e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
Volume:02/Issue:11/November -2020 Impact Factor- 5.354 www.irjmets.com

EVALUATION OF 2- HYDROXY 4-METHOXY BENZALDEHYDE FROM PETROLEUM ETHER EXTRACT OF *HEMIDESMUS INDICUS* (L) BY USING THIN LAYER CHROMATOGRAPHY AND GC-HRMS TECHNIQUES

Madhuri M. Deshmukh[*1], Chhaya S. Ambad[*2], Manoj R. Kadam[*3], N. G. Kashid[*4]

[*1]Dept. of Botany Dr. Babasaheb Ambedkar Marathwada University Aurangabad.

[*2]M.G.M. College of Biosciences Aurangabad.

[*3]Dept. of Chemical Technology Aurangabad.

[*4]Baburaoji Adaskar College Kaij., Dist. Beed.

ABSTRACT

Objective: The present work was focused on the evaluation of 2-Hydroxy 4-Methoxy Benzaldehyde by using phytochemical analysis, Thin Layer Chromatography and advanced technique such as GC-HRMS of Petrolium extract of *Hemidesmus indicus* (L) roots . All these used techniques are very effective ,rapid, accurate, economical and stability-indicating for the quantification of bulk and pharmaceutical formulation.

Methods: The method used for extraction was Soxhlet Extraction. Phytochemical screening was performed by Practical Pharmacognosy by Khandelwal. Both Thin Layer i.e. Analytical and Preparative Chromatography used for detection and comparative study of 2-Hydroxy 4-Methoxy Benzaldehyde.The mobile phase used was same in both Hexane: Ethyl acetate (7:3) ratio. Standard 2-Hydroxy 4-Methoxy Benzaldehyde purchased from Sigma Aldrich Pune. Standard showed maximum solubility in polar solvent Methanol.Most advance technique such as GC-HRMS used for evaluation and confirmation of 2-Hydroxy 4-Methoxy Benzaldehyde.

Results: The Perliminary analysis of extract revealed the presence of protein, alkaloids, flavonoids, phenols, terpenoids and cardiac glycosides.Thin Layer Chromatography showed the spot observed at 254nm and 366nm. RF value of obtained sample compared with standard it was obtained 0.65 and 0.68 under 254 and 366 nm, hence confirmed. The plate obtain prominent spots after spraying with iodine spray.Total eleven biocompounds detected in GC-HRMS in which 2-Hydroxy 4-Methoxy Benzaldehyde detected at 10.52 RT. Helium was used as carrier gas at 1 mL/min.

Conclusion: The developed TLC method was demonstrated to be effective, simple, specific, sensitive, accurate, precise, and economical and can be used for comparative study of compound with standard.GC-HRMS technique was significant in rapid detection and confirmation of all the biocompounds present in herbal exract.The technique based on Retention Time of specific molecule. It will effective in bulk production of syrup and tablet formulations.

Keywords: *Hemidesmus indicus* (L), 2-Hydroxy-4-Methoxy Benzaldehyde, Phytochemical Analysis, Comparative study,TLC,GC-HRMS.

I. INTRODUCTION

Hemidesmus indicus is belonging to the family Asclepiadaceae. It is laticiferous twining climber. It roots are endless, so it's named in Marathi as Anantmuli. It is widely used in Ayurvedic and Unani medicine [1] *Hemidesmus* roots are effective against certain dangerous diseases, in leprosy, leucoderma, asthama, bronchitis, it also effective against snake bite, piles, paralysis, diabetics and urinary dysfunctions [2,3,4]. Hemidesmus indicus also grown in different parts of world such as Malaysia, Indonesia, Pakistan, Bangladesh and Sri Lanka. [5,6 ,7,8,9]Pandhy *et al.,* studied tri terpenoids found in roots of *H. indicus*. *Hemidesmus indicus* found very effective hepatotoxicity against rifampicin and isoniazid-induced in rats [10,11] Pharmacognostical investigation and adulterants of *H. indicus* also evaluated by Prasad *et al.,*[12,13]. The phenolic compound found in roots 2-hydroxy 4-Methoxy Benzaldehyde proved to be responsible for sweet fragrance. *H. indicus* has antimicrobial, anti-inflammatory, antioxidant, antidysenteric, antipyretic, hepatoprotective, antileprotic action [14,15,16]

The *H. indicus* methanol extract showed maximum phytochemical. The methanol showed maximum Rf value in HPTLC analysis. So its extract should be studied by column chromatography, for isolation of biologically active compounds. [17].Antibacterial assay along with phytochemical analysis performed by [18]of

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
Volume:02/Issue:11/November -2020 Impact Factor- 5.354 www.irjmets.com

H. indicus for confirmation of antibacterial compounds which are present in this plant. It was also evaluated that *H. indicus* herb had effective antimicrobial activity.[19].

HPTLC analysis of ethyl acetate extract of *H. indicus* showed the presence of Ferulic acid. It is one of the important phytoconstituents found in root extract. The chemical constituents are found in *H. indicus* constituents are sarsaponin, pmethoxy smilacin, salicylic aldehyde, beta sitosterol, smilgenin, sarsapogenin, sitosterol, stigmasterol, fatty acids and tannins . *H. indicus* proved that the its roots found to be many biocompounds like 2-hydroxy-4-methoxy Benzaldehyde, hemidesmol, lupeol, amyrins, β-sitosterol, lupeol acetate ,α amyrin, β- amyrin, amyrin acetate, lupeol octacosonate, hemsidesmin were isolated[20]. It also showed tannin and resin in root extract of *H.indicus* [21]

Plate no. 1: *Hemidesmus indicus* entire plant **Plate no. 2:** *Hemidesmus indicus* Roots.

Table No:-1. Scientific Classification of Hemidesmus indicus (L).

Kingdom	Plantae
Sub-kingdom	Tracheobionta
Super-Division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Sub class	Asteridae
Order	Gentianales
Family	Apocynaceae
Genus	*Hemidesmus*
Species	*Indicus(L)*

II. MATERIALS AND METHODS

Collection and identification of plant material:- The roots and plant of *Hemidesmus indicus* plant was collected from Dhanwantray Botanical Garden Rahuri of Ahmadnagar district, Maharashtra from July to August 2020, identified by Prof. and Head of Department of Botany, Dr. Babasaheb Ambedkar University Aurangabad. The Accession number obtained (0698).Roots of Plant material properly shade dried followed by Trey Dryer at temperature 40°c, to avoid denaturation of phytoconstituents. Drying followed by crushing in to powdered form of uniform size and stored in sealed dry glass container for further use.

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
Volume:02/Issue:11/November -2020 Impact Factor- 5.354 www.irjmets.com

Preparation of extract

Dried powder of roots (50gm) extracted with petroleum ether (200ml) in soxhlet apparatus for about 8 Hrs. The ratio of powder to solvent (1:4). The extract was then concentrated and evaporated with rotary evaporator. After evaporation the extract is kept in small vials at freezing temperature until its use. Root Extracts tested for their qualitative analysis by preliminary phytochemical analysis. All the tests were carried out by Using Practical Pharmacognosy book of Khandelwal.[28]There were some preliminary qualitative biochemical analysis performed out by using practical bool of Kokate. Practical pharacognosy books[29].The target was to evaluate and compare phenolic compound 2-hydroxy 4-Methoxy Benzaldehyde with standard.

Thin layer Chromatography:- Both Preparative and Analytical TLC performed for evaluation and comparision of 2-hydroxy-4-Methoxy Benzaldehyde. Preparative TLC performed on a sheet of aluminium foil coated with a thin layer of adsorbent silica gel, 60 F254 (Merck).

Petrolium Ether extract again redissolved in 1ml of Pet.Ether for TLC purpose. Two spots of sample extracts and single spot of 2-Hydroxy 4-Methoxy Benzaldehyde also spotted carefully on TLC plate with separate capillary tubes. It kept in solvent system (Mobile phase) for few minutes, still sample run properly on TLC. This plates was viewed in UV chamber and Rf values were recorded.The mobile phase used was same in both Hexane: Ethyl acetate (7:3) ratio.RF value of obtained sample compared with standard it was obtained 0.65 and 0.68 under 254 and 366 nm.These Rf values obtained from the phytochemicals provide the important information about their polarity and important clues for the separation of these phytochemical in the separation process. Different Rf values of the compound also reflect an idea about their polarity by the use of the various solvent systems for TLC studies could be important for the selection of the appropriate solvent system. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

The Analytical TLC plates also prepared for confirmation of comparative studies of 2-Hydroxy 4-Methoxy Benzaldehyde.It was prepared by using Silica gel 'G' as about 20 gm of silica gel was weighed and mixed in 50ml distilled water to obtain homogenous suspension. Suspension was spread uniformly over the plate which was air dried until the transparency of the layer disappeared. The plates were dried in hot air oven at 110°C for about 60 min. Samples were applied usually 1-10µl volumes to the origins of a TLC plate 2c.m above its bottom with the help of capillary tubes. The retention factor (Rf) values the compound was calculated for each extracts using the following formula.

Rf = Distance traveled by the solute/ Distance traveled by solvent

Standard Preparation:

Standard stock solution containing 10 mg mL-1 of 2 hydroxy-4 Methoxy Benzaldehyde was prepared by dissolving 10 mg of 2-hydroxy-4-Methoxy Benzaldehydein 10mL methanol. The pure compound applied on TLC plated along with samples. It is allow running in selected solvent system. Then RF value calculated. The obtained RF values were same for both.

GC-HRMS analysis :-

Crude petroleum ether extract of *Hemidesmus indicus roots* were used for GC-HR MS investigation for confirmation of 2-Hydroxy 4-Methoxy Benzaldehyde .Determination of same compound performed from roots of *Decalepis hamiltonii and H. indicus.*[22]

The GC-MS analysis was carried out using a Agilent, 7890, FID detector, Head Space injector Combipal autosampler. Column temperature program Initial temperature 1200C for 3 min. Ramp: 80C/min to 2700C. Again isothermal for 3 min, then ramp at 100C/min. to 2800C, isothermal for 12 min., Column used was HP5, The injector temperature was 2000C, detector temperature was 2800C.

Helium was used as carrier gas at 1 mL/min. Mass spectral scan range was set at 10 - 2000 amu, Mass resolution – 6000. A gas chromatograph coupled with mass spectrometer (GC-HRMS) is a combined analyzer that has a superior ability in analysing organic compounds qualitatively and quantitatively. The components in the extract were identified based on the mass spectra of latest NIST library data having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. (SAIF IITB).

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
Volume:02/Issue:11/November -2020 Impact Factor- 5.354 www.irjmets.com

III. RESULT AND DISCUSSION

Roots of H. indicus were extracted in petroleum ether and extract percentage found to be 2.50%. Petroleum ether extract contains lipids, fatty acids, sterols, steroid components of plant.[23]

Table No. 2. Biochemicals Activity of *Hemidesmus indicus*

TESTS	OBSERVATION
Test for Carbohydrates.	
Molischs Test.	--
Fehlings Test.	--
Benedicts test	--
Barfoed test	--
Test for pentose sugar.	--
Test for hexose sugar.	--
Test for mucilaes	--
Test for Proteins.	
a.Biuret test.	++
b. Millons test	++
Test for Alkaloids	
a.Dragendorffs test	++
b. Mayers Test	+++
c. Hagers reagent	++
d. Wagners test	++
Test for Flavonoids	
a.Shinoda Test	++
b. Sulphuric Acid	++
c. Lead Acetate Test	++
d.Zinc powder Test	++
Test for phenols	
a.5%$FeCl_3$ Sol^n	++
b. Lead acetate sol^n	++
c. Gelatin Sol^n	++
d.dil. Iodine	++
e. dil. HNO_3	++
Test for Saponin	
Foam test	--
Test for Amino acids	
a.Ninhydrin test	++
Test for Tryosine	--
Test for Tryptophan	--
Test for Terpenoids	++
Test for Tannins	--
Test for Steroids	--
Test for cardiac glycosides	--

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
Volume:02/Issue:11/November -2020 Impact Factor- 5.354 www.irjmets.com

(+) indicates less concentration, (++) moderate , (+++) high concentration and (-) indicates absence of that chemical constituent in the plant sample.

The Pet Ether extract showed presence of protein, alkaloids, flavonoids, phenols, terpenoids and cardiac glycosides. But absence of carbohydrates, mucilage, steroids, tannin,tryptophan, tyrosine in tested extract of Pet. Ether. Evaluation of phytochemical composition of Licorice, Indian Ginseng, Indian Madder and Indian Sarasaparilla also performed by Rekha and Parvati.[24].*H. indicus* roots also used in medicated talcum powder.[25].

Thin Layer Chromatography :-

The solvent system selected for pet. Ether extract was Hexane: Ethyl acetate (7:3) ratio. The spot observed at 254nm and 366nm were as given below. The plate obtain prominent spots after spraying with iodine spray. The plate dried at 105°C in hot air oven till the colour of the band appears.The Rf values were as given in (table-3). The sample spot obtained on 0.65 and 0.68_matched with spot of standard 2- Hydroxy 4-Methoxy Benzaldehyde.The same compound also studied from root culture of *H. indicus.*[26]. Antihyperglycemic and antioxidant and antidyslipidemic properties of *Hemidesmus indicus* root extracts studies on alloxan induced experimental diabetes in rats.[27].

Table No- 3. TLC of Pet. Ether Extract of *H. indicus.*

Observed at 254nm		**Observed at 366nm**	
Rf value	**colour**	**Rf value**	**colour**
0.05	Light black	0.05	Light blue
0.10	Light black	0.09	Light blue
0.20	Light black	0.20	Light blue
0.47	Deep black	0.29	Light blue
0.65	Blueish green	0.34	Light blue
0.71	Light black	0.45	Light blue
-	--	0.51	Light blue
-	---	0.68	Bluish green

GC-HRMS analysis :-

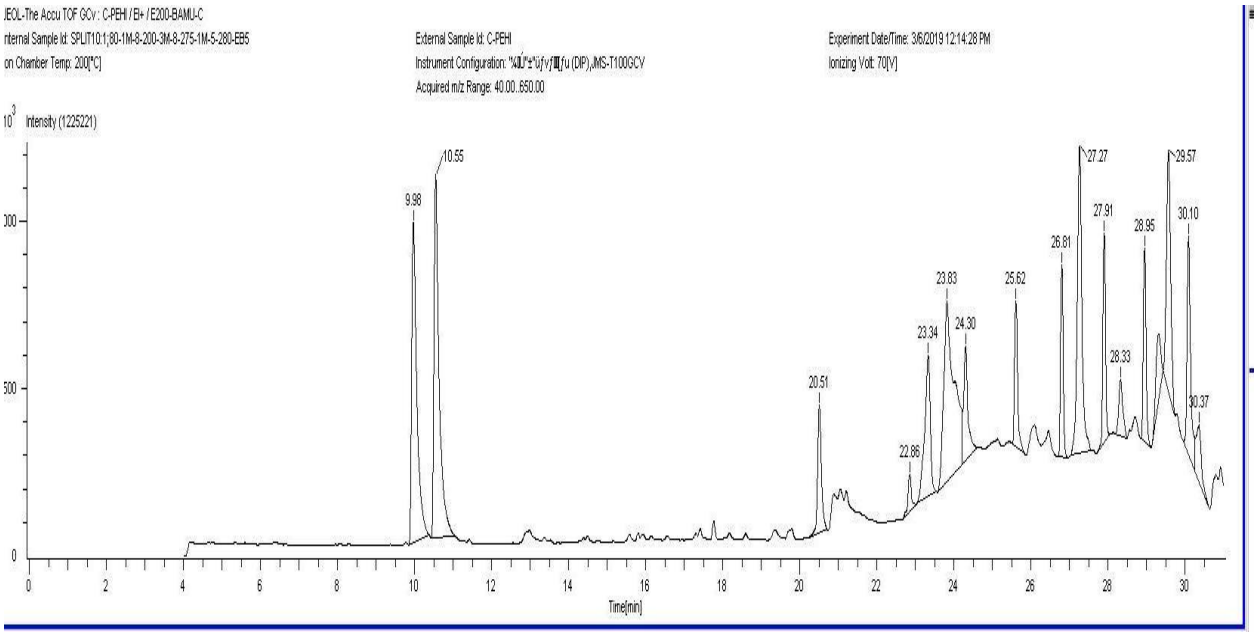


Figure No. 1. Graph of *Hemidesmus indicus* Petrolium Ether extract by GC-HRMS

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
Volume:02/Issue:11/November -2020 Impact Factor- 5.354 www.irjmets.com

Table No. 4. Total Ion Chromatogram of Pet. Ether extract of *H. indicus.*

#	Time [min]	Type	Peak Width(FWHM) [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
	9.98	BB	0.1277	8713311.23	955064.91		9.85	33409	10.37	63488
	10.55	BB	0.1256	10091730.81	1084136.95		10.44	53305	11.06	58747
	20.51	BB	0.0995	2620168.66	381909.65		20.25	55819	20.71	79214
	22.86	BB	0.0799	654692.96	110977.54		22.67	108536	23.01	152905
	23.34	BB	0.1458	4278163.41	420147.25		23.05	157220	23.55	192772
	23.83	BV	0.3603	10032225.20	537117.77		23.60	194536	24.22	274386
	24.30	VB	0.1054	2677455.60	340829.41		24.22	274386	24.61	323652
	25.62	BB	0.0793	2336828.92	436113.75		25.52	334795	25.84	304263
	26.81	BB	0.0779	2811888.68	572970.76		26.69	296225	26.96	293460
	27.27	BB	0.1195	7734881.56	918191.89		27.04	301735	27.56	313687
	27.91	BB	0.0782	3127088.88	624982.62		27.74	309967	28.06	364664
	28.33	BB	0.1074	1237467.43	169048.53		28.18	364441	28.49	351638
	28.95	BB	0.0807	2981397.04	576327.48		28.85	351451	29.12	325343
	29.31	BB	0.1648	1754905.58	201653.39		29.15	337292	29.43	556235
	29.57	BB	0.1182	5444364.35	715622.82		29.43	556235	29.73	425207
	30.10	BV	0.0868	4155950.68	655592.06		29.96	343208	30.26	252886
	30.37	VB	0.1621	1599186.60	168941.72		30.26	252886	30.61	146521

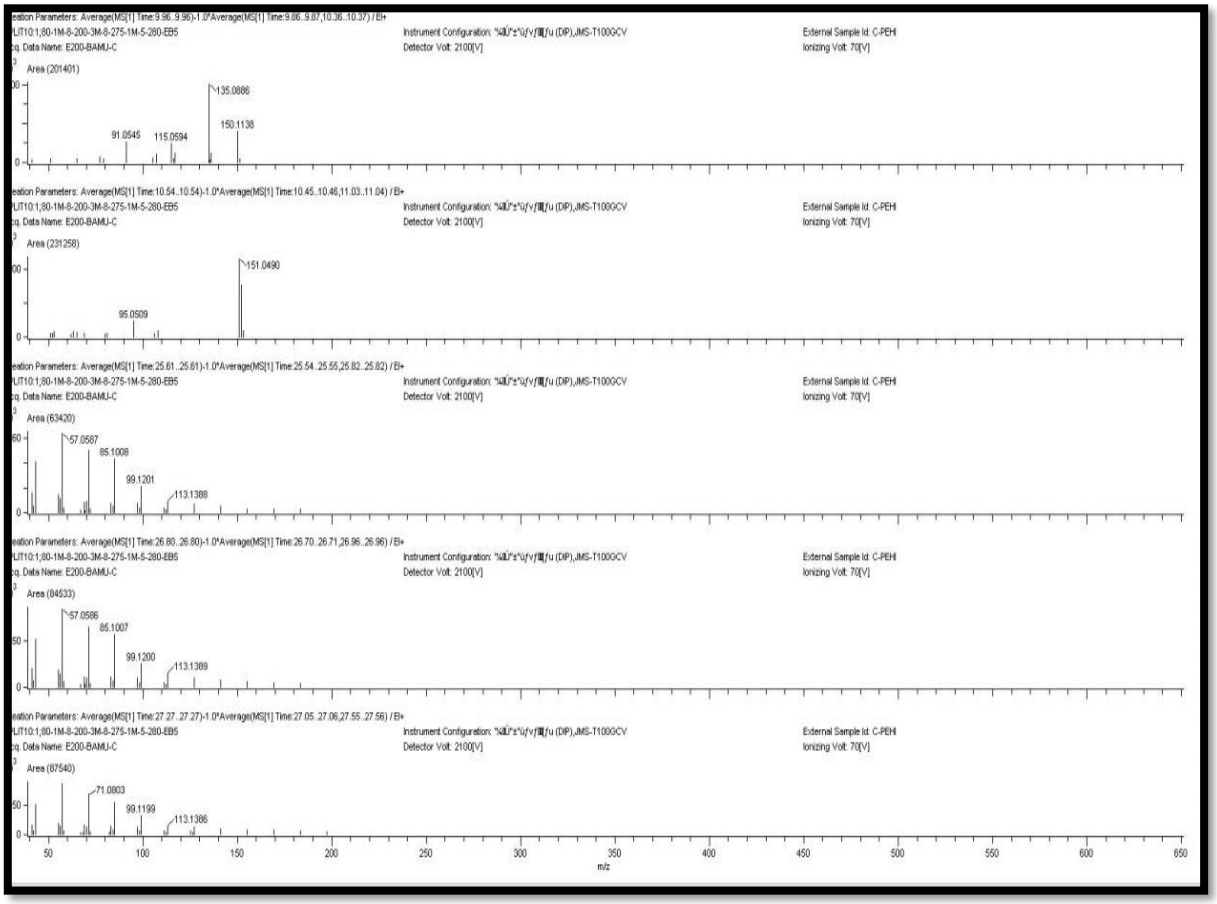


Fig.2. Fragmentation pattern-1. found in PEHI extract.

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
Volume:02/Issue:11/November -2020 Impact Factor- 5.354 www.irjmets.com

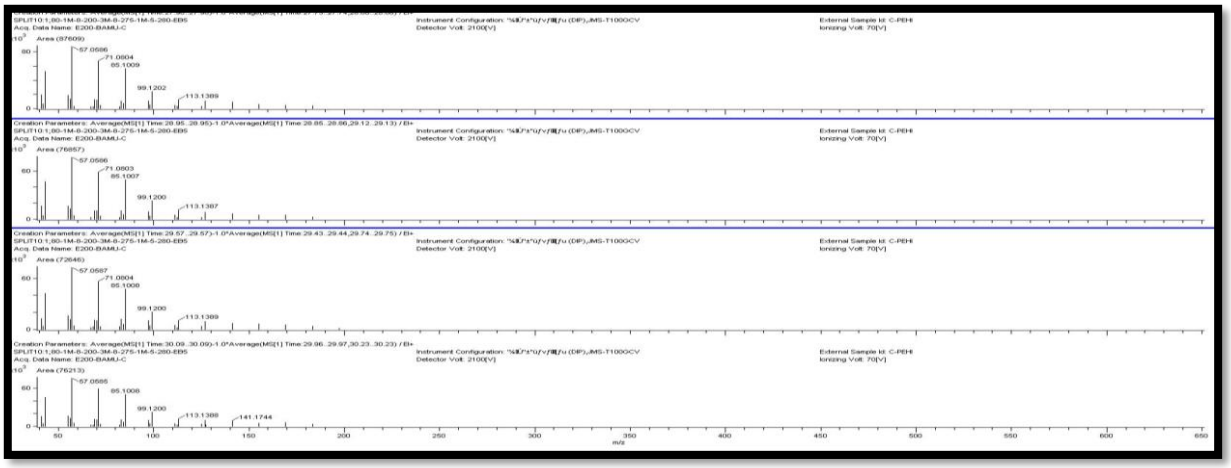
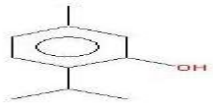
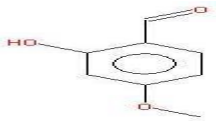
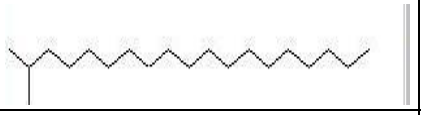
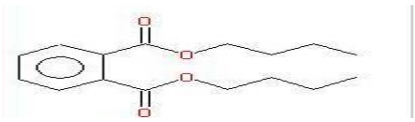
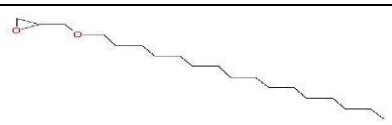
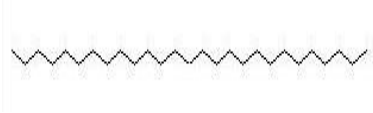
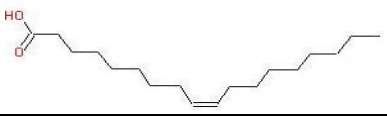
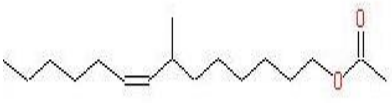
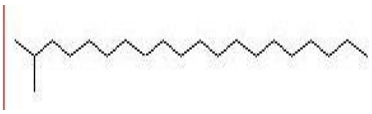


Fig.No.3. Fragmentation Pattern-2. found in GC-HRMS of Pet Ether extract of HI.

Table No.5. Compounds obtained in Pet. Ether extract of *H. indicus.*

S.N	Name of compound	M.F	M.W	Structure	RT
1	Thymol	C10H14O	150		9.9
2	**2-hydroxy-4-methoxy Benzaldehyde**	**C8H8O3**	**152**		**10.5**
3	Nonadecane,2-methyl	C20H42	282		20.5
4	Dibutyl phthalate	C16H22O4	278		22.8
5	n-hexadecanoic acid	16H32O2	256		23.3
6	Oxirane	C19H38O2	298		23.34
7	Heptacosane	C27H56	380		24.2
8	Oleic acid	C18H34O2	282		27.9
9	7-methyl-Z-tetradecen-1-ol acetate	C17H32O2	268		28.9

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
Volume:02/Issue:11/November -2020 Impact Factor- 5.354 www.irjmets.com

10	Heptacosane	C27H56	380		29.3
11	Eicosane,2-methyl	C21H44	296		29.5

The Pet. Ether extract also showed presence of target compound 2-hydroxy-4-methoxy Benzaldehyde. In the Pet. Ether extract the concentration of 2-hydroxy-4-methoxy Benzaldehyde was highest among all. The second highest concentration compound was Thymol having mol. Formula of (C10H14O) with MW 150 and RT 9.9 min. The total main biomolecules obtained in Pet. Ether extract was eleven.Formula of (C10H14O) with MW 150 and RT 9.9 min. The total main biomolecules obtained in Pet. Ether extract was eleven.HR-GCMS showed presence of 2-Hydroxy 4-Methoxy Benzaldehyde on RT of 10.5 with molecular weight of 152amu, having molecular formula $C_8H_8O_3$.

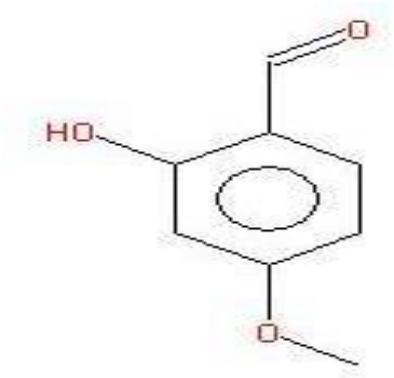


Fig. No.4. Chemical structure of of 2-Hydroxy 4-Methoxy Benzaldehyde

IV. CONCLUSION

In the present study 11 compounds from the Pet. Ether extract of *Hemidesmus indicus* (L).were identified by Gas-chromatography with High Resolution Mass spectrometry (GC-HRMS) analysis. Most of the identified phyto-components used for antimicrobial, antioxidant, anticancerous activities. The research findings have shown that the Pet. ether extract is extensively rich in secondary metabolites, terpenes and fat soluble vitamins. These findings have provided scientific basis to the ethno medical usage of the plant. However, isolation of the individual phytochemical constituents, subjecting it to biological activity and toxicity profile will give fruitful results. This plant can therefore be explored for further research studies based on isolation and characterization of bioactive components.

ACKNOWLEDGMENT

The authors extend their sincere thanks to Sigma Aldrich Pune (India), for providing pure standard 2-Hydroxy 4-Methoxy Benzaldehyde for comparative study. We also extend our thanks to the Head of Department and Pro. Vice Chancellor Dr. Wakte P.S. Department of Chemical Technology Dr. Babasaheb Ambedkar Marathwada University Aurangabad, for providing the

necessary facilities in laboratory.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

MK and CA designed the study. MD performed the experiment and analyzed the data and reviewed it. NK supervised the experiment, reviewed the data, and supported for writing the research paper.

CONFLICT OF INTERESTS

Authors declare that they have no conflict of interest exists in this investigation.

V. REFERENCES

[1] Anonymous. The Wealth of India, Publication and Information Directorate, CSIR, New Delhi, 1997; p. 181.

[2] Vaidya K and Kulkarni P.H.A study of an Ayurvedic formula viz "Jivak", Vol 7, Deerghaya International.1991; 20.

[3] Nadkarni, A.N.Indian Material Medica, 1989; Vol. I, Popular Book Depot, Bombay, India.

[4] Alam, M.I., Auddy, B. and Gomes, A.Viper venom neutralization by Indian medicinal plant (*Hemidesmus indicus* and *Pluchea indica*) root extracts. *Phytotherapy Research*, 1996;10(1): 58-61.

[5] Sasidharan, N.Biodiversity Documentation for Kerala. Flowering Plants.2004; *Kerala Forest Research Institute, Peechi,* Kerala, India.

[6] Siddique, N.A. and Bari, M. A Plant regeneration from axillary shoot segments derived callus in *Hemidesmus indicus* (L.) R. Br. (Anantamul) an endangered medicinal plant in Bangladesh. *Journal of Plant Sciences*, 2010; 5(1): 61-67.

[7] Anonymous. The useful plants of India.1986; CSIR, New Delhi, India.

[8] Anonymous. The Wealth of India. Raw materials, 1997;Vol. III, V and X, CSIR, New Delhi, India.

[9] Nayar, T.S., Beegam, A.R., Mohanan, N. and Rajkumar, G. Flowering Plants of Kerala: 2006; A Handbook of Tropical Botanic Garden and Research Institute, Thiruvanathapuram,Kerala, India.

[10] Padhy, S. N., Mahato, S. B. and Dutta, N. L.Triterpenoids from the roots of *Hemidesmus indicus. Phytochemistry,* 1973; 12(1): 217-218.

[11] Prabakan, M., Anandan, R. and Devaki, T. Protective effect of *Hemidesmus indicus* against rifampicin and isoniazid-induced hepatotoxicity in rats. *Fitoterapia*,2000; 71(1): 55-59.

[12] Prasad, S., Wahi, S.P. and Mishra, L.C.Pharmacognostical investigation on Indian Sarasaparilla, its substitutes and adulterants, Part I, *Hemidesmus indicus* R. Br. *Indian Journal Pharmacy,*1964; 36: 81.

[13] Prasad, S. and Wahi, S.P.Pharmacognostical investigation on Indian Sarasaparilla Part II. Root and root-stock of *Hemidesmus indicus* R. Br. *Indian Journal of Pharmacy,1965;* 37: 35-39.

[14] Sreekumar, S. and Seeni, S.A process for the manufacture of medicated talcum powder from biotechnologically derived root extracts of *Hemidesmus indicus* R. Br.1999; Parent application field at regional patent office, Chennai, India.

[15] Sreekumar, S.; Seeni, S. and Pushpangadan, P.Production of 2-hydroxy 4-methoxy benzaldehyde using root cultures of *Hemidesmus indicus*. Biotechnol. Lett.,1998; 20:631-635.

[16] Sahoo, A.K.Plants used in ophthalmic drugs in Phulbani, Orissa. In Pushpangadan P, Nayman, U.L.F. and George, V. (Eds), Glimpses of Indian Ethnopharmacology1995; (pp:173-178). T.B.G.R.I., India.

[17] Das Manosi, Banerjii Avijit, Dutta Sreya , Saha Sanjoy , Mondal D.N and Hazra Jayram. Phyto-Pharmacognostical Evaluation And HPTLC Study On Anantamul (*Hemidesmus Indicus* . (R.Br.) Root.Int. J. Res. Ayurveda Pharm.2017; 8 (3).

[18] Mohan Ch., S. Sridhar, B. Mohan, SB. Krishnaveni and S. Karnakar Reddy. Antibacterial activity and phytochemical screening of *Hemidesmus indicus* L. B. BR. Int. J. Pure App. Biosci. 2015; 3 (2): 221-225.

[19] Mehmood Zafar, Dixit K Arunesh, Singh Ajay, S. Farooq, Chhimwal Sumanlata, Indian Herb *Hemidesmus indicus* –A Potential Source Of New Antimicrobial Compound. Medical chemistry , 2016; 6:12: 734-738.

[20] Chatterjee I. Chakravarty AK and Gomes A.*Daboia russelli*iand *Naja kaouthia*venom neutralization by lupeol acetate isolated from the root extract of Indian sarasaparilla *Hemidesmus indicus*R. Br., J. Ethnoparmacol. 106 (1) ; 2006 : 38-43.

[21] Murthi B.R and Seshadri T.R. A study of the chemical components of the roots of *Decalepis hamiltoni,* Part III. Comparison with *Hemidesmus indicus* (Indian Sarasaparilla). Proceeding Indian.Acad. Sci.1941; 13 ; 3999-4003.

[22] Nagarajan, S.; and Rao, J.M. Determination of 2-hydroxy 4- methoxy benzaldehyde in roots of *Decalepis hamiltonii* (Wight and Arn.) and *Hemidesmus indicus* R. Br. J.A.O.A.C. Int.,2003; 86:564-567.

[23] Nagarajan, S. and Rao, J.M.Triterpenoids from swallow roots : A convenient HPLC method for separation. J. Chromatographic Sci.,2007; 45:189-193.

[24] Rekha, S. and Parvathi, A. Evaluation of phytochemical constituents of the roots of Licorice, Indian Ginseng, Indian Madder and Indian Sarasaparilla. Int. J. Pharma. and Bio. Sci., 2012; 3(2b):357-362.

[25] Sreekumar, S. and Seeni, S. A process for the manufacture of medicated talcum powder from biotechnologically derived root extracts of *Hemidesmus indicus* R. Br. Parent application field at regional patent office, 1999; Chennai, India.

[26] Sreekumar, S.; Seeni, S. and Pushpangadan, P.Production of 2-hydroxy 4-methoxy benzaldehyde using root cultures of *Hemidesmus indicus*. Biotechnol. Lett.,1998; 20:631-635.

[27] Subramanian, S.; Aparna, A. and Thamizhiniyan, V. Antihyperglycemic, antioxidant and antidyslipidemic properties of *Hemidesmus indicus* root extracts studies in alloxan induced experimental diabetes in rats. I.J.P.S.R., 2012; 3(1): 227-234.

[28] Khandelwal K.R. The 9th Edition., Preliminary Phytochemical Screening, Chapter 40 in Practical Pharmacognosy Techniques and Experiments, Pune: Nirali Prakashan, 2002; 149-57pp. 2.

[29] Kokate CK, Purohit AP and Gokhale SB . Practical pharmacognosy,2003 Nirali Prakashan Pune.