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LC-MS Analysis of crude extract of root of Rivea Hypocrateriformis.

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ABSTRACT-*The aim of the present study is to investigate the extract [with the use of solvent Methanol, Water and chloroform (4:4:2)] of root ofRivea Hypocrateriformis Phytochemical compounds using LC-MS. The shade dried root of plant powder Rivea Hypocrateriformiswas extracted with mixture of Methanol, Water and chloroform (4:4:2) and crude was obtained. The LC-MS analysis was performed by using LCMSQTOF 1260 Infinity model with the Mass Hunter Quantsoftware. The LC-MS analysis shows different peaks with low and high molecular weight determining the presence of 54 Phytochemical compounds. The Phytochemical compounds in the root extract of Rivea Hypocrateriformishave been evaluated by using METLIN data base. The presence of these compounds may proceed to find out various therapeutic activities.*

Keywords-*Rivea Hypo crateri formis, LC-MS analysis, Mass Hunter Quant software, phy to chemical Compounds, METL INdata base.*

I. INTRODUCTION

For millennia, people around the world have healed the sick with herbal derived remedies, and handed down through generations. Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illness [1]. In developing countries, the practice of medicine still relies heavily on plant and herbal extracts for the treatment of human ailments. Dietary agents consist of a wide variety of biologically active compounds that are ubiquitous in plants, and many of them have been used as traditional medicines [2-4]. An estimate of the World Health Organization (WHO) states that around 85-90% of the world's population consumes traditional herbal medicines [5]. Screening of active components from plants has direct to the development of new medicinal drugs which have efficient protection and treatment role against various diseases [6]. LCMS, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [7].Rivea Hypocrateriformis is an important medicinal plant in the Indian system of Medicine. It is commonly known as 'Phang' which grows in Monsoon session. The leaves of the Phang are used for making traditional food in India, Pakistan and Afghanistan. Root is usedas a potent medicine for venom. According to Ayurveda's, if the poison of the scorpion spreads in the body, the poison will be removed only by holding the fresh root of the plant.The root is supposed to be useful in treatment of hysteria and nervous disorders. Therefore this is an attempt to determine the Phytochemical compounds present in the root by extract [with the use of solvent Methanol, Water and chloroform (4:4:2)] using LC-MS technique.

II. METHODS AND MATERIAL

(A) Collection and Identification of Plant materials

Dry roots of Rivea Hypocrateriformis were collected from Khavad which is located in Mehsana District, Gujarat, India. This Rootwas identified and authenticated by Dr.K.J.Bhatt, Assistant ProfessorBotany Department, Pramukh Swami Science andH.D.Patel Arts College, Kadi. The roots were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags till use.

(B) Preparation of extracts

The dried roots were extracted with Methanol, Waterand chloroform using Soxhlet extractor. Obtained extract concentrated with rotary evaporator till dry powder was obtained. The final concentrated extract is analyzed by using LC-MS.

(C) Liquid Chromatography-Mass spectrometry (LC-MS) analysis

The LC-MS analysis was performed by using LCMSQTOF 1260 Infinity model with the MassHunter Quantsoftware. The ZORBAX 300 C18 Colum (4.6*100 mm, internal diameter of the Colum 3.5 mm)was used for analysis at room temperature. Acetonitrile and water with 0.1 % concentration of formic was used as Mobile Phase at constant flow rate of 1 ml/min at 90:10 ration for 2 minute and after 5 minute ration was 70:30, then up to 12 minute ratio was 0:100 and up to

15 minute ratio was 90:10.Total run time was 15 Minutes. And during this time 10µl sample was used. The mass range is 162-786amu.The data of LC-MS were screen in METLIN Data Base.

III.RESULTS AND DISCUSSION

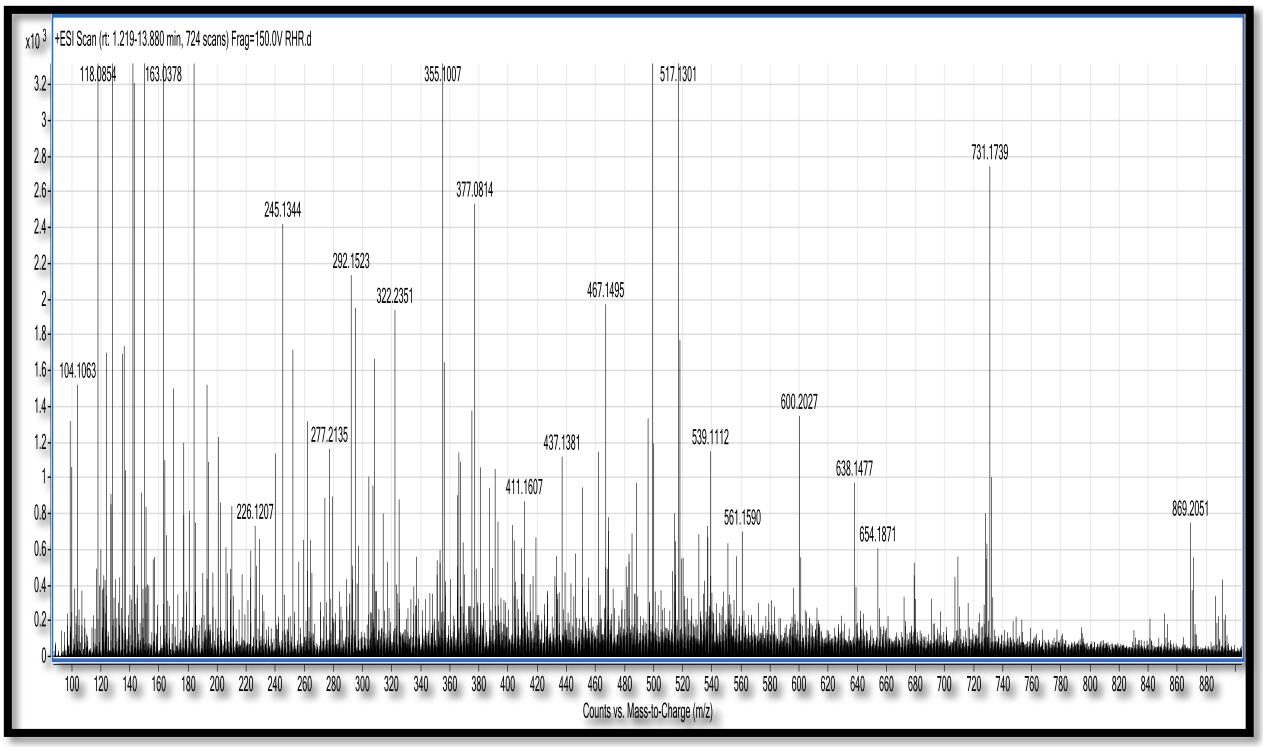

Fig. 1: The LC - MS Chromatogram of crude extract of root of Rivea Hypocrateriformis

Fig. 1: The LC - MS Chromatogram of crude extract of root of Rivea Hypocrateriformis
LC–MS chromatogram of the Methanol, Waterand chloroform (4:4:2) extract of root of Rivea Hypocrateriformis (Fig. 1) clearly shows 54 peaks indicating the presence of 54 Phytochemical compounds. The identification of the phytochemical compounds was based on the peak area, retention time and molecular formula. The table 1 shows the compound name with its molecular formula, mass and Retention time. The results reveal the presence of Dulciol B(464.2207), GlcAbeta-Cer(d18:1/18:0)(741.5756), Macrophylline(239.1524), PS(22:2(13Z,16Z)/14:1(9Z))(785.52), (4E,6E,d14:2) sphingosine(241.2044), TomentolideA(402.1466),3-hexanoyl-NBDCholesterol(662.4399),Meteloidine(255.1471),Cochlearine(261.1364),Symlandine(381.2151),Calophylloli de(416.1623),alpha-Viniferin(678.1876),N- Acetylmuramoyl Ala(364.1469),6-C-Glucopyranosylpilloin(476.1302),N-Acetylpuromycin(513.2319),PE(22:4(7Z,10Z,13Z,16Z)/P-18:1(9Z))(777.5691), Isocyclomorusin(418.1417), Blestriarene B(480.1569), His His Lys(420.2245), Lucuminic acid(446.1413), 6-C- Glucopyranosylpilloin(476.1298), Glu-P- 2(184.075), Mangostenone B(462.2049),Darlingine(219.1259),Pyrimethanil(199.1104),Glu-P- 2(184.0744), N-Acetylpuromycin(513.2314), 3'-Deoxymaysin(560.1501),Proansamitocin(443.231),8E-Tetradecenylacetate(254.2245),AspArgAsp(404.1661),pentadecanal(226.2292),Sparfloxacin(392.1656),Serratan idine(295.1782),3-Hydroxycoumarin(162.0309), (2xi,3xi,6E)-3,7- Dimethyl-6- octene-1,2,3,8- tetrol(204.1362), (-)- menthyl beta-D- glucoside(318.2044), Selegiline(187.1367), 16-hydroxy hexadecanoic acid(272.2347), N-(2-Methylpropyl)acetamide(115.1003), 2-hexyl- decanoic acid(256.2399), 2,4-Undecadienal(166.1361), Peruvianoside II(494.1408), Polyethylene, oxidized(244.1314), 1-Palmitoyl Lysophosphatidic Acid(410.2428), Glu His(284.1115), Crotamiton(203.1317), Streptidine(262.1391), Oleandrose(162.0895), 3S,7S-dimethyl-tridecan-2S- ol(228.2443), Tigloidine(223.1578), Methoprene acid(268.2037), C11:1n-3(184.1466), and Methyl jasmonate(224.1412).The spectrum sketch out of LC-MS confirmed the presence of 54 components with the retention time
7.992,22.351,3.592,22.369,8.104,7.182,22.359,4.34710.16,11.842,8.894,9.694,6.872,8.97,8.287,22.357,2.328,9.444,8.473,10.695,9.473,10.053,2.144,2.438,1.853,7.257,8.434,7.257,11.842,13.328,6.096,13.339,7.771,8.269,2.209,10.989,1.924,7.041,11.118,1.998,14.763,4.464,6.482,11.842,13,8.287,3.279, 8.912, 3.002, 13.641min respectively which is shown in Figure. 1.

Table 1: Phytochemical compounds identified in crude extract of root of Rivea Hypocrateriformis by LC-MS analysis.

Sr. No.	Peak Name	Molecular Formula	Mass	RT
1	Dulciol B	C28 H32 O6	464.2207	7.992
2	GlcAbeta-Cer(d18:1/18:0)	C42 H79 N O9	741.5756	22.351
3	Macrophylline	C13 H21 N O3	239.1524	3.592
4	PS(22:2(13Z,16Z)/14:1(9Z))	C42 H76 N O10 P	785.52	22.369
5	(4E,6E,d14:2) sphingosine	C14 H27 N O2	241.2044	8.104
6	Tomentolide A	C25 H22 O5	402.1466	7.182
7	3-hexanoyl-NBD Cholesterol	C39 H58 N4 O5	662.4399	22.359
8	Meteloidine	C13 H21 N O4	255.1471	4.347
9	Cochlearine	C15 H19 N O3	261.1364	10.016
10	Symlandine	C20 H31 N O6	381.2151	11.842
11	Calophyllolide	C26 H24 O5	416.1623	8.894
12	alpha-Viniferin	C42 H30 O9	678.1876	9.694
13	N-Acetylmuramoyl-Ala	C14 H24 N2 O9	364.1469	6.872
14	6-C-Glucopyranosylpilloin	C23 H24 O11	476.1302	8.97
15	N-Acetylpuromycin	C24 H31 N7 O6	513.2319	8.287
16	PE(22:4(7Z,10Z,13Z,16Z)/P-18:1(9Z))	C45 H80 N O7 P	777.5691	22.357
17	Isocyclomorusin	C25 H22 O6	418.1417	2.328
18	Blestriarene B	C30 H24 O6	480.1569	9.444
19	His His Lys	C18 H28 N8 O4	420.2245	8.473
20	Lucuminic acid	C19 H26 O12	446.1413	10.695
21	6-C-Glucopyranosylpilloin	C23 H24 O11	476.1298	9.473
22	Glu-P-2	C10 H8 N4	184.075	10.053
23	Mangostenone B	C28 H30 O6	462.2049	2.144
24	Darlingine	C13 H17 N O2	219.1259	2.438
25	Pyrimethanil	C12 H13 N3	199.1104	1.853
26	Glu-P-2	C10 H8 N4	184.0744	7.257
27	N-Acetylpuromycin	C24 H31 N7 O6	513.2314	8.434
28	3'-Deoxymaysin	C27 H28 O13	560.1501	7.257
29	Proansamitocin	C25 H33 N O6	443.231	11.842
30	8E-Tetradecenyl acetate	C16 H30 O2	254.2245	13.328
31	Asp Arg Asp	C14 H24 N6 O8	404.1661	6.096
32	pentadecanal	C15 H30 O	226.2292	13.339
33	Sparfloxacin	C19 H22 F2 N4 O3	392.1656	7.771
34	Serratanidine	C16 H25 N O4	295.1782	8.269
35	3-Hydroxycoumarin	C9 H6 O3	162.0309	2.209
36	(2xi,3xi,6E)-3,7-Dimethyl-6-octene-1,2,3,8-tetrol	C10 H20 O4	204.1362	10.989
37	(-)-menthyl beta-D-glucoside	C16 H30 O6	318.2044	1.924
38	Selegiline	C13 H17 N	187.1367	7.041
39	16-hydroxy hexadecanoic acid	C16 H32 O3	272.2347	11.118
40	N-(2-Methylpropyl)acetamide	C6 H13 N O	115.1003	1.998

41	2-hexyl-decanoic acid	C16 H32 O2	256.2399	14.763
42	2,4-Undecadienal	C11 H18 O	166.1361	4.464
43	Peruvianoside II	C23 H26 O12	494.1408	6.482
44	Polyethylene, oxidized	C12 H20 O5	244.1314	11.842
45	1-Palmitoyl Lysophosphatidic Acid	C19 H39 O7 P	410.2428	13
46	Glu His	C11 H16 N4 O5	284.1115	8.287
47	Crotamiton	C13 H17 N O	203.1317	3.279
48	Streptidine	C8 H18 N6 O4	262.1391	8.912
49	Oleandrose	C7 H14 O4	162.0895	3.002
50	3S,7S-dimethyl-tridecan-2S-ol	C15 H32 O	228.2443	13.641
51	Tigloidine	C13 H21 N O2	223.1578	8.563
52	Methoprene acid	C16 H28 O3	268.2037	11.689
53	C11:1n-3	C11 H20 O2	184.1466	4.479
54	Methyl jasmonate	C13 H20 O3	224.1412	8.25

IV. CONCLUSION

In the present study, 54 phytochemical constituents have been identified from the ethanol extract of roots of *Rivea Hypocrateriformis* by Liquid Chromatogram - Mass Spectrometry (LC - MS) analysis. The presence of these phytochemical constituents justifies the use of this plant for various ailments by traditional practitioners. Isolation of individual photochemical constituents and subjecting it to biological activities are being undertaken...

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