UHPLC-ESI-QTOF-MS Analysis on *Canthium Coromandelicum* Stem

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Abstract

A rapid ultra high-performance liquid chromatography coupled with crossover triple quadrupole time of flight mass spectrometry (UHPLC- ESI -QTOF-MS/MS) method has been developed for the identification of debasement products. According to the distinctive fragmentation patterns, the presence of 79 compounds with retention time between 1.05 to 26.81 minutes was found.

In Canthium coromandelicum stem, 22 amino acids, 10 fatty acids, 6 alkaloids, 6 steroids, 2 flavonoids, 2 terpenoids, 2 phenolic, 4 lipids, 3 anthraquinone glycosides, sugars, vitamins were distinguished. These outcomes demonstrated that the contemporary technique has been utilized for quality control of Canthium coromandelicum, exceptionally for recognizable proof, verification and portrayal in medication arrangements.

Keywords: *Canthium coromandelicum*, UHPLC-ESI-QTOF-MS, Secondary metabolites.

Introduction

Natural products are overwhelmingly seen by numerous individuals as being protected and more ground than customary drugs[9]. *Canthium coromandelicum* (family*:* Rubiaceae) is a prickly sub scandent bush with spreading branches disseminated all throughout India in clean backwoods, coromandel coast and in dry fields. Its leaves are straightforward, little, obovate, inverse with interpetiolar stipules and axillary spines. The leaves and roots are huge herbals; they are astringent, sweet, thermogenic, diuretic, febrifuge, obstructing, anthelmintic and as tonic utilized in ruined states of the hack, looseness of the bowels, strangury, fever, leucorrhoea, intestinal worms and general weakness[11].

It is utilized against snake nibbles by custom[8]. Macerated leaf glue is applied remotely to treat scabies and ringworm diseases two times every day[2]. The concentrated leaves separate is utilized for twisted mending in creatures. Critical cell reinforcement and diuretic movement was shown by concentrates of leaves[5]. The major ancestral gatherings in south Tamil Nadu have utilized some *Canthium* species as huge medications for the treatment of diabetes[3].

Present-day insightful advances, for example, UHPLC-ESI-QTOF-MS have empowered numerous scientists to methodically dissect the confounded network of phytochemical compounds. UHPLC provides high-resolution division of enormous quantities of constituents[12] while the QTOF-MS detector provides structural information from the separated chromatographic peaks[1,6].

Hence, the advancement of such scientific techniques would improve the extent of ensuing investigations. The regular goal is to improve the explicitness, affectability and selectivity of identification over single-stage-instruments. MS can be achieved in a number of scan modes, in which a product ion (daughter scan), a precursor ion (parent scan), loss of neutral fragment (neutral loss scan) or a selected fragmentation reaction (selected-reaction monitoring, multiple-reaction monitoring) can be observed. This strategy is utilized to recognize and subjective to low degrees of plant extracts. The current study was meant to explore the phytochemical constituents of *Canthium coromandelicum* stem utilizing UHPLC-ESI- Q-TOF-MS investigation

Material and Methods

Collection and processing of the plant material: Healthy, illness-free plant samples of *Canthium* were collected from natural habitats at Sivanthipuram, Tirunelveli District, Tamil Nadu, India. The specimen was recognized by Dr. M. Johnson, Assistant Professor of Botany, St. Xavier's College (Autonomous), Palayamkottai. The voucher specimen (XCH 26878) was deposited in St. Xavier's College Herbarium, Palayamkottai for additional reference. The entire plant tests were washed completely in running faucet water to eliminate the dirt particles followed by sterile refined water. The washed plants were blotched utilizing the smudging paper. The fresh leaves and stem of *C. coromandelicum* were spread out at room temperature in shade. The shade dried example was powdered utilizing the electric homogenizer and afterwards put away in a cooler for additional utilization.

Preparation of plant extracts: The dried, powdered stem of *C. coromandelicum* was progressively extricated with petrol ether, chloroform and methanol utilizing the Soxhlet extractor for 72 hours at a temperature not surpassing the limit of the dissolvable. The concentrates were sifted utilizing Whatmann channel paper (No.41) and afterward amassed in vacuum at 40ºC utilizing Rotary evaporator. The deposits acquired were put away in a cooler at 20º C until additional investigation.

UHPLC-ESI-QTOF-MS Analysis

Sample Preparation: A methanolic extracts of *Canthium coromandelicum* stem was dissolved in 1mL of methanol

and centrifuged at 3000 rpm for 5 min. This solution was utilised as test sample for UHPLC examination.

UPLC-DAD–ESI-Q-TOF-MS/MS: UPLC–MS/MS analyses were completed utilising a ultra-performance liquid chromatography apparatus equipped with PDA detector (Waters, USA) and a symmetry C18 column (150 mm×2.1 mm, particle size 1.7 µm) (Waters, USA). The mobile phases were: A. water with 0.1% formic acid and B. ethanol (100%). The peaks of the phenolic compounds were checked at 280 nm. Mass spectroscopic analysis of phenolic compounds in the sample was performed using a SYNAPT mass spectrometer, outfitted with an electrospray ionization source working in both positive and negative ion modes.

Methanolic extracts of *Canthium coromandelicum* stem were tentatively characterized by comparison with their UV–Vis absorption spectra and comparison of MS/MS fragmentation pattern with reference standards and literature reports.

Data analysis method in the MetID software: The initial phase in the examination of the information comprises of a correlation between the information record that contains the metabolite compounds (metabolite sample) and the information document that contains just the parent drug (control sample). In this investigation, all detectable mass signals are extracted from the MS level data using the Molecular Feature Extraction (MFE) algorithm.

At that point, related compound isotope masses and adduct masses are grouped together into discrete molecular features and chemical noise is taken out. The compound lists of the metabolized sample and the control are then compared. The algorithms can identify and qualify new metabolites or can simply qualify metabolites found by another algorithm. The results of all metabolite identification algorithms are weighed and combined into a final identification relevance score. Metabolites are qualified when their last score is over a characterized pertinence edge. The outcomes from all calculations are populated in an outcomes table.

Result and Discussion

UHPLC-ESI-QTOF-MS profile of *Canthium coromandelicum*: Distinctive chromatographic and electrophoretic methodologies have been utilized to advance the partition of mixes. These procedures have been applied for the basic assessment of metabolites. Due to the characteristics of these samples (polar, non-volatile, good ionization), ultra high performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry (UHPLC-ESI-MS/MS) has shown great promise due to its high reliability, unmatched reproducibility and high sample throughput[4,7,10].

The UHPLC– ESI–Q-TOF/MS BPC (base peak chromatogram) of a representative extract (optimized by using response surface methodology) is to illustrate the position of the peaks over the chromatographic run.

The compounds were assigned while considering their retention times and by comparing MS and MS2 data (accurate mass, isotopic distribution and fragmentation pattern in positive as well as negative mode) of the compounds detected. The current investigation manages UHPLC-ESI-QTOF/MS profile of methanol concentrate of stem of *Canthium coromandelicum* which can be utilized for identification, verification and portrayal. The outcomes are organized in table 1.

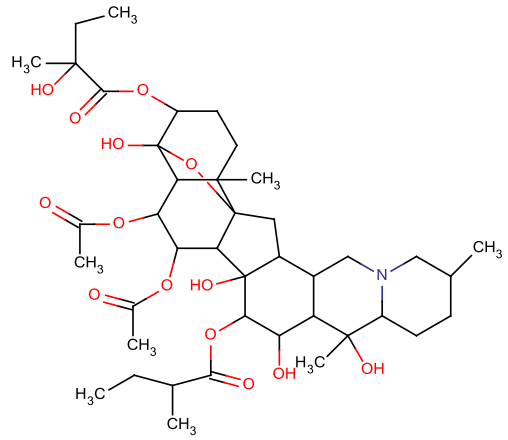
This sort of investigation is accounted for without precedent for *C. Coromandelicum.* Methanolic extract of *Canthium coromandelicum* stem verified 79 compounds with retention time between 1.05 to 26.81 minutes. In *Canthium coromandelicum* stem, 22 amino acids, 10 fatty acids, 6 alkaloids, 6 steroids, 2 flavonoids, 2 terpenoids, 2 phenolic, 4 lipids, 3 anthraquinone glycosides, sugars and vitamins were also observed.

Table 1
Identification of metabolites in *Canthium coromandelicum* stem by UHPLC–ESI–QTOF–MS

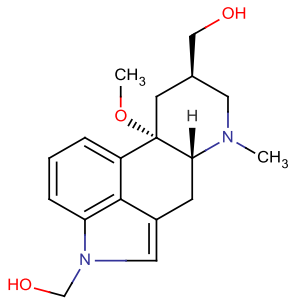
Peak	Compound Labeled	Mass	m/z	RT (min)	DB Diff (ppm)
1	Trolamine	149.1039	150.1113	1.05	8.75
2	Carnitine	162.1108	162.1106	1.084	13.62
3	Methyl(2-furoylamino)acetic acid	183.0549	184.0624	1.134	-9.76
4	Methyl N-(a-methylbutyryl)Glycine	173.1044	156.1013	1.161	4.35
5	Gabapentin	171.1265	194.1158	1.185	-3.34
6	2E,4E,6E,8E Decatetraenedioic acid	194.0591	177.0559	4.381	-6.34
7	4- hydroxylevamisoleglucuronide	396.1003	397.1086	4.382	-2.96
8	tetrahydro-a-(1-naphthyl methyl)-2-Furanpropionic acid	284.1449	289.1237	5.603	-13.01
9	N-Carbamylglutamate	190.0584	191.0655	5.818	3.01
10	5-(3-Methyltriazen-1-yl)imidazole-4-carboxamide	168.0761	151.0728	5.82	-0.97
11	Hydrocortisone-17-Butyrate	432.2517	415.2478	6.429	-1.28
12	Hydroxysalmeterol	431.2704	432.2774	6.436	-7.53
13	Trp Asp Asp	434.1391	439.1186	6.529	10.73

14	Netilmicin	475.2968	476.3044	6.661	8.00
15	GlnArgArg	458.2702	459.2773	6.662	2.62
16	GPCho(16:0/2:0[U])	538.3397	520.33	6.859	20.83
17	Aldicarb	190.0799	195.0586	7.374	-11.97
18	Dihydrofissinolide	514.2594	519.2395	8.811	-5.37
19	Sulfolithocholylglycine	513.2758	514.2834	8.818	0.38
20	Val Pro Lys	342.2269	325.2241	9.489	-0.48
21	Epirubicin	543.1678	544.1752	9.784	11.6
22	Gly Met Trp	392.1528	397.1329	10.09	-2.4
23	cis-5-Tetradecenoylcarnitine	370.2918	370.2923	10.29	10.69
24	Valproic acidGlucuronide	320.1456	325.1245	10.44	4.72
25	N-Acetylprimaquine	301.1795	324.1686	10.99	-1.51
26	NicotinamideRiboside	255.0992	256.1065	10.99	-4.13
27	AsnGlnAsn	374.1545	397.1447	11.21	1.2
28	Swietenine	568.272	573.2506	11.34	-6.73
29	Amikacin	585.2859	568.2811	11.35	-0.33
30	KetoprofenGlucuronide	430.1323	435.113	11.42	-13.83
31	Gestrinone	308.1799	313.1585	11.56	-7.35
32	C17 Sphinganine	287.2797	288.2872	11.74	9.66
33	Protorifamycin I	639.3062	640.3136	11.86	-2.91
34	Protoveratrine A	793.4262	816.4175	11.89	-1.66
35	2,4-dimethyl-2-eicosenoic acid	338.3128	343.2925	11.92	16.82
36	Dinorpromazine	256.1022	279.0911	12.30	4.94
37	1alpha-butyl-1beta,25-dihydroxyvitamin D3	472.3842	455.3824	12.92	15.69
39	Cetylpyridinium	304.2985	304.2983	13.66	6.43
40	Nervonic acid	366.3465	371.3252	13.68	8.89
41	Arg Pro Gly	328.1866	311.1836	13.89	-2.15
42	2-[3-Carboxy-3-(methylammonio)propyl]-L-histidine	270.1334	252.1225	14.18	-2.06
43	Mitoxantrone	444.2015	445.2094	14.37	-1.42
44	Ergoline-1,8- dimethanol, 10-methoxy-6-methyl	316.1797	321.1582	15.11	-3.23
45	Lys GlnLeu	387.2504	392.2291	15.29	-5.73
46	Praziquantel	312.1843	335.1743	15.74	-1.83
47	3-Hydroxydodecanedioic acid	246.1463	229.1431	16.45	1.67
48	GPCho(11:0/11:0[U])	594.417	576.4068	17.08	-5.89
49	13-hydroxytridecanoic acid	230.1884	235.1674	17.12	-0.72
50	16-iodo-hexadecanoicAcid	382.1342	365.1316	17.25	7.09
51	Tetranor Iloprost	306.1841	311.1631	17.28	-3.33
52	Amastatin	474.2695	457.2644	17.30	-1.15
53	2-docosanamidoethanesulfonic acid	447.3415	452.3203	17.32	-7.36
54	PhePhe	312.1463	295.1437	17.47	3.63
55	Duartin	360.1561	365.1351	17.65	3.26
56	ArachidonoylDopamine	439.3169	462.3088	17.65	-18.85
57	Fexofenadine	501.2931	484.2897	17.66	-10.34
58	2-propyl-3-Hydroxyethylene pyran-4-one	180.0777	163.0749	17.80	5.5
59	1-Methyl-4-nitroimidazole	127.0361	150.0257	18.38	16.14
60	Lactone of PGF-MUM	296.1617	301.1408	18.38	2.39
61	2E,4E,8E,10EDodecatetraenedioic acid	222.089	205.0856	18.38	1.11
62	PerindoprilGlucuronide	544.2601	545.2674	19.37	5.65
63	Lys Arg	302.2074	325.1974	19.60	-2.57
64	LeuArg Asp	402.2234	425.2129	19.62	-1.88
65	Arg Ile Asp	402.2236	403.231	19.62	-2.24
66	1alpha,25-dihydroxy-24a,24b-dihomo-22-thiavitamin D3	462.3147	485.3034	19.70	4.4
67	(24R)-24-fluoro-1alpha,25-dihydroxyvitamin D2 /(24R)-24-fluoro-alpha,25-dihydroxyergocalciferol	446.3168	429.315	19.78	6.4
68	1alpha,25-dihydroxy-3-deoxy-3-thiavitamin D3 3-oxide /1alpha,25-dihydroxy-3-deoxy-3-thiacholecalciferol	436.2996	441.2798	19.88	3.58

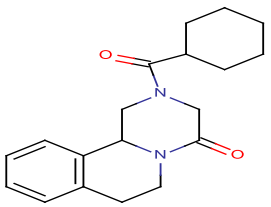
69	(Z)-N-(2-hydroxyethyl)hexadec-7-enamide	297.2639	280.2609	19.89	9.67
70	Distigmine	416.2417	398.231	20.50	1.55
71	Flurandrenolide	436.2271	441.207	20.96	-2.36
72	5-oxo-7-octenoicacid	156.0761	161.0547	21.11	16.18
73	N-(2-hydroxyethyl)palmitamide	299.2804	282.2773	21.44	6.84
74	Vinpocetine	350.1976	351.2051	22.66	5.29
75	Thr Ile Leu	345.2195	350.1982	22.67	19.78
76	TrpLeu Ile	430.2604	413.2563	25.59	-5.66
77	3beta,6alpha,7alpha-Trihydroxy-5betacholan-24-oic Acid	408.2846	391.2822	25.59	7.28
78	3-methyl-2,5-dioxo-3-Pyrrolidineacetic acid	171.0527	176.0314	26.81	2.49
79	Hydantoin-5-propionicAcid	172.0499	177.0286	26.81	-8.93



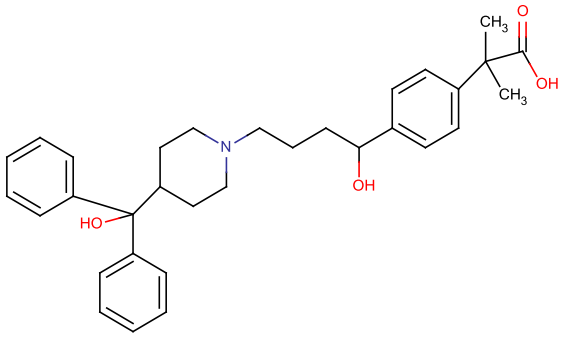
Protoveratrine A



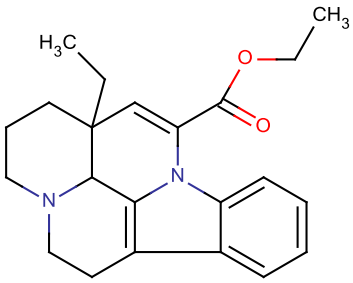
Ergoline-1,8-dimethanol,10-methoxy-6-methyl



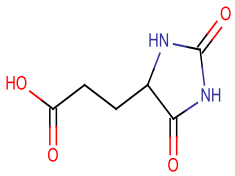
Praziquantel



Fexofenadine



Vinpocetine

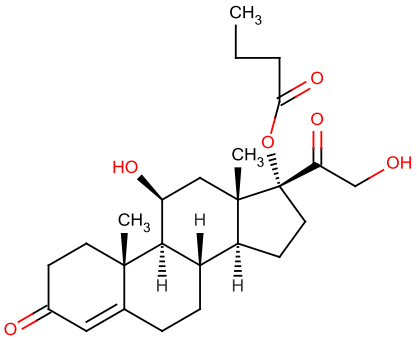


Hydantoin-5-propionic acid

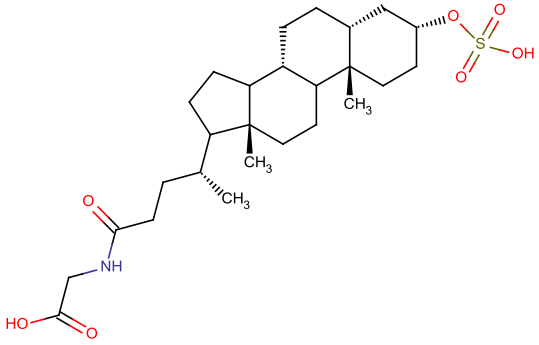
There are six alkaloids in *Canthium coromandelicum* stem observed with retention time 11.89, 15.11, 15.74, 17.66, 22.66 and 26.81minutes monitored at m/Z values 816.4175, 321.1582, 335.1743,484.2897, 351.2051 and 177.0286 corresponding to Protoveratrine A, Ergoline-1,8-dimethanol, 10-methoxy-6-methyl, Praziquantel, Fexofenadine, Vinpocetine and Hydantoin-5-propionic acid respectively.

In *Canthium coromandelicum*, six steroids were seen with retention time 6.42, 8.81, 11.56, 12.92, 20.96 and 25.59 minutes. The m/Z esteems for the corresponding retention

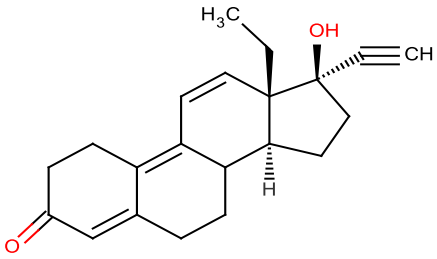
time and compounds are 415.24(Hydrocortisone-17-Butyrate), 514.28 (Sulfolithocholyl-glycine), 313.15 (Gestrinone), 455.38 (1alpha-butyl-1beta,2,5-dihydroxyvitamin D3), 335.17(Praziquantel), 441.21 (flurandrenolide) and 391.28 (3beta, 6alpha, 7alpha-Trihydroxy-5betacholan- 24-oic Acid) respectively.



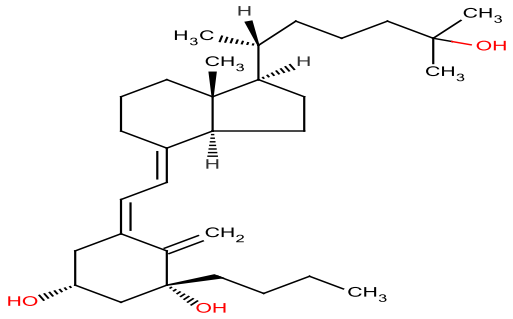
Hydrocortisone-17- Butyrate



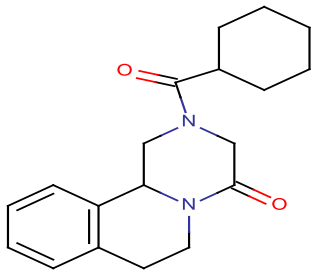
Sulfolithocholyl –glycine



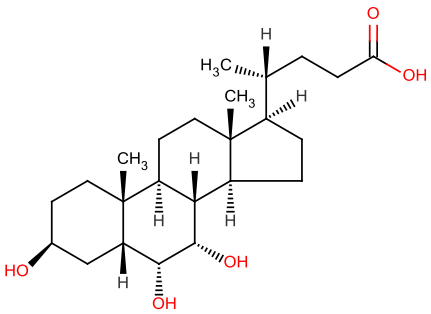
Gestrinone



1alpha-butyl-1beta, 2,5- dihydroxyvitamin D3

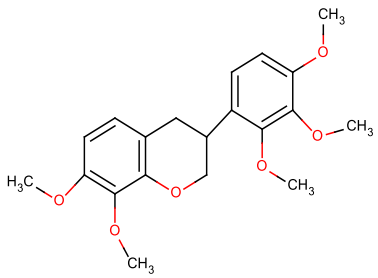


Praziquantelflurandrenolide

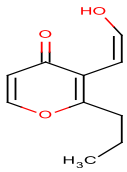


beta,6alpha,7alpha- Trihydroxy-5 betacholan- 24-oic Acid

The flavonoids were observed at 17.65 and 17.80 minutes, the m/z value are 365.14, 163.07 and corresponding to duartin and 2-propyl-3- hydroxyethylene pyran-4-one respectively.

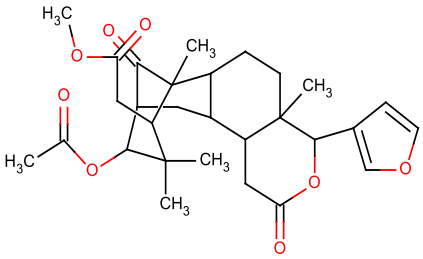


Duartin

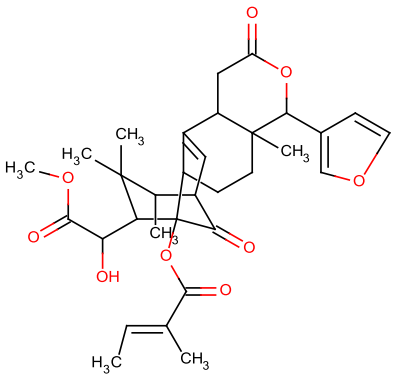


2-propyl-3- hydroxyethylene pyran-4-one

The two triterpenoids were observed at 8.811 and 11.34 minutes, the m/z values are 519.24 and 573.25 corresponding to dihydrofissionolide and swietenine respectively.



Dihydrofissionolide



Swietenine

Conclusion

This study relates to the synthetic arrangement of *Canthium coromandelicum* stem in UHPLC-ESI-QTOF/MS strategy. It was developed and validated for exploring the potential chemical markers for quality control inside the spicies with comparative chemical ingredients due to origin from the same genus or same plant species.

The methanolic extract showed 79 compounds, 22 amino acids, 10 fatty acids, 6 alkaloids, 6 steroids, 2 flavonoids, 2 terpenoids, 2 phenolic, 4 lipids, 3 anthraquinone glycosides, sugars, vitamins. These discoveries propose the possibility of additional improvement in the confinement of remedially helpful mixes.

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