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GC-MS Analysis of a medicinal plan:t *Hemidesmus indicus*

Botany

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ABSTRACT

The aim of present study was to screen the total phytochemicals present in the roots. One medicinal plant was selected for scientific validation of ethnomedicinal knowledge collected from local people of Jharkhand. The GC-MS study summarized the presence of a total 47 phytochemical constituents in methanolic extracts of *Hemidesmus indicus*. These constituents may be responsible for pharmacological activities.

KEYWORDS:

Medicinal Plants, Phytochemicals, Ethnomedicine, GC-MS Analysis

Introduction

The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. Medicinal herbs are significant source of synthetic and herbal drugs. In the commercial market, medicinal herbs are used as raw drugs or extracts. Isolated active constituents are used for applied research. For the last few decades, phytochemistry has been making rapid progress and herbal products are becoming popular. Medicinal herbs are considered to be chemical

factories as they contain multitude of chemical compounds like alkaloids, flavanoids, glycosides, saponins, resins, oleoresins, sesquiterpene lactones and oils. Several bioactive constituents have been isolated and studied for pharmacological activity [1].

Hemidesmus indicus R.Br. belongs to the family Asclepiadaceae widely used in folk medicine is a slender, laticiferous, twining, sometimes prostrate or semi-erect shrub. Roots are woody and aromatic. The stem is numerous, slender, terete, thickened at the nodes. The leaves are opposite, short-petioled, very variable, elliptic-oblong to linear-lanceolate. The flowers are greenish outside, purplish inside, crowded in sub-sessile axillary cymes [2].

This article briefly describes the extraction methodologies used in the preparation of herbal extracts and reviews the utility of chromatography-mass spectrometry for the analysis of their active components.

Materials and methods:

Collection of plant material: Roots of *Hemidesmus indicus* was collected from the Biodiversity Park, Namkum, Ranchi. The roots were washed thoroughly 2-3 times with running tap water and kept in shade to dry after final washing with autoclaved water.

Solvent Extraction: The methanolic extracts were prepared. For the plant cold extraction was performed. The dried powders of plant mix with desirable amount of methanol (1:10 ratio) were placed in shaker incubator for 48 hours. The extract was filtered; the filtrate obtained was dried and dried extract was re dissolved in Dimethylsulphoxide (DMSO) to form stock solutions, which were filter sterilized (0.2µm) before testing.

GC-MS Analysis: The GCMS analysis of Plant extracts was performed using GC-MS SHIMADZU MS 2010 equipped with AB innowax column (60 × 0.25 mm id, film thickness 0.25 µm). Initially, oven temperature was maintained at 50×C for 3 min and temperature was gradually increased up to 280°C at 30 min and 0.2 µl of sample was injected for analysis. Helium was the carrier gas. The flow rate of helium gas was 1.2 ml/min. The sample injector and mass transfer line temperature were set at 270 and 280°C and split ratio is 20 throughout the experiment periods. The ionization mass spectroscopic analysis was done with 70 eV. Mass spectra were recorded across the range of 40 to 1000 m/z for the duration of 35 min. Identification of components was based on comparison of their mass spectra with those of Wiley and NIST libraries and those described by Adams [3] as well as on comparison of their retention indices with literature [4, 5].

RESULTS AND DISCUSSION

GC-MS Analysis:

Phytochemical analysis of this plant was done through gas chromatography and mass spectroscopy. In the GC-MS analyses, total 47 compounds were identified in the Methanolic extract of *Hemidesmus indicus* .The identification of phytochemicals is based on the peak area (which represents the percentage of that compound), molecular weight and molecular formula. The chromatogram are present in Figure 1. LUP-20(29)-EN-3-YL ACETATE (32.13%), OCTADEC-9-ENOIC ACID (13.00%), l-(+)-Ascorbic acid 2,6-dihexadecanoate(5.20%),5-(HYDROXYMETHYL)-2 FURALDEHYDE(4.78%), Vanillin lactoside (4.56%), MOME INOSITOL (4.11%) , are the major components whereas some other minor components are also present (Table 1, Fig. 2).

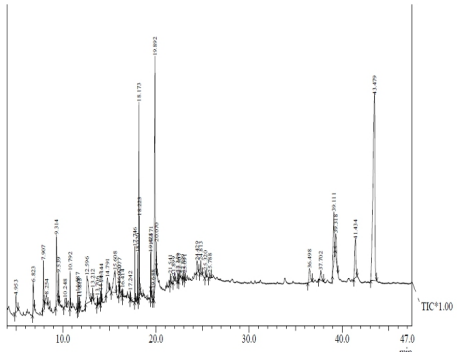

Fig. 1: Chromatogram (GC/MS) of the methanolic extract of *Hemidesmus indicus*

Table 1: Chemical constituents present in the methanolic extract of *Hemidesmus indicus*

Peak	R.Time	Area	Area%	Name of compounds
1	4.953	3279736	1.28	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
2	6.823	4595955	1.80	1,3,5-TRIAZINE-2,4,6-TRIAMINE
3	7.907	4269872	1.67	2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL-4H-PYRA
4	8.254	2519330	0.99	1,2,3-PROPANETRIOL
5	9.314	12191723	4.78	5-(HYDROXYMETHYL)-2-FURALDEHYDE
6	9.539	3071326	1.20	1,2,3-PROPANETRIOL, 1-ACETATE
7	10.248	1557459	0.61	Heptanoic acid, 6-oxo-

8	10.792	2274744	0.89	Benzaldehyde, 2-hydroxy-4-methoxy-
9	11.587	858992	0.34	TETRADECANE
10	11.697	708776	0.28	ISOQUINOLINE, 3-METHYL-, 2-OXIDE
11	11.813	459612	0.18	BENZALDEHYDE, 4-HYDROXY-3-METHOXY-
12	12.596	11634798	4.56	Vanillin lactoside
13	13.212	871638	0.34	Phenol, 3,5-bis(1,1-dimethylethyl)-
14	13.706	508291	0.20	(3,4-DIMETHOXYPHENYL) (METHOXY)METHANOL
15	14.061	522120	0.20	9-Eicosene, (E)-
16	14.144	838262	0.33	Hexadecane
17	14.791	4177411	1.64	1,3,4,5-TETRAHYDROXY-CYCLOHEXANECARBOXY
18	15.608	10501952	4.11	MOME INOSITOL
19	16.020	420106	0.16	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol
20	16.077	378975	0.15	Tetradecanoic acid
21	16.414	404072	0.16	Heptadecane
22	17.242	506266	0.20	8-Octadecanone
23	17.746	2623370	1.03	Hexadecanoic acid, methyl ester
24	18.020	2159883	0.85	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydro
25	18.173	13285622	5.20	l-(+)-Ascorbic acid 2,6-dihexadecanoate
26	18.223	4974245	1.95	1,2-BENZENEDICARBOXYLIC ACID, DIBUTYL ESTE
27	19.425	1800796	0.71	9,12-OCTADECADIENOIC ACID (Z,Z)-, METHYL EST
28	19.471	2038514	0.80	9-Octadecenoic acid (Z)-, methyl ester
29	19.688	551586	0.22	Methyl stearate
30	19.892	33178971	13.00	OCTADEC-9-ENOIC ACID
31	20.070	1711241	0.67	Octadecanoic acid
32	21.541	868563	0.34	(R)-(-)-14-Methyl-8-hexadecyn-1-ol
33	21.899	570710	0.22	cis-1,2-Cyclododecanediol
34	22.349	588347	0.23	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester
35	22.507	745328	0.29	(R)-(-)-14-Methyl-8-hexadecyn-1-ol
36	22.899	268936	0.11	OCTADECANAL
37	23.091	367496	0.14	BIS(2-ETHYLHEXYL) PHTHALATE
38	24.429	2317005	0.91	(R)-(-)-14-Methyl-8-hexadecyn-1-ol
39	24.813	1609059	0.63	PREGNA-5,16-DIEN-20-ONE, 3-HYDROXY-, (3.BETA.)
40	25.320	846347	0.33	9-OCTADECENAMIDE

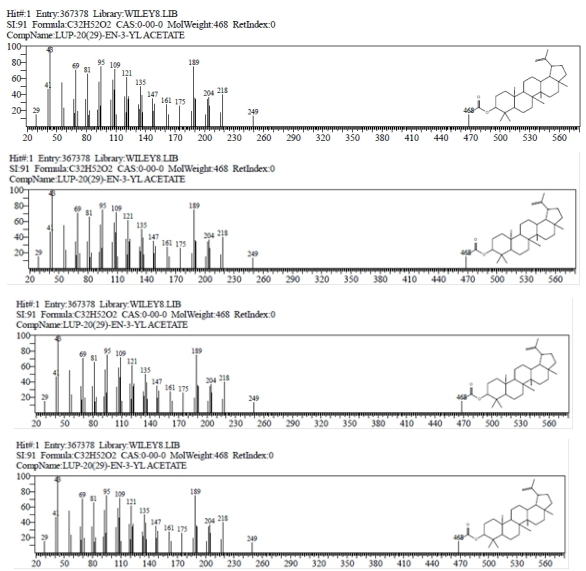

Fig. 2: Chemical structure of some major compounds *Hemidesmus indicus.*

This study highlighted the presence of many secondary metabolites in the root parts of *Hemidesmus indicus*, provide an overview of the different classes of molecules present that have led to their pharmacological activities. This study confirmed that the plant extract could be used for the treatment of various diseases. The GC-MS analysis of extracts showed the presence of various types of anticancer compounds like 2-hydroxy, 4-methoxy benzoic acid having anti-carcinogenic activity.

Cancer is a disease recognised by seven hallmarks: unlimited growth of abnormal cells, self sufficiency in growth signals, insensitivity to growth inhibitors, evasion of apoptosis, sustained angiogenesis, inflammatory microenvironment and eventually tissue invasion and metastasis [6,7,8].

From the year 1981–2002 reports showed that approximately 60% of anticancer agents are derived from natural products. Herbal drugs do not only serve as drugs but also provide a rich source of novel structures that may be developed into novel anticancer agents [9].

Plant-based compounds are well known for the development of several clinically useful anti-cancer drugs i.e. including taxol, vinblas-tine, vincristine, the camptothecin derivatives, topotecan and irinotecan and etoposide derived from epipodophyllotoxin [10].

These molecules might act as cancer-blocking agents, preventing initiation of the carcinogenic process and as cancer-suppressing agents, inhibiting cancer promotion and progression [11]. In addition a number of other mechanisms are also involved in the process. Kumar and Pandey [12] have described the mechanisms by which flavonoids can exert their anticancer activity.

The DNA replication may be due to inhibition of DNA topoisomerase II, a key enzyme in DNA replication. The arrest the cell growth in cell cycle, as they reduces the rate of cell division by preventing the entry of cell into prophase and subsequent phases which was concluded from the antimitotic and antiproliferative results that may be considered as an alternate mechanism might be also depended upon the morphology of cell lines and mechanism of action of the plant extract [13].

The antioxidants are also known to play a key role in reducing cancer cell proliferation and tannins are known as strong lipid peroxidation inhibitors [14, 15]. Hence, due to the presence of tannins and related compounds as the major compounds in plant extracts may play anti-cancer activities.

CONCLUSIONS

From the results of the present study it could be concluded that Hemidesmus indicus could be used as sources of potent agents for anticancer drug development. As such these plants could be further investigated for the individual components of the methanolic extract is recommended to try to determine the ingredient(s) that may be responsible for its anticancer effects and the mechanism of growth inhibition, which may be used as natural and low cost drugs to fight cancer with minimal or no side effects.

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