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Identification and characterization of phytoconstituents of ethanolic root extract of *Clitoria ternatea* L. utilizing HR-LCMS analysis

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ABSTRACT

Medicinal plants act as a vital source in improving health and overcoming the side effects of modern-day medicine. Many evidence-based reports are present in the literature about the benefits of medicinal plants. *Clitoria ternatea* L. belongs to the family Fabaceae and is known to be one of the important Ayurvedic medicinal plant whose uses are specified mainly for the modification of nervous system activities. '*Medhyarasayana*' is one of the Ayurvedic formulations which is used to promote the intellectual capacity, revive the body and nervous tissue, *Clitoria ternatea* serves as a major constituent of '*Medhyarasayana*.' Identification and characterization of active metabolites of *C. ternatea* will help to isolate the important phytoconstituents responsible for the central nervous system effects, isolated components can be utilized in future for the formulation of new medicine for various neurodegenerative disorders. In the present study, the phytochemical evaluation of the ethanolic root extract of *C. ternatea* (EECT) was performed using the HR-LCMS technique. Preliminary qualitative phytoconstituents analysis showed the presence of tannins, alkaloids, saponins, steroids, carbohydrate, protein, flavonoids and triterpenoids in the ethanolic root extract. Almost 42 compounds were identified when the EECT subjected to HR-LCMS analysis.

Introduction

Medicinal plants are considered as amusing resources of ingredients that can be used in drug discovery and development as they are a very vital source to improve health and to overcome adverse effects of allopathic medicine. Many evidence-based reports are present in the literature about the benefits of medicinal plants and their biochemical and molecular effects (1). Worldwide a huge percentage of the population utilize medicinal plants and herbs for their health purpose. Therefore, scientific scrutiny of their phytoconstituents, therapeutic potential, biological properties and safety will be valuable in making wise decisions about their use. (2, 3) Ayurveda is one of the most popular Indian traditional health care systems which labels several herbal preparations which are well-known to uphold health and endurance. 'Rasayana' is the common term representing one of such herbal preparations which is ultimate for the progress of tissue functions in addition to their role as micronutrients (4). '*Medhyarasayana*' is an Ayurvedic preparation made from the selected plant extracts to revitalize the brain by acting on the nervous system (5).

Clitoria ternatea L. belongs to the family Fabaceae, is a perennial twining herb with terete steam. It possess two varieties- white-flower and blue flower varieties (6). The local name is 'Shankhpushpi' and this is one of the '*Medhyarasayana*' ingredients and is reported to promote intellectual capability, revive the body and nervous tissue and because of all these properties it has been widely used as a brain tonic (6). Scientific studies also reported other medicinal properties including antidepressant and anticonvulsant (7), anti-inflammatory, analgesic and antipyretic (8), local anesthetic (9), purgative (10) and anti-diabetic (11) activity. It is also used for the treatment of snakebite and scorpion sting in India (12). In the present study, the phytochemical evaluation of the ethanolic root extract of *C. ternatea* (EECT) was performed using the HR-LCMS technique.

Materials and Methods

Collection and Preparation of *Clitoria ternatea* root extract

Fresh roots of the white variety of wild *C. ternatea* were collected from Kerala, India. Authentic

identification was carried by taxonomist Prof.P.Jayaraman, Director, Plant Anatomy and Research Centre, West Tambaram, Chennai. India. A voucher specimen (SES.CLBM.NO. 1458) has been deposited at the Herbarium of Department of Pharmacognosy, C.L.Baid Metha College of Pharmacy, Chennai, India. The collected materials were shade dried at room temperature to remove moisture, then coarsely powdered by using an electric grinder. The powdered materials were stored in an air-tight container and used for further extraction.

Extraction procedure

Extraction of roots was carried out using ethanol by continuous hot extraction method using Soxhlet apparatus. The obtained extract was concentrated by gentle heating followed by using rotarat vacuum evaporator. The concentrated extract was then weighed, calculated the percentage yield and stored. The extract was subjected to various preliminary phytochemical tests and HR-LCMS analysis (13). The qualitative phytochemical tests were performed for alkaloids, flavonoids, glycosides, phenolics, terpenoids, saponins, carbohydrate, protein, amino acids and triterpenoids (14, 15).

High-Resolution Liquid Chromatography and Mass Spectrometry (HR-LCMS) analysis

The HR-LCMS analysis of the extract was carried out in Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Mumbai. Methanol was used as the solvent for the preparation of extract and this process was done before subjecting the extract for analysis. Agilent high-resolution liquid chromatography and mass spectrometry model- G6550A (0.01% mass resolution) was used to prepare the chemical fingerprints of the subjected extract. The acquisition method was set to be Mass- minimum range 50 dalton (M/Z) and maximum 1000 Dalton (M/Z). The scanning was done with a rate of each spectrum per second (16).

Hip sampler G4226A-model with ancillary speed 100 µl/min, ejection speed 100 µl/min, flush out factor 5 µl and 8 µl injection volume was used for HR-LCMS. (15) Acquisition time was 30 min with initial 2 min of the flow of solvent. The solvent composition used for HR-LCMS was 95: 5-100% water and 100% Acetonitrile. Column details –Hypersil GOLD C18 100 x 2.1mm-3MICRON.

Identification of components

Interpretation on mass spectrum HR-LCMS was carried out by comparing the spectrum of unknown components with known components spectrum. For comparison, we have utilized the SAIF -IIT Bombay database, where they have been stored more than 62000 patterns of the spectrum. The name, molecular weight and structure of the components of the trial materials were determined.

Results and Discussion

The percentage yield of (EECT) was found to be 10.4%w/w. Preliminary phytochemical evaluation of EECT showed the presence of tannins, alkaloids, saponins, steroids, carbohydrate, protein, flavonoids and triterpenoids (Table 1).

HR-LCMS analysis of EECT showed different major peaks indicating the presence of various phytochemical constituents. The characterization and identification of constituents were done by performing a comparison with the HRLC-MS spectrum of SAIF library compounds. The HR-LCMS study was performed for both positive and negative mode of ionization, the respective chromatogram is represented in Fig. 1 and Fig. 2. The fingerprint obtained was interpreted and mentioned (Table 2, 3). Positive ionization ESI of EECT showed 24 compounds and negative ionization ESI of EECT showed 18 compounds. The MS zoom spectrum of few important compounds identified by both positive and negative ionization ESI are also represented (Fig. 3, Fig. 4).

Neuropharmacologic effects of various crude root extract of *C. ternatea* were reported by different researchers. It was reported that the oral intubation of CT aqueous root extract had shown a significant increase in learning and memory of postnatal and young adult Wistar rats (17). In another study, there are reports the *in vitro* effects of 200 mg/ml of *C. ternatea* aqueous root extract on proliferation, differentiation and growth of anterior subventricular zone neural stem cells derived from prenatal and postnatal rat pups (18). Acetylcholine (ACh) and Acetylcholinesterase (AChE) activity modification in connection with memory and cognitive enhancement of laboratory rodents upon administration of various root extracts of *C. ternatea* was reported by various researchers (19, 20). Anti-depressant and anti-anxiety effects of different root extracts have been studied and reported by different scientists (21, 22).

Even though the preclinical trial on rodents with various crude root extract of *C. ternatea* reported promising results on nervous system, a detailed study on isolated compounds from the root was not done so far. The present study imparts light on various constituents in root ethanolic extract. As per the results of the present study, the identified compounds like Chelidonine, Gibberellin, Elephantopin, Deoxy sapponone B 7,3'-dimethoxy ether acetate, 3 hydroxy-3'4'-dimethoxy flavone, Tubernoic acid, Pectolinarin, Isotectorigenin 7-methyl ether, Mucronulatol, Biochanin A dimethyl ether and different amino acids may be responsible for the reported effects produced by the root. For the confirmation, a detailed fractionation and constituent's isolation research study have to be performed on its roots. A well-designed constituent isolation and preclinical studies with those isolated compounds will confirm the safety and efficacy of *C. ternatea* against different neurological disorders.

Conclusion

The present study investigated and specified the various active metabolites found in the ethanolic root extract of *Clitoria ternatea* by carrying out different qualitative phytochemical screening and HR-LCMS analysis. The results serve as a potential resource to explore the isolation, purification and pharmacological screening of

Table 1. Preliminary phytochemical screening of ethanolic root extract of *Clitoria ternatea* L.

Sl.no.	Test	Extract	Inference
I	**Test for carbohydrates**		
	Molisch's test	+	Presence of carbohydrates
	Benedicts test	+	Presence of carbohydrates
	Fehlings test	+	Presence of carbohydrates
II	**Test for tannins and phenolics**		
	Lead acetate test	+	Presence of phenolics and tannins
	Ferric chloride test	+	Presence of phenolics and tannins
III	**Test for steroids**		
	Salkowski's test	+	Presence of steroids
	Libermann Burchard test	+	Presence of steroids
IV	**Test for triterpenoids**		
	Isoprenoid test	+	Presence of triterpenoids
V	**Test for flavones and flavonoids**		
	Shinoda test	+	Presence of flavanoids
	Aqueous sodium hydroxide test	+	Presence of flavanoids
VI	**Test for alkaloids**		
	Mayer's test	+	Presence of alkaloids
	Hager's test	+	Presence of alkaloids
	Dragendroff's test	+	Presence of alkaloids
	Wagner's test	+	Presence of alkaloids
VII	**Test for Glycosides**		
	Liebermann's test	+	Presence of glycosides
	Borntrager's test	+	Presence of anthroquinone glycosides
VIII	**Test for Proteins**		
	Millon's test	+	Presence of proteins
	Biuret test	+	Presence of proteins
	Ninhydrin test	+	Presence of proteins
IX	**Test for Saponins**		
	Foam/Froth test	+	Presence of saponins

Sample Name	EECT	Position	P1-B2	Instrument Name	QTOF	User Name	
Inj Vol	5	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	EECT.d	ACQ Method	30mins_+ESI_10032014	Comment		Acquired Time	3/7/2020 12:39:50 PM
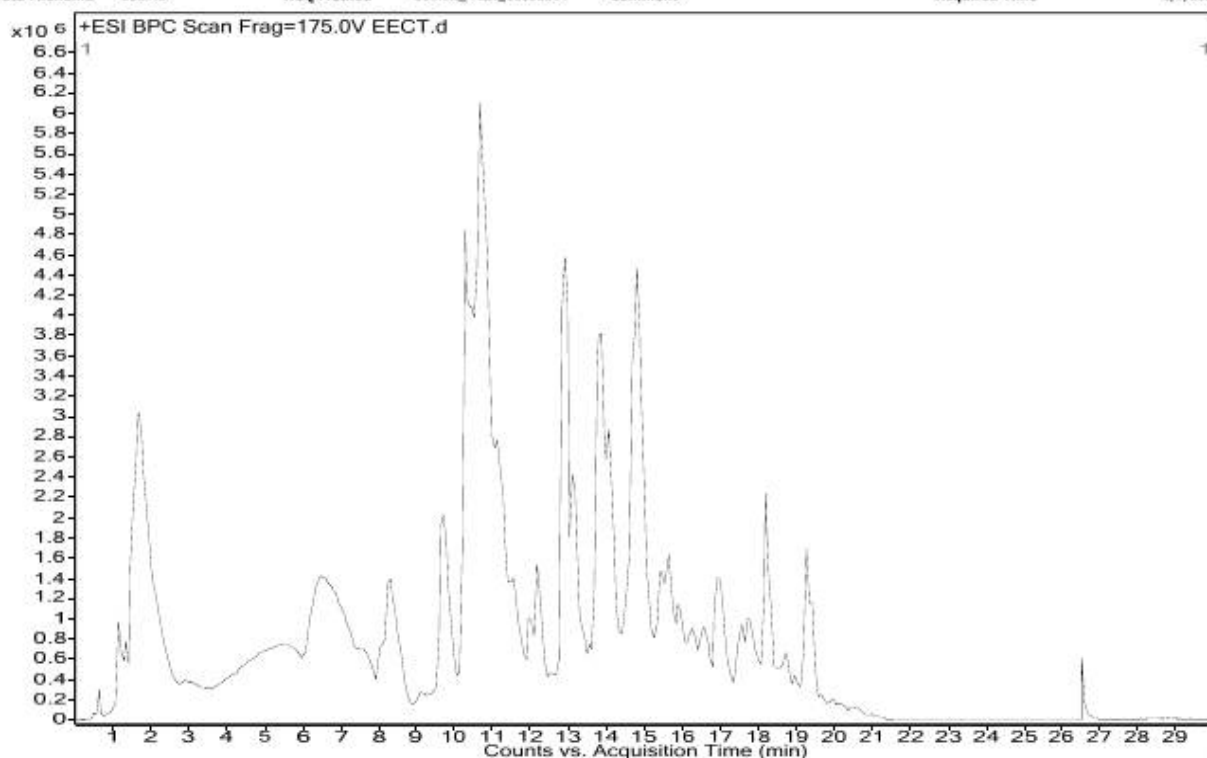
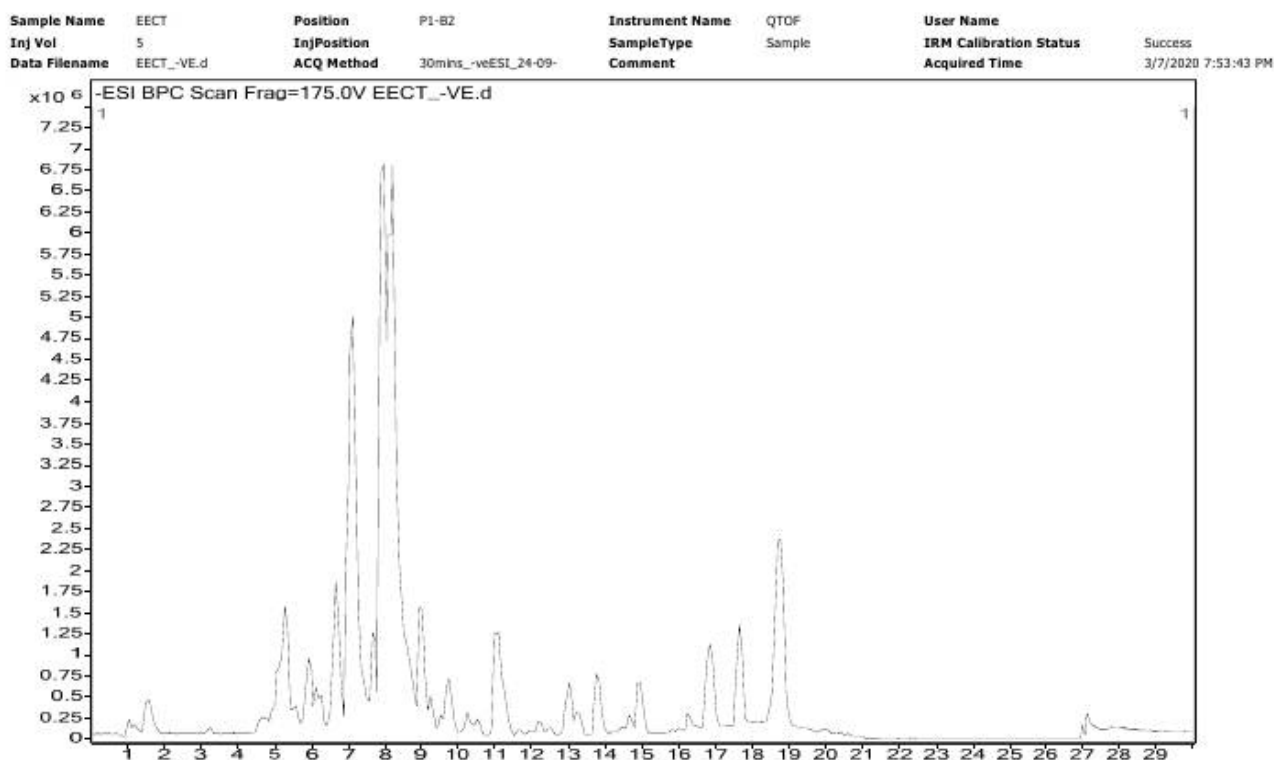**Fig. 1.** HR-LCMS chromatogram (Positive ESI) of ethanolic root extract *of Clitoria ternatea* L.

Fig. 2. HR-LCMS chromatogram (Negative ESI) of ethanolic root extract *of Clitoria ternatea* L.

Table 2. HR-LCMS analysis of ethanolic root extract *of Clitoria ternatea* L. at Positive ESI

Sl. No.	Compound	Retention time	Mass	Molecular formula	DB diff (ppm)	Hits (DB)
1	Chelidonine (+)	1.245	353.1258	C20 H19 N O5	1.49	63
2	Retusin dimethyl ether	1.586	312.0993	C18 H16 O5	1.46	27
3	Elephantopin	1.638	360.1199	C19 H20 O7	2.83	31
4	Sebacic acid	1.874	202.1222	C10 H18 O4	-8.33	2
5	Mycophenolic acid	1.986	320.1271	C17 H20 O6	-3.53	31
6	Deoxysappanone B 7,3'-dimethyl ether acetate	6.05	356.1247	C20 H20 O6	3.57	21
7	7-[2 trifluoromethyl-4-(2-hydroxyphenyl) -1,3-dioxan-cis-5-yl]-hept-5Z-enoic acid	6.439	374.1351	C18 H21 F3 O5	-2.75	21
8	Deoxysappanone B 7,3'-Dimethyl ether acetate	6.713	356.124	C20 H20 O6	3.43	21
9	3-hydroxy-3',4'-dimethoxyflavone	8.097	298.0831	C17 H14 O5	3.5	16
10	Isotectorigenin, 7-Methyl ether	8.231	328.094	C18 H16 O6	1.79	7
11	Tuberonic acid	9.675	226.1201	C12 H18 O4	1.69	20
12	Gibberellin A29	10.232	348.1583	C19 H24 O6	-3.03	29
13	Anisodamine	10.492	305.1619	C17 H23 NO4	2.59	27
14	8-(1-Hydroxyethyl)etodolac	10.621	303.1467	C17 H21 NO4	1.3	47
15	Triptonide	11.506	358.1407	C20 H22 O6	2.55	36
16	Naloxol	12.009	329.1622	C19 H23 NO4	1.5	49
17	Butorphanol	12.846	219.2221	C12 H29 NO2	-10.41	1
18	2-Isoprenyl-3-hydroxy-5-methyl-a-pyrone	13.091	194.0939	C11 H14 O3	2.06	13
19	Lys Ser Lys	14.17	361.224	C15 H31 N5O5	23.0	3
20	LTB4 ethanol amide	15.637	379.2733	C22 H37 NO4	-2.86	7
21	Cer(d18:0/16:0)	17.631	539.5262	C34 H69 NO3	2.87	1
22	Anandamide (20:2, n-6)	18.23	351.3129	C22 H41 NO2	2.45	1
23	(Z)-N-(2-hydroxyethyl)icos-11-Enamide	19.369	353.3286	C22 H43 NO2	2.33	1
24	Docosanamide	19.372	339.351	C22 H45 NO	-4.3	1

Table 3. HR-LCMS analysis of ethanolic root extract *of Clitoria ternatea* L. at Negative ESI

Sl. No.	Compound	Retention time	Mass	Molecular formula	DB diff (ppm)	Hits (DB)
1	Pectolinarin	4.894	622.187	C29H34 O15	3.6	2
2	Glycogen	5.282	666.216	C24H42 O21	8.78	3
3	5-Formiminotetrahydrofolic Acid	5.95	472.181	C20H24N8 O6	-0.06	14
4	Levan	6.54	504.16	C18 H32 O16	11.9	9
5	Maltotriose	6.54	504.163	C18 H32 O16	11.81	6
6	Tyr Gln Glu	6.622	438.1768	C19H26N4 O8	-3.98	16
7	Sappanone A 7-methyl Ether	6.97	298.0845	C17 H14 O5	-1.3	7
8	Isotectorigenin, 7-Methyl ether	6.982	328.095	C18 H16 O6	-2.6	2
9	6,4'-Dimethoxyflavon	7.05	282.0894	C17 H14 O4	-0.76	16
10	Mucronulatol((+/-))	7.151	302.116	C17 H18 O5	-2.14	12
11	Elephantopin	7.894	360.1233	C19 H20 O7	-6.66	33
12	Epiafzelechin trimethyl Ether	7.962	316.132	C18 H20 O5	-4.6	13
13	Neu5Acalpha2-6Galbeta1-4Glcbeta-Sp	7.96	702.233	C25H42N4O19	14.96	5
14	Biochanin A, dimethyl Ether	8.202	312.10	C18 H16 O5	-7.2	7
15	25-O-Deacetylrifabutin N-oxide	9.7	820.4247	C44H60N4O11	1.43	2
16	Telmisartan	10.997	514.2466	C33H30N4O2	-18.91	4
17	Cys Tyr Arg	14.815	440.185	C18H28N6O5S	-3.54	48
18	DL-8-hydroxy stearic acid	18.598	300.2679	C18 H36 O3	-4.69	53
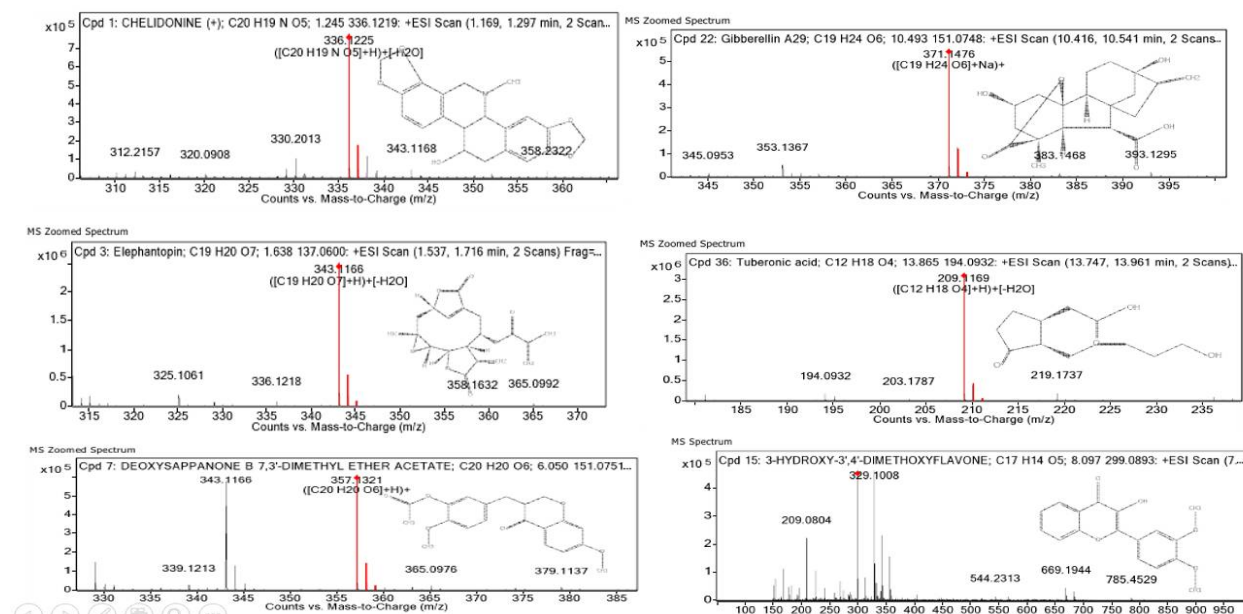
Fig. 3. HR-LCMS- MS Zoomed Spectrum of different compounds detected from ethanolic root extract *of Clitoria ternatea* L. at Positive ESI.
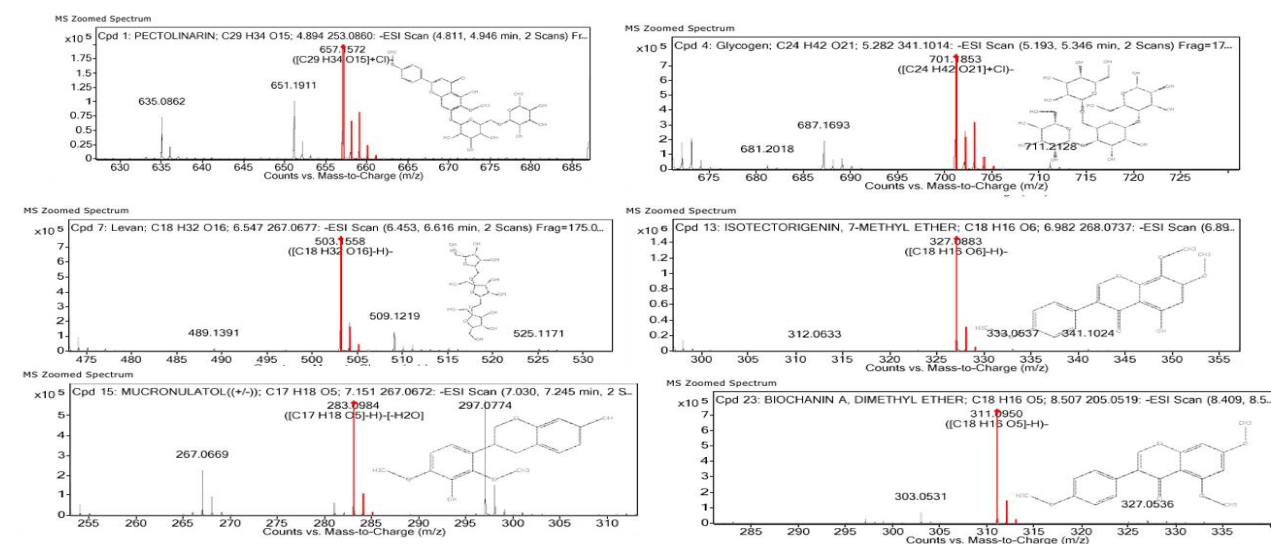
Fig. 4. HR-LCMS- MS Zoomed Spectrum of different compounds detected from ethanolic root extract *of Clitoria ternatea* L. at Negative ESI.

various secondary active metabolites from this traditionally well-known medicinal plant.

Authors' contributions

PM guided JKN in planning, designing and conducting the research experiment to obtain the data. PM and JKN participated in the manuscript draft and have thoroughly checked and revised the manuscript. The author(s) read and approved the final manuscript.

Conflict of interests

The authors declared that they have no conflict of interest.

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