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GC-MS ANALYSIS AND ANTIOXIDANT POTENTIAL OF PETROLEUM EXTRACT OF SEEDS OF *PSORALEA CORYLOFOLIA*

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ABSTRACT

Plants show various medicinal properties due to presence of phytoconstituents. Gas chromatography-mass spectroscopy is a technique which is used to identify compounds in a plant extract on the basis of retention time and their mass. In the current investigation, petroleum ether extracts of seed of *Psoralea corylifolia* (family Leguminosae) was subjected for GC-MS analysis. Total 51 compounds were identified out of which many are significant as having antioxidant potential such as ficusin, vitamin E, stigmasterol, gamma sitosterol etc. The extract was also subjected for their free radical scavenging potential by using DPPH assay. Results revealed the presence of good antioxidant activity of the petroleum extract.

Keywords: Phytoconstituents, GC-MS, Psoralea corylifolia, DPPH etc.

1. INTRODUCTION

Over the course of history, therapeutic plants, often known as medical herbs, have seen widespread application in the treatment of a great variety of ailments. It is estimated that there are 750,000 different species of plants on earth, yet only 1% to 10% of those are cultivated for human consumption and medical purpose [1, 2]. Plants are not only the origin of many effective medications, but they will also continue to play an essential role in the screening process for new chemicals of biological and pharmacological significance [3]. Medicinal plants demonstrate a wide variety of pharmacological qualities, including antioxidants [4], anti-diabetes [5], antibacterial, antiviral [6], anticancer [7], and anti-ulcer activity [8]. The fact that medicinal plants contain bioactive secondary metabolites enables them to treat a variety of illnesses that are prevalent in humankind [9].

In the current climate, having an knowing of the chemical components of herbs that have therapeutic potential should not only open the way for the development of advanced drugs, but it should also have a significant roles in the search for new sources of plant components that can be produced profitably [10]. By analysis of various metabolites present in traditional medical practises and medicinal plants, gas chromatography and mass spectrometry are the methods that are utilised [11, 12]. Chromatography of Gases Mass spectroscopy is a system that has been hyphenated and is a technique that is wellsuited for the goal of identification and quantification. It is also the technique that is employed the most frequently. It is possible to determine the unidentified compounds present in a system through the process of interpretation as well as by comparing the spectra to those of reference spectra [13]. Research in the fields of biology and chemistry can benefit greatly from the use of spectrometry conjunction mass in with gas chromatography [14].

Nowadays, GC-MS techniques have been widely employed for the phytoanalysis of medicinal plants since this method has proven to be a trustable methodology for the analysis of phytoconstituents. This is because the GC-MS technique was first developed in the [15]. This metabolite plays a vital role in health care systems [16]. Antioxidants compounds found are in lower concentrations which have capability of lowering oxidation process in body [17]. Antioxidants are substances that shield cells from the potentially harmful effects of reactive oxygen species (ROS), which include superoxide, hydroxyl radicals, singlet oxygen, peroxyl radicals, and others. Antioxidants are also known as free radical scavengers. These are promising agents against oxidative stress [18]. DPPH, or 1,1-diphenyl-2picrylhydrazyl, is a free radical that is rather stable. When its solutions absorb hydrogen from a similar donor, the deep purple coloration (maximum 515-517 nm) that is characteristic of these solutions is lost. Several studies have been done using DPPH as antioxidant system [19]. In the current investigation, petroleum ether extract of seeds of *Psoralea corylifolia* (family Leguminosae) was subjected to DPPH free radical scavenging activity and its phytoconstituents were determined using GC-MS technique.

2. MATERIAL AND METHODS

2.1. Collection of plant material

In the month of February, seed samples of *Psoralea corylifolia* were gathered from the grounds of the University of Rajasthan in Jaipur. The University of Rajasthan's Department of Botany was responsible for the authentication of the herbarium specimens that were deposited there.

2.2. Preparation of plant material

After being washed with tap water, the seeds were allowed to dry at room temp. before being converting into a fine powder using an electric mixer and afterwards being kept in airtight boxes. It was mixed with pet ether and kept at 25°C in incubating shaker for 72 hours. Then, it was filtered. Filtrate was dried and used for further experiment.

2.3. Antioxidant activity assay

For determination of DPPH radical scavenging potential of the extracted sample, 1,1-diphenyl 2-picryl-hydrazil (DPPH) method proposed by Alothman *et al.*, (2009) was applied. The mixing of 100 μ l aliquot form plant extract was done in 3.9 ml taken from 0.1 mM DPPH (methanolic) solution. Then blend was subjected to vortex and left for incubation in the dark for 30 min. Its OD was calculated at 515 nm while methanol was utilized as negative control.

The radical scavenging activity was determined by the ratio = $(Ab_{control} - Ab_{sample} / Ab_{control}) \times 100$

Where $Ab_{control}$ is presenting the absorbance of the DPPH solution and absorbance of the DPPH solution with sample is denoted by Ab_{sample} .

Linear plot of concentration versus % inhibition was plotted and by this IC_{50} values were determined. The antioxidant potential of each extract was showed in form of IC_{50} (stated as the quantity of concentration necessary to prevent DPPH radical development by 50%), find out with the help of inhibition curve.

2.4. GC-MS analysis

The GC-MS examination was carried out at JNU's Advanced Instrumentation Research Facility (AIRF), which is located in Delhi. On a GC-MS QP2010Ultra, an analysis was done using GC-MS. The GC/MS equipment had a Rtx-5MS column with dimensions of 30 0.25 mm 0.25 mdf. This column was made up of 5% diphenyl and 95% dimethyl poly siloxane, and it was set to run in electron impact mode at 70 eV. The injection volume was 1 l, and the carrier gas that was used was helium. The helium flow rate was constant at 1.21 ml/min, and the injection volume was 1 l. (split ratio of 10:1). The temperature of the oven was initially set to be 1000 degrees Celsius for two minutes, after which it was programmed to increase to 250 degrees Celsius at a rate of 100 degrees Celsius per minute for five minutes, and then it was programmed to increase to 2800 degrees Celsius at a rate of 200 degrees Celsius per minute for twenty-one minutes. The operational conditions for the MS were as follows: interface Temperature of 270.000C, Ion Source Temperature of 230.000C, Solvent Cut Time of 3.50 minutes, Scan Speed of 3333, mass scan (m/z)-40-650, and threshold of 1000. In order to identify and quantify the compounds being studied by GC-MS, electron impact ionisation at 70 eV was used to analyse the data, and total ion count (TIC) was utilised to evaluate the data. A calculation was made to determine the relative percentage amount of each compound that was present in the GC-MS spectrum by comparing the average peak area of each individual compound to the overall area of those compounds. For processing mass spectra and chromatograms, the programme known as Turbo mass 5.2 was utilised.

2.5. Identification of components

The components in the extracts were able to be identified by comparing their retention data and mass spectra fragmentation patterns with those that were saved on the computer library and also with published literatures. This was done in order to determine which components were present. For the purpose of matching the components that were identified from the plant material, the library sources NIST08.LIB [20] and WILEY8.LIB [21] were utilised.

3. RESULTS AND DISCUSSION

The vast majority of mammalian species possess an innate defence mechanism that can prevent and repair damage caused by free radicals. In the biochemical system, the superoxide radical and the H2O2 molecule combine with one another to generate a singlet oxygen and hydroxyl radical. These two radicals have the ability to target and destroy practically all known biochemicals [22]. It is possible that the hydroxyl radical that is created will cause sugar fragmentation, base loss, and DNA strand leaking [23]. The majority of ROS are hydroxyl radicals, which are responsible for oxidation of lipids and significant amounts of cellular damage [24]. The findings of this study make it abundantly clear that the PEPN not only eliminates the harmful effects of free radicals but also prevents new free radicals from being produced. Due to the presence of hydroxyl groups, naturally occurring phenolic compounds have been demonstrated to possess free radical scavenging characteristics. This was previously documented [25]. In addition to this, phenolic compounds are powerful hydrogen donors, which gives them their antioxidant properties [26].

In the present investigation, petroleum extract of seeds of the collected plant was subjected for evaluation of antioxidant potential by DPPH free radical scavenging assay. The test solution along with standard antioxidant drug ascorbic acid was evaluated at different concentrations (20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, 100 mg/L). results revealed that the plant extract showed good antioxidant potential ranging from $18.22\pm0.66\%$ to $36.97\pm0.83\%$ with a lower IC₅₀ value (146.89 mg/L).

The antioxidant potential of the crude extract was good and due to presence of various phytochemicals which were detected by GC-MS technique. There are additional important advantages for using GC-MS, which makes it the best method for identifying the bioactive components of long chain hydrocarbons, alcohols, and acids employed in the analysis of herbal medicines. GC-MS is also the greatest technology for analysing herbal medications [27]. In the GC-MS analysis, total 51 compounds were identified out of which (Z/E)-DL-Bakuchiol covered the maximum area (57.62%). Bakuchiol has been found to show good free radical scavenging activity against proteins and lipids [28].

Ficusin (5.02%) has also been found to be antioxidant compound in previous study. Along this, vitamin E (0.11%) and gamma-tocopherol (0.50%) were also detected in GC-MS analysis which are well known antioxidants for human beings [29].

Besides these, various phytosterols including sitosterol, stigmasterol etc were also identified in the plant which are important therapeutic compounds.

Table 1: Free radical scavenging activity of petroleum ether extract of seeds of Psoralea corylifolia

Concentration (mg/L)	20	40	60	80	100	Regression equation	IC ₅₀ (mg/L)
Test	18.22±0.66	21.34±0.27	27.71±0.32	34.88±0.21	36.97±0.83	Y=0.2552x+12.512	146.89
Ascorbic acid	36.77±0.41	41.23±0.56	47.82±0.47	54.62±1.12	61.15±0.48	Y=0.3107x+29.673	65.42

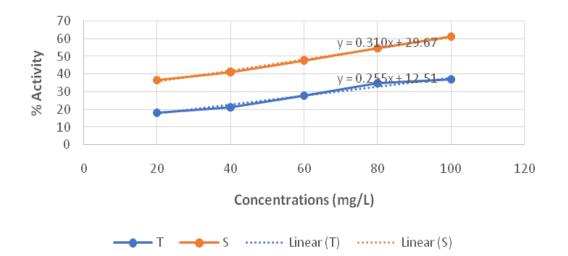




Table 2: Name of compour	nds identified in pet ether e	xtract of Psoralea corylifolia	ov GC-MS study

Peak #	R.Time	Area	Area%	Name
1	14.088	913454	0.16	1-Heptadecene
2	14.315	38797275	6.81	Isopsoralen
3	14.950	206273	0.04	9-Heptadecanone
4	15.017	28619707	5.02	Ficusin
5	15.464	6713416	1.18	HEXADECANOIC ACID, METHYL ESTER
6	15.957	5801080	1.02	4-[3,7-DIMETHYL-3-VINYL-1,6-OCTADIENYL]PHEN
7	16.125	1768547	0.31	HEPTADECANOIC ACID, ETHYL ESTER
8	16.174	594875	0.10	2-Bromotetradecane
9	16.290	2812299	0.49	4-[3,7-DIMETHYL-3-VINYL-1,6-OCTADIENYL]PHEN
10	16.409	15367490	2.70	Isopropyl palmitate
11	17.104	12438365	2.18	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
11	17.163	15868397	2.78	9,12-Octadecadienovl chloride, (Z,Z)-
12	17.103	1200913	0.21	OCTADECANOIC ACID, METHYL ESTER
15	17.624	328518202	57.62	4-[3,7-DIMETHYL-3-VINYL-1,6-OCTADIENYL]PHEN
15	17.780	1925033	0.34	ETHYL (9Z,12Z)-9,12-OCTADECADIENOATE #
16	17.825	965238	0.17	cis,cis,cis-7,10,13-Hexadecatrienal
17	17.895	653584	0.11	Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol, .alphaalpha.
18	18.007	29493397	5.17	trans, trans-9, 12-Octadecadienoic acid, propyl ester
19	18.062	25309618	4.44	Dichloroacetic acid, tridec-2-ynyl ester
20	18.134	775453	0.14	CYCLOHEXENE, 1-METHYL-4-(1-METHYLETHYLID
21	18.200	472986	0.08	(R-1,T-4)-P-MENTH-8-EN-1-OL
22	18.272	3917246	0.69	ISOPROPYL STEARATE
23	18.322	736595	0.13	3-BUTEN-2-ONE, 1-(2,3,6-TRIMETHYLPHENYL)-
24	18.504	535400	0.09	Isoamyl laurate
25	18.598	710249	0.12	Cyclohexane, 1,5-dimethyl-2,3-divinyl-
26	19.152	4312249	0.76	4-[3,7-DIMETHYL-3-VINYL-1,6-OCTADIENYL]PHEN
27	19.718	1387287	0.24	1-Heneicosanol
28	19.938	599641	0.11	Eicosanoic acid, isopropyl ester
29	20.395	860910	0.15	Hexadecanoic acid, (2,2-dimethyl-1,3-dioxolan-4-yl) methy
30	21.300	1098342	0.19	Octacosanol
31	21.499	519107	0.09	DOCOSANOIC ACID
32	21.733	971098	0.17	Methanesulfonic acid, 17-cyano-10,13-dimethylhexadecah
33	22.075	2253730	0.40	Tetratetracontane
34	23.488	4844369	0.85	Tetratetracontane
35	23.656	1021435	0.18	(9Z,12Z)-(E)-3,7-Dimethylocta-2,6-dien-1-yl octadeca-9,1
36	24.161	1418286	0.25	Tetratetracontane
37	24.656	2828864	0.50	.gammaTocopherol
38	24.841	4695764	0.82	6,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-2,6,10,15,1
39	24.887	3736323	0.66	Tetratetracontane
40	25.259	644363	0.11	Vitamin E
41	25.713	1205949	0.21	Tetratetracontane
42	26.551	4174319	0.73	STIGMASTA-5,22-DIEN-3-OL
43	26.679	596953	0.10	2-Methylhexacosane
44	27.204	1860882	0.33	.gammaSitosterol
45	28.652	688811	0.12	5-(7A-ISOPROPENYL-4,5-DIMETHYL-OCTAHYDRO-I
46	29.973	713608	0.13	Phytyl palmitate
47	30.310	1868229	0.33	5,11,17,23-TETRATERT-BUTYLPENTACYCLO[19.3.1.1
48	30.936	422710	0.07	5-(7A-ISOPROPENYL-4,5-DIMETHYL-OCTAHYDRO-I
49	32.218	437770	0.08	Isopimaric acid, TMS
<u>50</u> 51	33.498 33.937	1095872 754258	0.19 0.13	Undec-10-ynoic acid, octadecyl ester Phytyl decanoate
10	55.757	570126221	100.00	i ny tyr decanoate
		570120221	100.00	

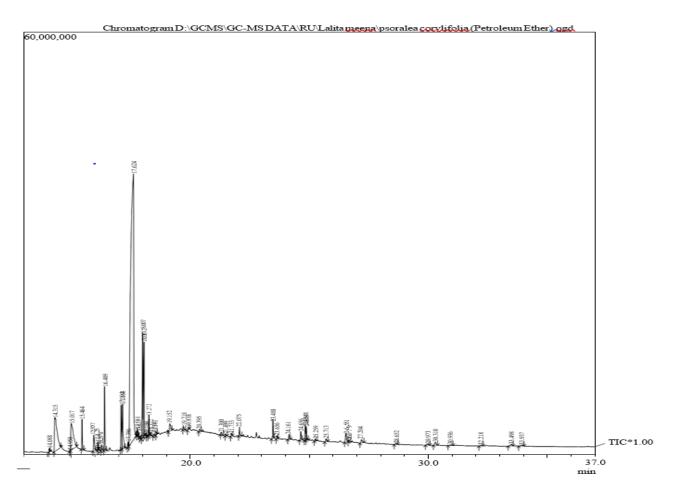


Fig. 2: Spectra of pet ether extract of seeds of Psoralea corylifoliaObtained by GC-MS study

4. CONCLUSION

It can be concluded from the present study, that the pet. ether extract of seeds of *Psoralea corylifolia* have good antioxidant potential which is due to presence of important phytoconstituents.

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Conflict of interest

None declared

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