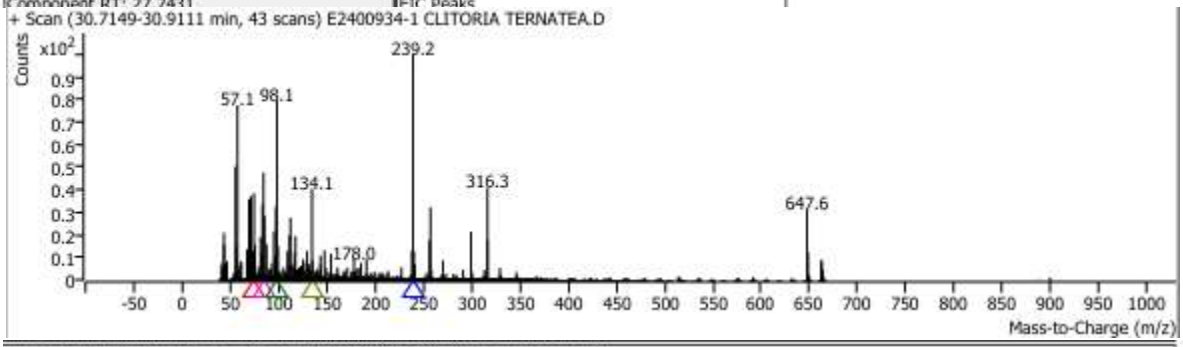
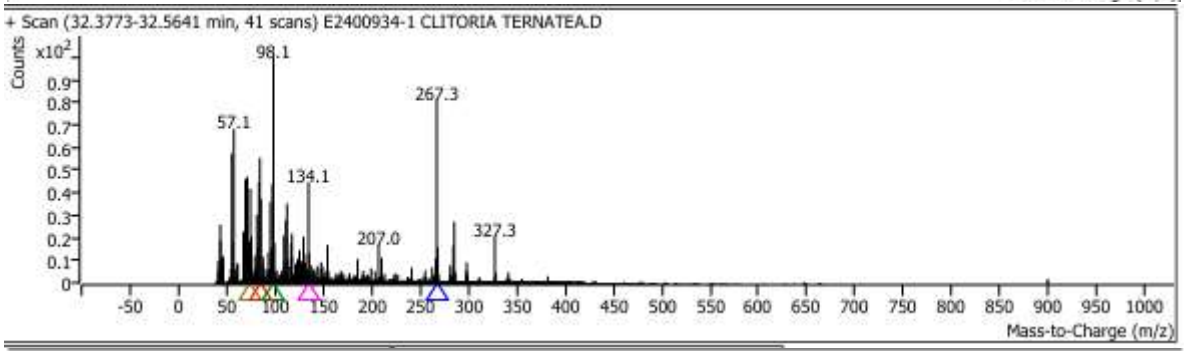
x10^2
284.3
241.2
73.0
129.1
185.1
312.3
Mass-to-Charge (m/z)

C


+ Scan (30.7149-30.9111 min, 43 scans) E2400934-1 CLITORIA TERNATEA.D
Counts
x10^2
239.2
57.1
98.1
134.1
316.3
647.6
178.0
Mass-to-Charge (m/z)

D


+ Scan (32.3773-32.5641 min, 41 scans) E2400934-1 CLITORIA TERNATEA.D
Counts
x10^2
98.1
267.3
57.1
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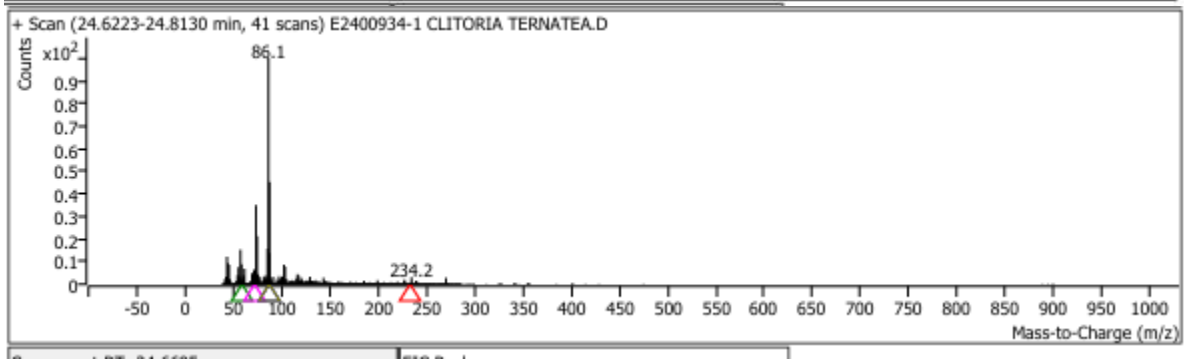
327.3
Mass-to-Charge (m/z)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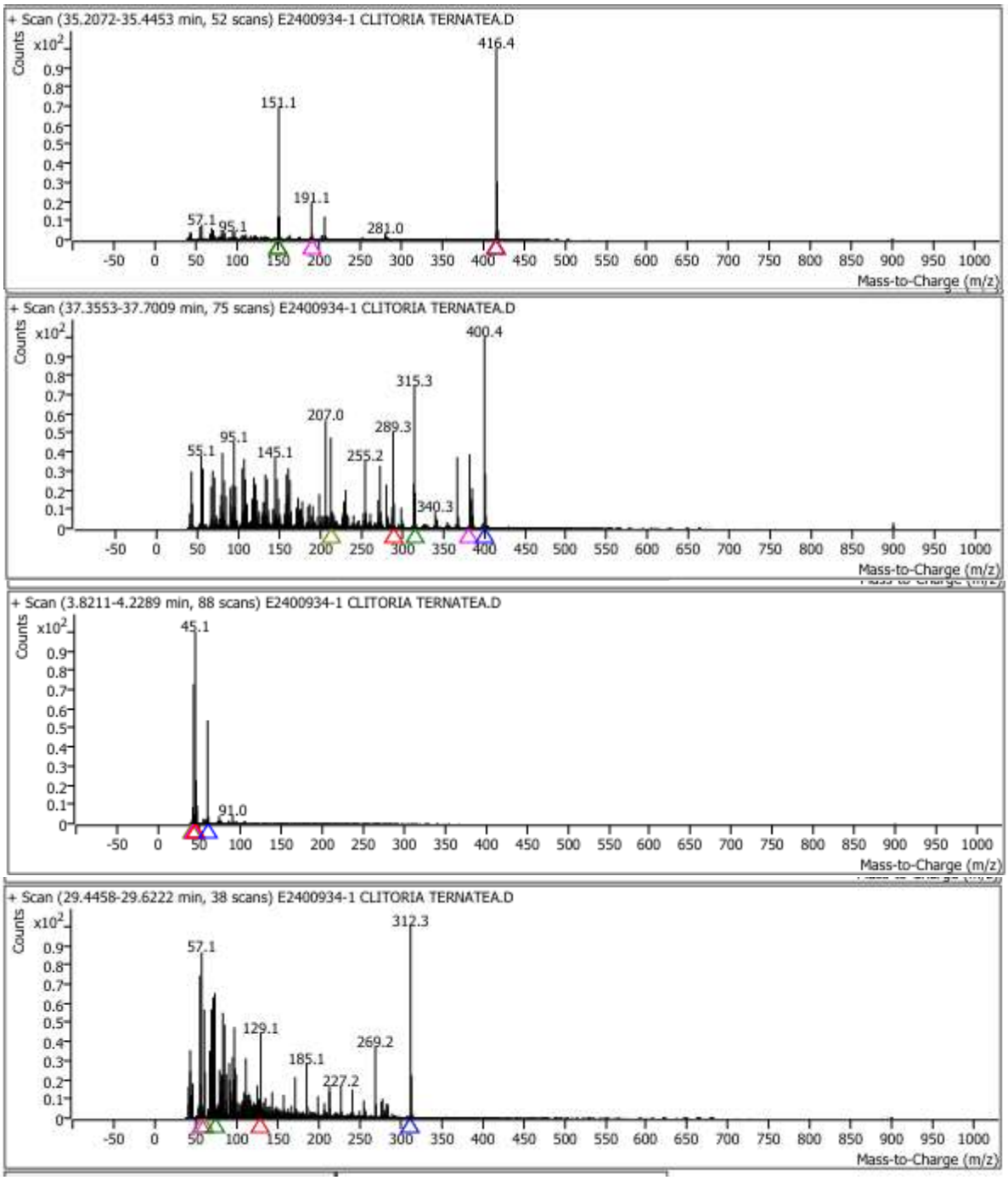
Counts
x10^2
86.1
234.2
Mass-to-Charge (m/z)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Counts
x10^2
416.4
151.1
191.1
57.1
95.1
281.0
Mass-to-Charge (m/z)
+ Scan (37.3553-37.7009 min, 75 scans) E2400934-1 CLITORIA TERNATEA.D
400.4
315.3
207.0
289.3
95.1
55.1
145.1
255.2
340.3
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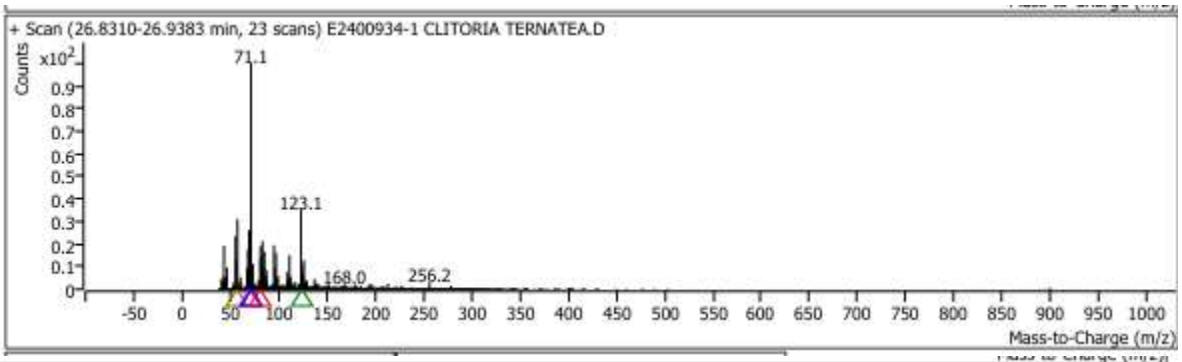
45.1
91.0
+ Scan (29.4458-29.6222 min, 38 scans) E2400934-1 CLITORIA TERNATEA.D
312.3
57.1
129.1
269.2
185.1
227.2

F

G

H

J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Counts
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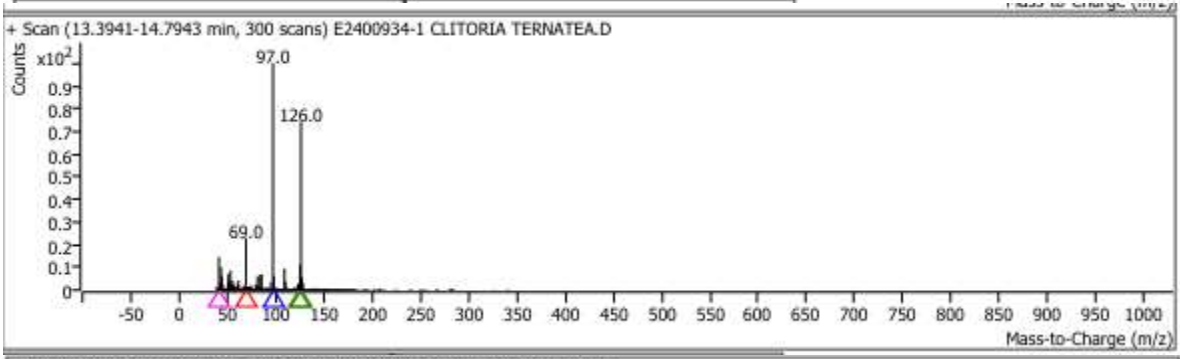
71.1
123.1
168.0
256.2
Mass-to-Charge (m/z)

M

K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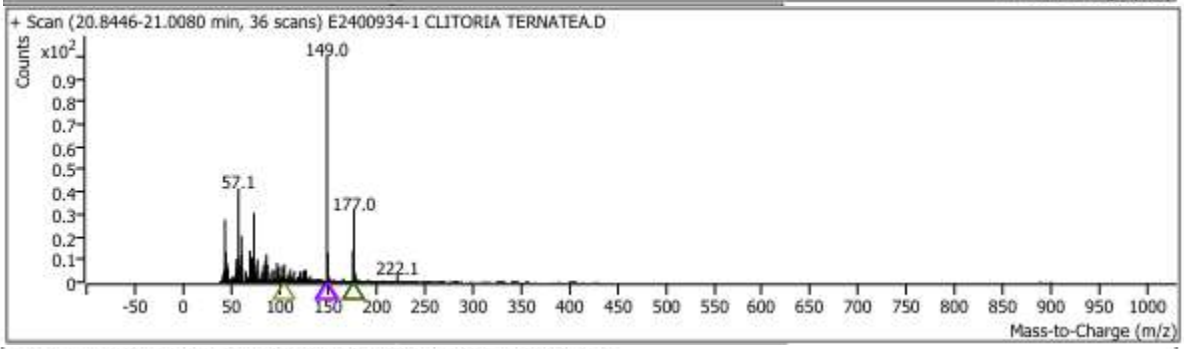
Counts
x10^2
97.0
126.0
69.0
Mass-to-Charge (m/z)

L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Counts
x10^2
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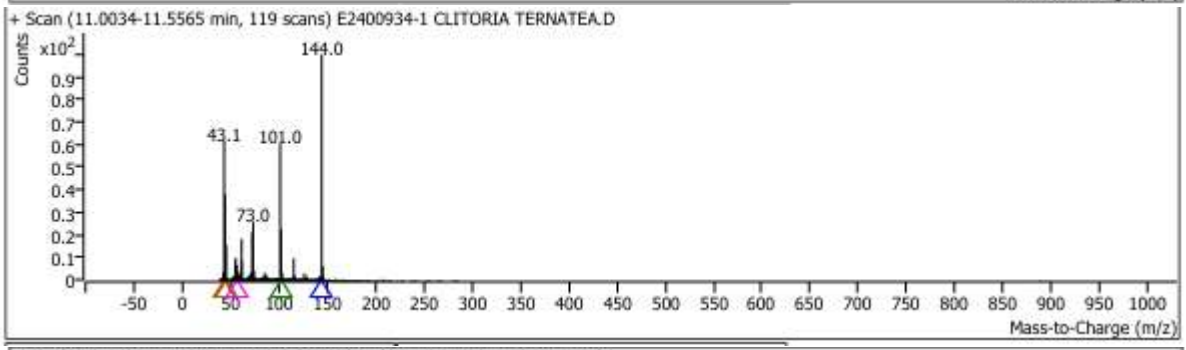
57.1
177.0
222.1
Mass-to-Charge (m/z)

I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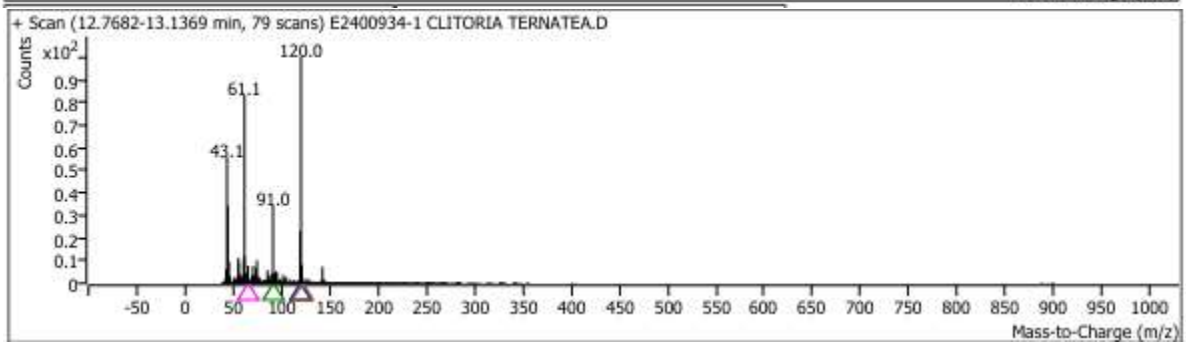
144.0
43.1
101.0
73.0
Mass-to-Charge (m/z)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Counts
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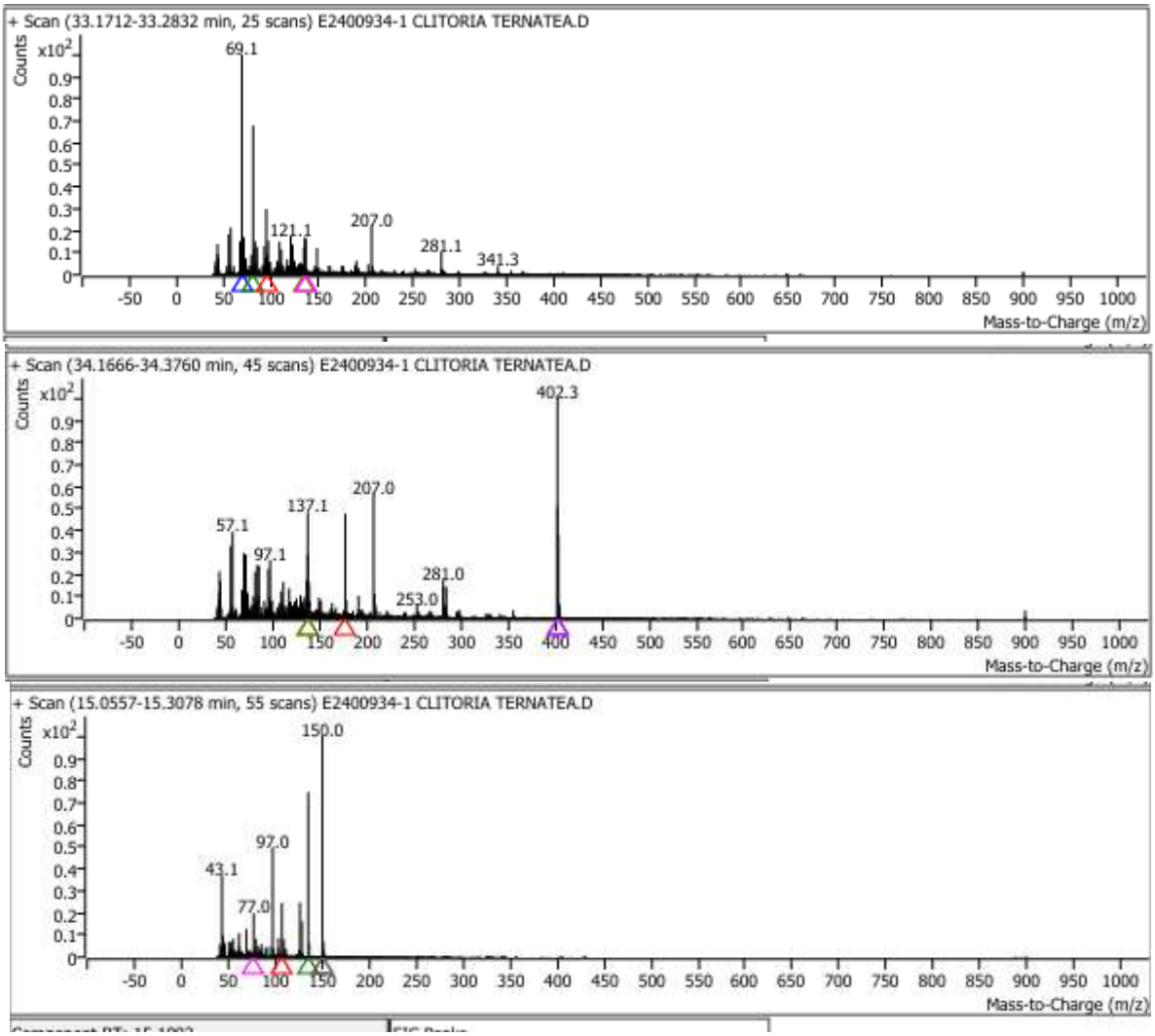
120.0
61.1
43.1
91.0
Mass-to-Charge (m/z)

O

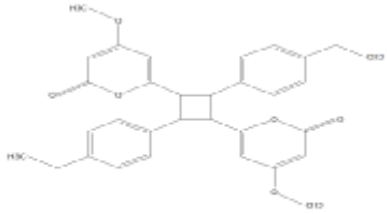


P

Q

FIG.3: GC-MS MASS SPECTRA OF A FEW COMPOUNDS FROM ETHANOLIC EXTRACT OF *CLITORIA TERNATEA* FLOWER. (a) Mass spectra of Cyclobutane, 1R,3E-bis(4-methoxy-2-oxo-2H-pyran-6-yl)-2Z,4E-bis(4-ethylphenyl) (C32H32O6), (b) D-Alanine, N-(2,5-ditrifluoromethylbenzoyl)-pentyl ester (C17H19F6NO3) , (c) Hexadecenoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (C19H38O4), (d) Octadecanoic acid, 2,3-dihydroxypropyl ester (C21H42O4), (e) Lidocaine (C14H22N2O) ,(f) delta.-Tocopherol, O-methyl- (C28H48O2) , (g) Campesterol (C28H48O) ,(h) Acetic acid (C2H4O2) , (i) Eicosanoic acid(C20H40O2), (j) Phytol (C20H40O),(k) 5-Hydroxymethylfurfural (C6H6O3), (l) Diethyl Phthalate (C12H14O4), (m) Alanine, N-methyl-n-propoxycarbonyl-,dodecyl ester (C18H35NO4), (n) Benzofuran, 2,3-dihydro (C8H8O), (o)Squalene (C30H50), (p) delta.-Tocopherol (C28H48O2), (q) Phenol, 5-ethenyl-2-methoxy- (C10H12O2).

A



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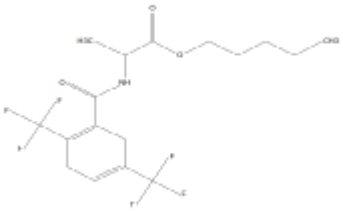

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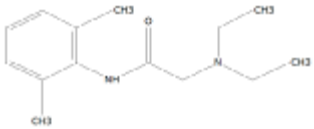
D

E



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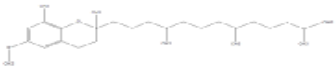

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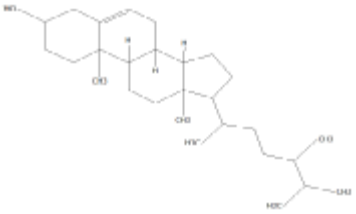

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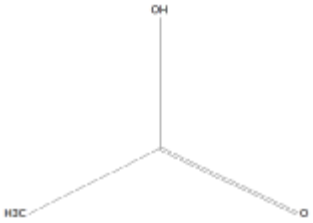

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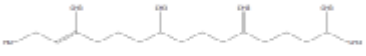

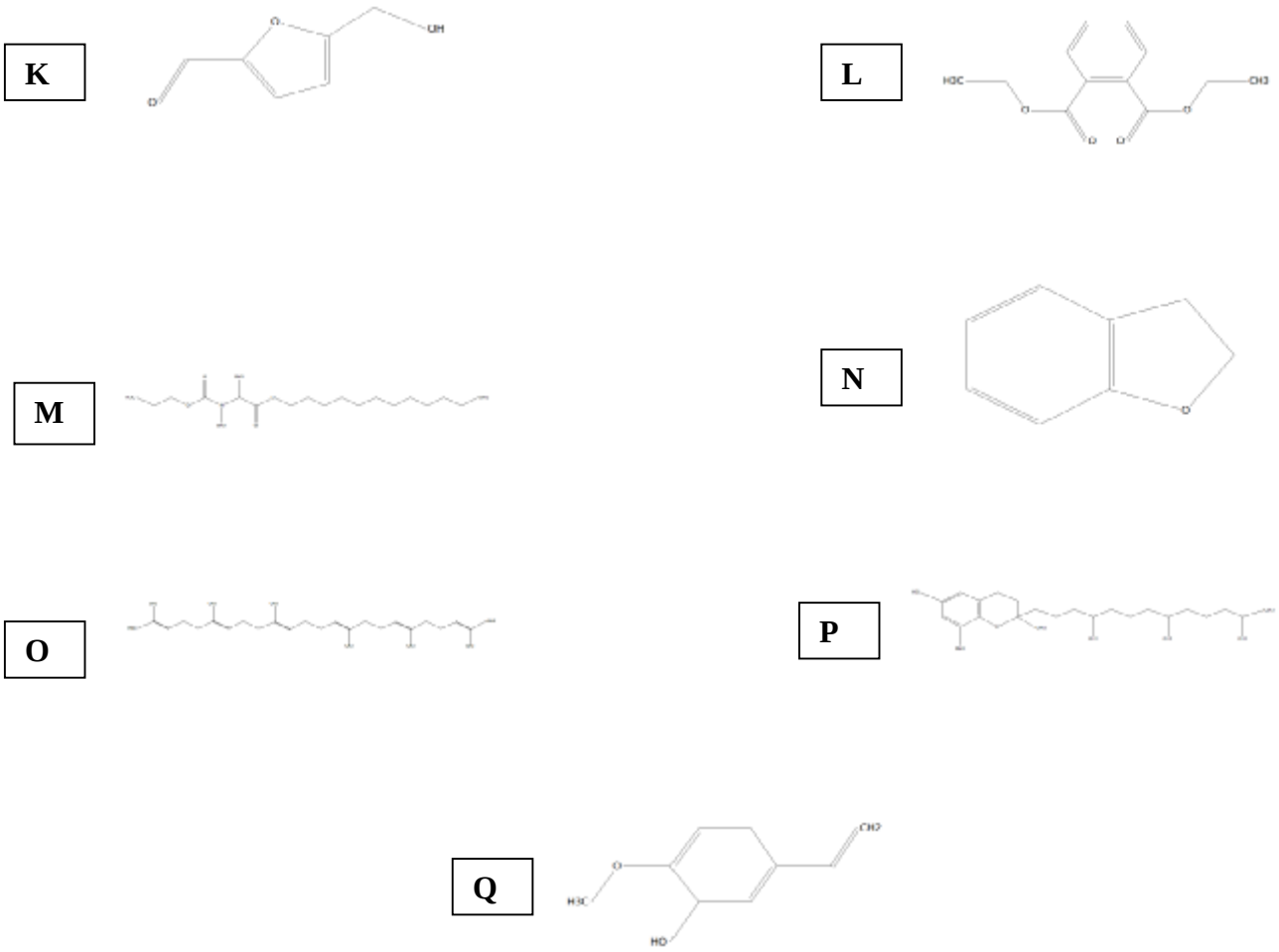

FIG.4: GC-MS CHEMICAL STRUCTURE OF A FEW COMPOUNDS FROM ETHANOLIC EXTRACT OF *CLITORIA TERNATEA* FLOWER.(a) Mass spectra of Cyclobutane,1R,3E-bis(4-methoxy-2-oxo-2H-pyran-6-yl)-2Z,4E-bis(4-ethylphenyl) (C32H32O6), (b) D-Alanine, N-(2,5-ditrifluoromethylbenzoyl)- pentyl ester (C17H19F6NO3) , (c) Hexadecenoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (C19H38O4), (d) Octadecanoic acid, 2,3-dihydroxypropyl ester (C21H42O4), (e) Lidocaine (C14H22N2O) ,(f) delta.-Tocopherol, O-methyl- (C28H48O2) , (g) Campesterol (C28H48O) ,(h) Acetic acid (C2H4O2) , (i) Eicosanoic acid(C20H40O2), (j) Phytol (C20H40O), (k) 5-Hydroxymethylfurfural (C6H6O3), (l) Diethyl Phthalate (C12H14O4), (m) Alanine, N-methyl-n-propoxycarbonyl-,dodecyl ester (C18H35NO4), (n) Benzofuran, 2,3-dihydro (C8H8O), (o) Squalene (C30H50), (p) delta.-Tocopherol (C28H48O2), (q) Phenol, 5-ethenyl-2-methoxy- (C10H12O2).

CONCLUSION:

Based on the results obtained in the present investigation, it may be concluded that the biological activities of the identified phytocomponents used for anti-cancer, anti-microbial, anti-inflammatory, anti-bacterial, anti-bacterial, anti-fungal, immunomodulatory, neuroprotective, skin wound healing effect, analgesic and anti-oxidant activities. Therefore, *Clitoria ternatea* is recommended as a source of phytopharmaceutical value.

ACKNOWLEDGEMENT:

All authors contributed equally.

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