

LC-MS Analysis of crude extract of leaves of *Rivea Hypocretariformis*.

Dharmesh Patel*¹, Jay patel¹, Viral patel¹, Zalak patel¹, Hiral Patel¹ Dr. Chintan V. Pandya¹, Dr. Aditee Pandya¹

*Department of chemistry, H.V.H.P Institute of post graduate studies and research, Kadi.
KSV University, Gandhinagar, Gujarat, India.*

Abstract— *The aim of the present study is to investigate the crude extract of Leaves of *Rivea Hypocretariformis* 46 Phytochemical compounds using LC-MS. The shade dried powder of Leaves of *Rivea Hypocretariformis* was extracted with mixture of Methanol, Water and chloroform (4:4:2) and crude was obtained. The LC-MS assessment was done by using LCMSQTOF 1260 Infinity model and using the MassHunter Quant software. The LC-MS analysis shows different peaks with low and high molecular weight determining the presence of 46 phytochemicals compounds. The phytochemicals compounds in the Leaves extract of *Rivea Hypocretariformis* have been screened by using METLIN Data base. The presence of these compounds may continue to find out different therapeutic activities.*

Keywords- *Rivea Hypocretariformis, Ethanol extract; LC-MS analysis; phytochemical Compounds.*

I. INTRODUCTION

For thousand of the years, people around the world have healed the sick with herbal consequent remedy, and handed down through generation. Conventional medicine is the sum total of information, skills and practices based on the theories, beliefs and experiences aboriginal to different cultures that are used to maintain health, as well as to avert, diagnose, treat physical and mental illness [1]. In upward countries, the practice of medicine still relies heavily on plant and herbal extracts for the treatment of human ailments. Dietary agents consist of a spacious variety of biologically active compounds that are everywhere in plants, and many of them have been used as traditional medicines [2, 3, 4]. An approximation 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 improvement of new medicinal drugs which have efficient defense and treatment role against different 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 unidentified organic compounds in a complex mixture can be determined by analysis and also by matching the spectra with reference spectra [7]. *Rivea Hypocretariformis* is an important medicinal plant in the Indian system of Medicine. It is commonly 'Phang' which grows in Monsoon session. The leaves of the Phang are used for making traditional food in India, Pakistan and Afghanistan. Leaves are used as a potent medicine for Leaves is use as venom. The leaves are supposed to be useful in treatment of sexual and nervous disorders. Therefore this is an attempt to determine the Phytochemical compounds present in the Water and chloroform (4:4:2) extract of leaves of *Rivea Hypocretariformis* by LC-MS technique

II. METHODS AND MATERIAL

(A) Collection and Identification of Plant materials :

Dry Leaves of *Rivea Hypocretariformis* were collected from Khavad which is located in Mehsana District, Gujarat, India. This Leaves was identified and authenticated by Dr.K.J.Bhatt, Assistant Professor- Botany Department, Pramukh Swami Science And H.D.Patel Arts College, Kadi. The foliage 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 Leaves were extracted with Methanol, Water and chloroform using Soxhlet extractor. The extract which is acquired is concentrated with rotary evaporator till dry powder was obtain. 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 Quant software. 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. Total LC-MS running time was 15 minutes. The detection employed in METLIN Data Base.

III.RESULTS AND DISCUSSION

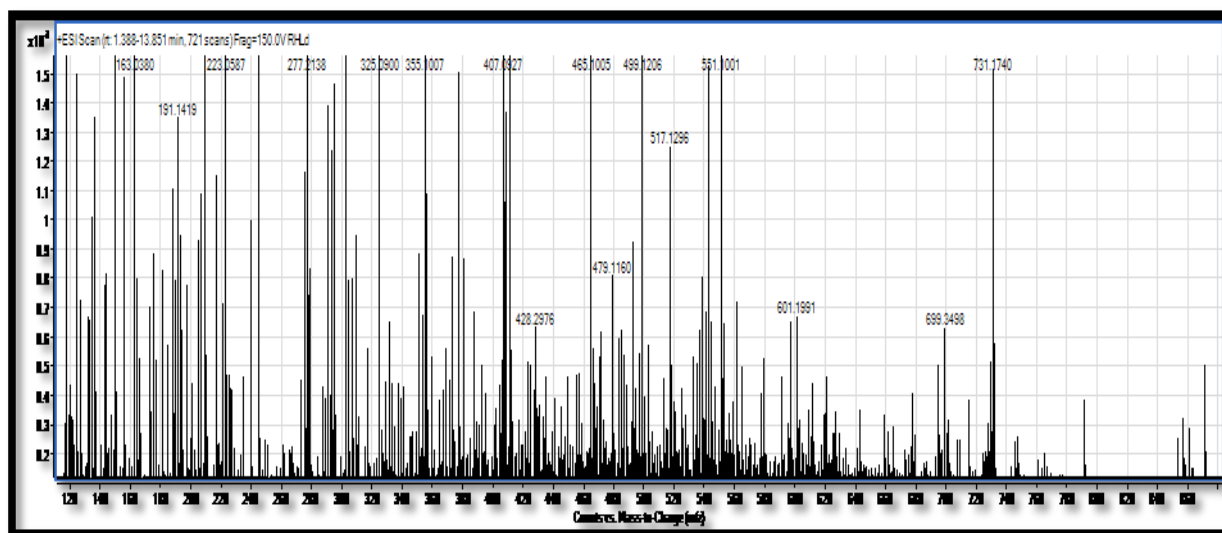


Fig. 1: The LC - MS Chromatogram of crude extract of Leaves of Rivea Hypocretariformis

LC-MS chromatogram of the Methanol, Water and chloroform (4:4:2) extract of leaves of *Rivea Hypocretariformis* (Fig. 1) clearly shows 46 peaks indicating the presence of 46 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 7-methyl-decanoic acid (186.1624), Symplandine (381.215), PG(17:1(9Z)/22:6 (4Z,7Z,10Z,13Z, 16Z, 19Z) (806.5102), Etoposide glucuronide(764.2159), Isoliensinine(610.3045), Papyriferic acid(604.3979), 8-Hydroxyluteolin 8-glucoside (464.0933), Septentriodine(700.3586), 8-trans-[2-(6-Benzoyloxy-4-hydroxy-2-methoxy-3-methylphenyl)ethenyl]-5-methoxyflavan-7-ol(538.2001), Scilliroside(604.2872), 3-hexanoyl-NBD Cholesterol(662.4411), Flavidulol C(514.3074), 8-Hydroxyluteolin 8-glucoside(464.0931), Tamarixin (478.1086), GlcAbeta-Cer(d18:1/18:0)(741.5736), D-Urobilin(588.2919), 10E,12Z-Tetradecadienyl acetate (252.2088), 1alpha-fluoro-25-hydroxy-16,17,23,23,24,24-hexadehydrovitamin D3 (412.2783), Peruvianoside II(494.1414), 10-undecenal(168.152), Diospyrin(484.1218), Lanceotoxin A(620.2818), 2-tridecenal(196.1824), Cyclic de-hypoxanthine futasine(294.0739), 7Z-Undecenyl acetate(212.1779), 10E,12Z-Tetradecadienyl acetate(252.2092), neoisomenthol(156.1521), 14,14,14-Trifluoro-11E-tetradecenyl acetate (308.1959), undecan-2-one(170.167), 10E,12Z-Tetradecadienyl acetate(252.2091), 1-Monopalmitin(330.2756), PS(22:2(13Z,16Z)/ 14:1(9Z))(785.5203), 9-keto palmitic acid(270.2188), 9Z,11E-Hexadecadienal(236.2141), 3-Hydroxycoumarin (162.0309), 4E,6E,11Z-Hexadecatrienal(234.1985), MG(0:0/16:1(9Z)/0:0)(328.261), 2-Hydroxy-6-oxo-7-methylocta-2,4-dienoate(184.0739), 9-keto palmitic acid(270.2197), 4,4-Difluoropregn-5-ene-3,20-dione (350.205), 12-hydroxyjasmonic acid(226.1204), (4E,6E,d14:2) sphingosine(241.2042), 10E-Tridecen-2S-ol(198.1985), Pro Glu Tyr(407.1696), Lanceotoxin A(620.2813), C11:1n-3(184.1469). The spectrum sketch of LC-MS definite the presence of 46 components with the retention time 4.501, 11.842, 14.034, 8.128, 2.424, 12.764, 8.217, 12.794, 6.784, 12.508, 22.359, 13.737, 8.366, 9.304, 22.351, 13.796, 12.232, 12.287, 6.753, 9.028, 9.304, 12.287, 12.948, 9.286, 11.496, 12.009, 13.254, 11.639, 13.151, 11.496, 12.089, 22.369, 11.514, 14.678, 2.748, 12.232, 12.394, 7.257, 10.943, 11.22, 8.44, 8.104, 9.82, 3.286, 11.367, 5.366, min respectively which is shown in Figure.1.

Table. 1. Phytochemical compounds identified in crude extract of Leaves of Rivea Hypocretariformis by LC-MS analysis.

Sr. No.	PEAK Name	Molecular Formula	Mass	RT
1	7-methyl-decanoic acid	C11 H22 O2	186.1624	4.501
2	Symalandine	C20 H31 N O6	381.215	11.842
3	PG(17:1(9Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	C45 H75 O10 P	806.5102	14.034
4	Etoposide glucuronide	C35 H40 O19	764.2159	8.128
5	Isoliensinine	C37 H42 N2 O6	610.3045	2.424
6	Papyriferic acid	C35 H56 O8	604.3979	12.764
7	8-Hydroxyluteolin 8-glucoside	C21 H20 O12	464.0933	8.217
8	Septentriodine	C37 H52 N2 O11	700.3586	12.794
9	8-trans-[2-(6-Benzoyloxy-4-hydroxy-2-methoxy-3-methylphenyl)ethenyl]-5-methoxyflavan-7-ol	C33 H30 O7	538.2001	6.784
10	Scilliroside	C32 H44 O11	604.2872	12.508
11	3-hexanoyl-NBD Cholesterol	C39 H58 N4 O5	662.4411	22.359
12	Flavidulol C	C34 H42 O4	514.3074	13.737
13	8-Hydroxyluteolin 8-glucoside	C21 H20 O12	464.0931	8.366
14	Tamarixin	C22 H22 O12	478.1086	9.304
15	GlcAbeta-Cer(d18:1/18:0)	C42 H79 N O9	741.5736	22.351
16	D-Urobilin	C33 H40 N4 O6	588.2919	13.796
17	10E,12Z-Tetradecadienyl acetate	C16 H28 O2	252.2088	12.232
18	1alpha-fluoro-25-hydroxy-16,17,23,23,24,24-hexadehydrovitamin D3	C27 H37 F O2	412.2783	12.287
19	Peruvianoside II	C23 H26 O12	494.1414	6.753
20	10-undecenal	C11 H20 O	168.152	9.028
21	Diospyrin	C21 H24 O13	484.1218	9.304
22	Lanceotoxin A	C32 H44 O12	620.2818	12.287
23	2-tridecenal	C13 H24 O	196.1824	12.948
24	Cyclic de-hypoxanthine futasoline	C14 H14 O7	294.0739	9.286

25	7Z-Undecenyl acetate	C13 H24 O2	212.1779	11.496
26	10E,12Z-Tetradecadienyl acetate	C16 H28 O2	252.2092	12.009
27	neoisomenthol	C10 H20 O	156.1521	13.254
28	14,14,14-Trifluoro-11E-tetradecenyl acetate	C16 H27 F3 O2	308.1959	11.639
29	undecan-2-one	C11 H22 O	170.167	13.151
30	10E,12Z-Tetradecadienyl acetate	C16 H28 O2	252.2091	11.496
31	1-Monopalmitin	C19 H38 O4	330.2756	12.089
32	PS(22:2(13Z,16Z)/14:1(9Z))	C42 H76 N O10 P	785.5203	22.369
33	9-keto palmitic acid	C16 H30 O3	270.2188	11.514
34	9Z,11E-Hexadecadienal	C16 H28 O	236.2141	14.678
35	3-Hydroxycoumarin	C9 H6 O3	162.0309	2.748
36	4E,6E,11Z-Hexadecatrienal	C16 H26 O	234.1985	12.232
37	MG(0:0/16:1(9Z)/0:0)	C19 H36 O4	328.261	12.394
38	2-Hydroxy-6-oxo-7-methylocta-2,4-dienoate	C9 H12 O4	184.0739	7.257
39	9-keto palmitic acid	C16 H30 O3	270.2197	10.943
40	4,4-Difluoropregn-5-ene-3,20-dione	C21 H28 F2 O2	350.205	11.22
41	12-hydroxyjasmonic acid	C12 H18 O4	226.1204	8.44
42	(4E,6E,d14:2) sphingosine	C14 H27 N O2	241.2042	8.104
43	10E-Tridecen-2S-ol	C13 H26 O	198.1985	9.82
44	Pro Glu Tyr	C19 H25 N3 O7	407.1696	3.286
45	Lanceotoxin A	C32 H44 O12	620.2813	11.367
46	C11:1n-3	C11 H20 O2	184.1469	5.366

IV. CONCLUSION

In this study, 46 Phytochemical constituents have been recognized from the ethanol extract of leaves of *Rivea Hypocretariformis* by Liquid Chromatogram - Mass Spectrometry (LC - MS) investigation. 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.

V. ACKNOWLEDGEMENT

Author greatly thankful to Dr. Chintan Pandya for this encouragement and moral support, at all times and author also grateful to his family member for providing all help whenever needed. My heartiest thanks to Chemistry Department, H.V.H.P Institute of post graduate studies and research, Kadi and KSV University Gandhinagar for providing the laboratory facilities and for his cooperation and inspiration at all times

V. REFERENCES

- [1] Singh R., Verma PK, Singh G. Total phenolic, flavonoids and tannin contents in different extracts of *Artemisia absinthium*. J. Intercult. Ethnopharmacol 2012; 1: 101–104.
- [2] Dhore MA. Flora of Amravati district with special reference to the distribution of tree species. Ph.D. Thesis, Amravati University, Amravati.1986; 154-156.
- [3] Shivalingappa H, Satyanarayan ND, Purohit MG, Sharanabasappa A, Patil SB. Effect ethanol extract of *Riveahypocrateriformis* on the estrous cycle on the rat. J. Ethnopharmacol. 2002; 82: 1:11-17.
- [4] Evans SR. In: Frust PT, editor. An overview of hallucinogens: the flash of God. Illinois: Waveland Press. 1990; 3–54.
- [5] Brahmabhatt MR, Patel JM, Patel VB, Saluja AK. Analgesic and Anti-inflammatory activity of leaves of *Rivea hypocrateriformis*. J. PharmaPhytotherapy. 2010; 1:1:001-003.
- [6] Nataraj L, Dharmar R, Sellamuthu M. Phytochemical studies and Anti-inflammatory activity of the stem of *Rivea hypocrateriformis*. Food SciBiotechnol. 2010; 19(5):1251-1258.