



## GAS CHROMATOGRAPHY-MASS SPECTROMETRIC ANALYSIS OF ETHANOLIC EXTRACTS OF *GLYCYRRHIZA GLABRA* LINN. ROOTS.

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### ABSTRACT

*Glycyrrhiza glabra* is a plant with high medicinal and commercial value. Although several secondary metabolites have been reported from different species of this plant, there has been not much information available on the complete profile of phytochemical constituents in *Glycyrrhiza glabra* Linn. This study applies Gas Chromatography-Mass Spectrometry technique to determine the possible chemical components in the ethanolic extracts of *Glycyrrhiza glabra* roots and reports for the first time most extensive profile of the plant. In this analysis we found one hundred and twenty six compounds including flavonoids, terpenoids, saponins, essential oils, amino acids, and other nitrogen containing compounds, hydrocarbons, fatty acids and their esters. The most abundant phytoconstituents identified were 5-(Hydroxymethyl)-2-furancarboxaldehyde (23.15%), N-Methyl-4-(4-methyl-1-phthalazinylamino)-benzamide (7.18%), 2-Phenyl-furo[b]benzopyran-4(4H)-one (5.69 %) and 1, 2-Benzenedicarboxylic acid (5.31%). Most of the compounds in the list are said to possess medicinal properties which further justify the applications of this plant in the discovery of novel therapeutics.

**KEY WORDS:** *Glycyrrhiza glabra*, Ethanol extract, GC- MS, Phytochemical



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## INTRODUCTION

Medicinal plants are sources of important therapeutic aids for a large number of human ailments. *Glycyrrhiza glabra* commonly known as licorice is one of the medicinally important plants belonging to the family Fabaceae. It is native to Mediterranean region and parts of Asia and is also cultivated in Afghanistan and sub-himalayan tracts in India. Many ayurvedic preparations containing licorice such as yashtyadi churna, yashtimadhvadya taila, brihat ashwagandha gritha, pippalyadi taila and vridhihara lepa, having age long therapeutic uses in cough, respiratory disorders, hair fall, baldness, piles, gout, weakness in lower back and lower limbs, constipation and allergic rhinitis<sup>1</sup> are available in the Indian market<sup>2</sup>. Licorice extracts have been used for more than sixty years in Japan to treat chronic hepatitis and have therapeutic benefits against other viruses including *Herpes simplex*, SARS and HIV<sup>3</sup>. The parts of this plant used medicinally are mainly rhizomes and tap root, have many clinically proven activities such as anti-ulcer, anti-microbial, anti-asthmatic, anti-diuretic and anti-hepatotoxic activity<sup>4</sup>. Pharmacological activities of licorice are attributed mainly to its phytochemical constituents, which are still required to be studied broadly in order to identify the probable compounds of therapeutic use. It has been shown that in-vitro phytochemical screening methods could provide the necessary preliminary observations required to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations. Until now the chemical investigations on *Glycyrrhiza glabra* were concentrated mainly on flavonoids and terpenoids. Attempts of isolation and characterization of only few phytochemicals of these classes such as Glycyrrhizic acid<sup>5</sup> Licochalcone A<sup>6</sup>, Umbelliferone<sup>7</sup>, and some triterpenoids<sup>8</sup> have been generally done. Reports on detailed identification and characterization of biologically active compounds of *Glycyrrhiza glabra* are scarce and despite its well documented medicinal

uses, a complete profile of active phytochemicals of *Glycyrrhiza glabra* has not been determined yet. Hence, the present study was carried out to identify the possible phytochemicals present in ethanolic extracts of *Glycyrrhiza glabra* roots using Gas Chromatography Mass Spectrometric (GC-MS) analysis. This study is vital not only to explain the medicinal uses of *Glycyrrhiza glabra* but also to explore the possibilities of novel pharmaceutical compounds. It can be assumed to be the very first report giving such a detailed metabolic profile of this plant.

## MATERIALS AND METHODS

### *Plant material and preparation of crude ethanolic extract*

*Glycyrrhiza glabra* plant was obtained from Nursery of Medicinal plants, Forest department, Faridabad. The roots were harvested from the mature plant and shade dried for 5 days and subjected to extraction. The dried roots were pulverized to powder in a mechanical grinder. One gram of the powder was transferred into a flask and extracted with three volumes of 50% ethanol at 85°C for 4 hours with constant agitation in water bath shaker. The extract was filtered and re-extracted two times under same conditions. Each time the filtrate was collected in the same flask and finally concentrated to get viscous residue which was dissolved in 3ml of analytical grade methanol for GC-MS analysis.

### *Gas Chromatography- Mass Spectrometric Analysis (GC-MS)*

GC-MS analysis of this extract was performed using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS). The sample (2 µl) was injected into a RTX-5 column (60 m x 0.25 mm internal diameter, film thickness 0.25 µm) of GC-MS (model GC-MS-QP-2010 plus, Shimadzu Make). Helium was used as carrier gas at a constant column flow of 1.21 ml/min at 85.4 kpa inlet pressure. Temperature

programming was maintained from 80°C to 250°C with constant rise of 5°C/min and then held isothermal at 250°C for 10 min; further the temperature was increased by 30°C/min up to 310°C and again held isothermal at 320°C for 22 min. The injector and ion source temperatures were 270°C and 230°C, respectively. The crude extract dissolved in methanol (Chromatography grade, Merck, India) was injected with a split ratio of 1:20. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and the total GC/MS running time was 70 minutes.

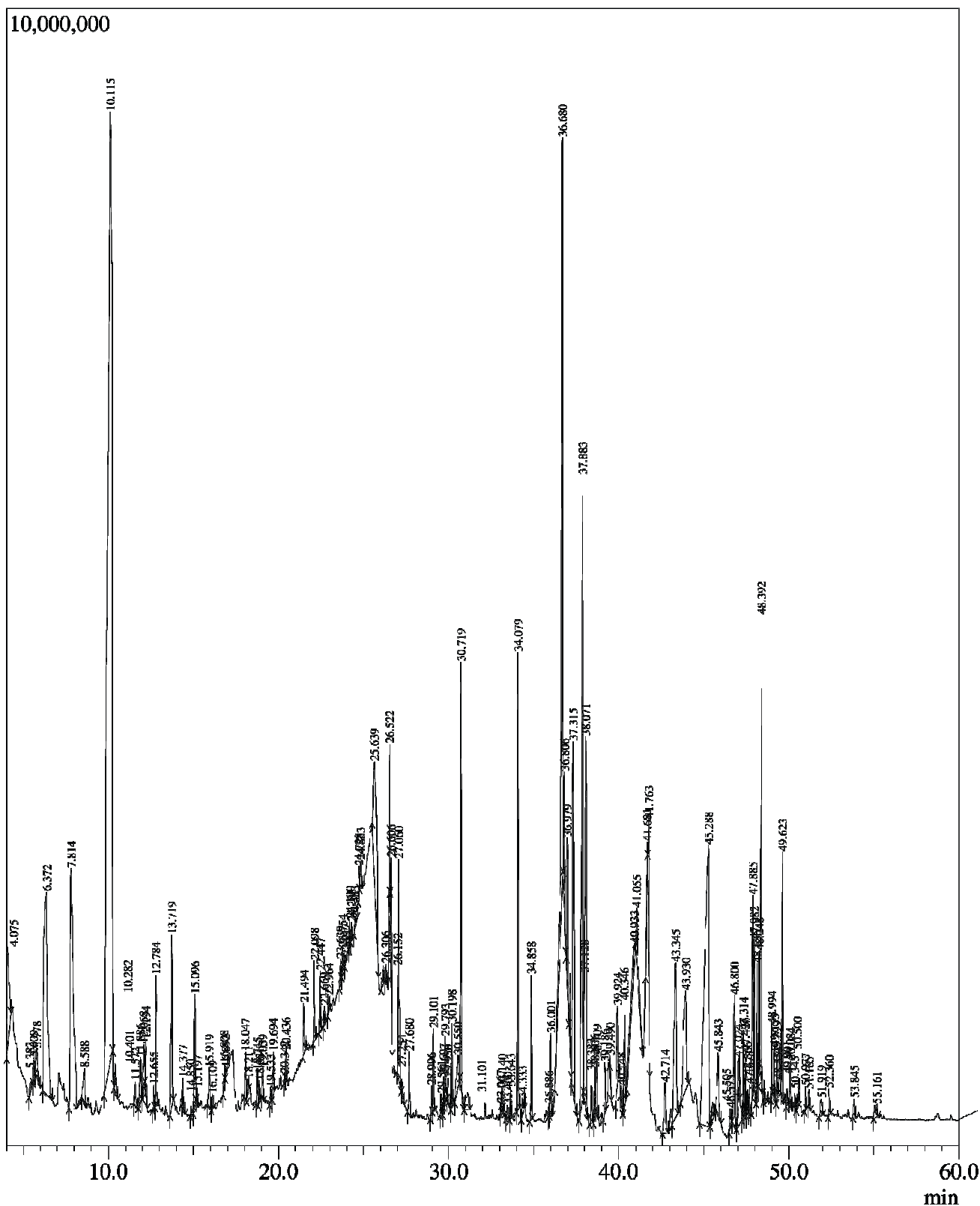
### ***Identification of compounds***

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns and WILEY- 8. The mass spectrum of unknown components were compared with the spectrum of the known

components stored in these libraries. The name, molecular weight and structure of the components of the test materials were ascertained.

## **RESULTS**

The GC-MS chromatogram (Figure-1) of ethanolic extracts of *Glycyrrhiza glabra* roots revealed one hundred and twenty six compounds which belonged to various classes of secondary metabolites. The peak report of the chromatogram obtained with details of peak number, retention time, area percentage, name of the identified phytocomponent, its molecular formula and molecular weight, are presented in Table1. The mass spectrum was obtained with mass/charge ratio on x-axis and relative intensity on y-axis.



**Figure 1**  
**The GC-MS Chromatogram of ethanolic extract of *Glycyrrhiza glabra* L. roots showing the peaks of the test compounds Vs retention time in minutes.**

Table 1

**Peak report of compounds identified in the ethanolic extract of *Glycyrrhiza glabra* roots.**

Peak no.	Retention Time	Area%	Name of the compound	Molecular formula	M.W
1	4.075	1.01	3,4-Furandimethanol	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	110
2	5.383		4,5-Dimethyl-1,3-dioxol-2-one	C <sub>5</sub> H <sub>6</sub> O <sub>3</sub>	114
3	5.609	0.11 0.13	4-Hydroxy-N-methylpiperidine	C <sub>6</sub> H <sub>13</sub> NO	115
4	5.778	0.29	2,5-Dimethyl-3(2H)furanone	C <sub>6</sub> H <sub>8</sub> O <sub>3</sub>	128
5	6.372	4.48	4H-Pyran-4-One, 2,6-Dimethyl-	C <sub>7</sub> H <sub>8</sub> O <sub>2</sub>	124
6	7.817	0.31	2,4,5-Trimethyl-1,3-Dioxolane	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	116
7	8.588	1.08	Cycloserine	C <sub>3</sub> H <sub>6</sub> N <sub>2</sub> O <sub>2</sub>	102
8	10.115	23.15	5-(Hydroxymethyl)-2-Furan carboxaldehyde	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126
9	10.282	0.17	N,1-Dimethyl-4-piperidinamine	C <sub>7</sub> H <sub>16</sub> N <sub>2</sub>	128
10	10.401	0.2	Glycerin monoacetate	C <sub>5</sub> H <sub>10</sub> O <sub>4</sub>	134
11	11.572	0.21	3-Isopropoxy alanine	C <sub>6</sub> H <sub>13</sub> NO <sub>3</sub>	147
12	11.886	0.34	2,8,4,6(Epoxyethanediyliidenoxy)[1,3]dioxino[5,4-d]-1,3-dioxin	C <sub>8</sub> H <sub>8</sub> O <sub>6</sub>	200
13	12.058	0.34	2-Methoxy-4-vinylphenol	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	150
14	12.194	0.28	Cis-Dimethyl morpholine	C <sub>6</sub> H <sub>13</sub> NO	115
15	12.655	0.1	2-Chloro-4-formyl-6-methoxyphenyl 4-morpholine carboxylate	C <sub>13</sub> H <sub>14</sub> ClNO <sub>5</sub>	299
16	12.784	0.51	3-Methyl-3-[(2-oxopropyl)amino]-2-butanone	C <sub>8</sub> H <sub>15</sub> NO <sub>2</sub>	157
17	13.719	1.08	1,2,4-Trimethylpiperazine	C <sub>7</sub> H <sub>16</sub> N <sub>2</sub>	128
18	14.377	0.14	N-Methyl-3-hydroxymethylpyrrolidin-2-one	C <sub>6</sub> H <sub>11</sub> NO <sub>2</sub>	129
19	14.85	0.07	Sarreroside	C <sub>30</sub> H <sub>42</sub> O <sub>10</sub>	562
20	15.096	0.75	6-Methoxy-8-methyl-8-azabicyclo[3.2.1]octan-3-ol	C <sub>9</sub> H <sub>17</sub> NO <sub>2</sub>	171
21	15.197	0.05	5-Ethyl-Furan-2-carboxylic acid,	C <sub>7</sub> H <sub>8</sub> O <sub>3</sub>	140
22	15.919	0.22	1-Dodecanol	C <sub>12</sub> H <sub>26</sub> O	186
23	16.109	0.07	2-Allyl-4-hydroxyprolin	C <sub>8</sub> H <sub>13</sub> NO <sub>3</sub>	171
24	16.808	0.09	3,7-Dimethylimidazo[1,2-a]pyrimidine-2,5(1H,3H)-dione	C <sub>8</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub>	179
25	16.892	0.03	3,5-Di-tert-butylphenol	C <sub>14</sub> H <sub>22</sub> O	206
26	18.047	0.2	Dodecanoic acid	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	200

27	18.211	0.07	Acetic acid, (2-isopropenylcyclopentylidene)-, methyl ester	C <sub>11</sub> H <sub>16</sub> O <sub>2</sub>	180
28	18.745	0.16	7-Methoxy-2-benzofuranyl methyl ketone	C <sub>11</sub> H <sub>10</sub> O <sub>3</sub>	190
29	18.881	0.1	Isonicotinic acid, 2-tetrahydrofurylmethyl ester	C <sub>11</sub> H <sub>13</sub> NO <sub>3</sub>	207
30	19.059	0.11	(3Z)-3-Ethyl-2-methyl-1,3-hexadiene	C <sub>9</sub> H <sub>16</sub>	124
31	19.533	0.07	1-(4-Amino-furazan-3-yl)-5-pyrrolidin-1-ylmethyl-1H-[1,2,3]triazole-4-carboxylic acid ethyl ester	C <sub>12</sub> H <sub>17</sub> N <sub>7</sub> O <sub>3</sub>	307
32	19.694	0.18	6-Methoxy-2-hydroxyquinoxaline-4-oxide	C <sub>9</sub> H <sub>8</sub> N <sub>2</sub> O <sub>3</sub>	192
33	20.342	0.03	2-Furan propionic acid	C <sub>7</sub> H <sub>8</sub> O <sub>3</sub>	140
34	20.436	0.11	beta.-Methyl-4-methoxycinnamic acid	C <sub>11</sub> H <sub>12</sub> O <sub>3</sub>	192
35	21.494	0.28	2-pyridine carboxylic acid	C <sub>14</sub> H <sub>19</sub> NO <sub>2</sub>	233
36	22.098	0.42	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	C <sub>10</sub> H <sub>12</sub> O <sub>3</sub>	180
37	22.447	0.28	Tetra decanoic acid	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228
38	22.669	0.09	Benzoic acid	C <sub>14</sub> H <sub>12</sub> O <sub>2</sub>	212
39	22.964	0.07	4-(7-Methoxy-3,3,7-trimethyl-oxepan-2-ylidene)-butan-2-one	C <sub>14</sub> H <sub>24</sub> O <sub>3</sub>	240
40	23.639	0.1	Linalool	C <sub>10</sub> H <sub>18</sub> O	154
41	23.754	0.11	Stypticin	C <sub>12</sub> H <sub>15</sub> NO <sub>4</sub>	237
42	23.917	0.06	2,6-Nonadienoic acid	C <sub>16</sub> H <sub>26</sub> O <sub>3</sub>	266
43	24.19	0.11	1-Tert-butyl-2-methoxy-4-methyl-3,5-Dinitrobenzene	C <sub>12</sub> H <sub>16</sub> N <sub>2</sub> O <sub>5</sub>	268
44	24.288	0.07	o-Nitrocumene, 2-IsopropylNitrobenzene	C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub>	165
45	24.483	0.06	Eicosanoic acid	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312
46	24.728	0.1	1,2-Benzenedicarboxylic acid,	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278
47	24.833	0.17	Salicylic acid	C <sub>14</sub> H <sub>12</sub> O <sub>3</sub>	228
48	25.639	2.4	5-Formyl-2-methoxyphenyl 4-morpholine carboxylate	C <sub>13</sub> H <sub>15</sub> NO <sub>5</sub>	265
49	26.152	0.12	Pseudoarsasapogenin-5,20-dien	C <sub>20</sub> H <sub>30</sub> O <sub>3</sub>	318
50	26.306	0.08	3-Deoxy-d-mannonic acid	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	180
51	26.522	0.79	Ascorbic acid	C <sub>38</sub> H <sub>68</sub> O <sub>8</sub>	652
52	26.606	0.47	Mome Inositol	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>	194
53	27.058	1.19	1-Nonadecene	C <sub>19</sub> H <sub>38</sub>	266

54	27.254	0.06	Benzenepropanoic acid, 2,5-dimethoxy-	C <sub>11</sub> H <sub>14</sub> O <sub>4</sub>	210
55	27.68	0.34	Hexadecanoic acid, 1-methylethyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298
56	28.996	0.12	9,12-Octadecadienoic acid, methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294
57	29.101	0.35	9-Octadecanoic acid	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296
58	29.561	0.09	Octadecanoic acid, methyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298
59	29.697	0.12	Linoleic acid, methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294
60	29.793	0.32	22-Tricosanoic acid	C <sub>23</sub> H <sub>44</sub> O <sub>2</sub>	352
61	29.95	0.12	N-[3-[6-Hydroxyhexyl]aminopropyl]aziridine	C <sub>11</sub> H <sub>24</sub> N <sub>2</sub> O	200
62	30.198	0.33	Hystrene S-97	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284
63	30.55	0.44	Hydrazine carboxaldehyde, 2,2-diethyl-, diethyl hydrazone	C <sub>9</sub> H <sub>22</sub> N <sub>4</sub>	186
64	30.719	2.18	Cyclotetracosane	C <sub>24</sub> H <sub>48</sub>	336
65	31.101	0.29	Undecanoic acid,11-amino-	C <sub>11</sub> H <sub>23</sub> NO <sub>2</sub>	201
66	33.067	0.02	1-Phenanthrenecarboxylic acid,	C <sub>21</sub> H <sub>34</sub> O <sub>2</sub>	318
67	33.14	0.09	5.alpha.-Androst-7-ene	C <sub>19</sub> H <sub>30</sub>	258
68	33.46	0.09	Phthalic acid, di(3-methylphenyl) ester	C <sub>22</sub> H <sub>18</sub> O <sub>4</sub>	346
69	33.643	0.14	7-methyl coumarins	C <sub>10</sub> H <sub>8</sub> O <sub>2</sub>	160
70	34.079	1.95	Tetracosan-1-ol	C <sub>24</sub> H <sub>50</sub> O	354
71	34.333	0.11	Tetrahydro pyran-4-carboxylic acid	C <sub>20</sub> H <sub>21</sub> NO <sub>3</sub>	323
72	34.858	0.6	2-Hydroxy-benzoic acid (1-methyl-2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide	C <sub>16</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub>	295
73	35.886	0.05	n-(4-hydroxybutyl)phthalimide	C <sub>12</sub> H <sub>13</sub> NO <sub>3</sub>	219
74	36.001	0.38	2-Methyl-6-phenyl-2,3,4,5-tetrahydro-3-pyridazinone	C <sub>11</sub> H <sub>12</sub> N <sub>2</sub> O	188
75	36.68	5.31	1,2-Benzenedicarboxylic acid	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390
76	36.806	0.64	4-(4-Methoxyphenyl)-6-phenyl-2-pyrimidinol	C <sub>17</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub>	278
77	36.979	0.72	3(2h)-Benzofuranone-3-18ol,6-methoxy-2-(3-phenyl-2-propenylidene)-	C <sub>18</sub> H <sub>14</sub> O <sub>3</sub>	278
78	37.128	0.3	1,2,3,4-Tetrahydroisoquinolin,	C <sub>27</sub> H <sub>30</sub> N <sub>2</sub> O <sub>3</sub>	430
79	37.315	0.3	cis-9-Tricosene	C <sub>23</sub> H <sub>46</sub>	322
80	37.883	5.69	2-phenyl-furo[b]benzopyran-4(4h)-one( flavone)	C <sub>17</sub> H <sub>10</sub> O <sub>3</sub>	262
81	38.071	2.67	4methyl-herniarin	C <sub>11</sub> H <sub>10</sub> O <sub>3</sub>	190

82	38.383	0.22	n-(2,4-dimethyl-phenyl)-3-oxo-butynamide	C <sub>12</sub> H <sub>15</sub> NO <sub>2</sub>	205
83	38.606	0.22	2,3-dihydro-7-methoxy-8-(3-methylbut-2-enyl)-2-phenyl-4h-1-benzopyran-4-one	C <sub>21</sub> H <sub>22</sub> O	322
84	38.709	0.25	LicoisoflavoneB	C <sub>20</sub> H <sub>16</sub> O <sub>6</sub>	352
85	39.186	0.3	beta.-Methylumbelliferone (Hymechromone)	C <sub>10</sub> H <sub>8</sub> O <sub>3</sub>	176
86	39.49	0.1	7-Acetoxy-4-methylcoumarin	C <sub>12</sub> H <sub>10</sub> O <sub>4</sub>	218
87	39.924	0.61	7-Hydroxy-8-(.gamma.,.gamma.-dimethylallyl)	C <sub>20</sub> H <sub>20</sub> O <sub>3</sub>	308
88	40.148	0.07	Benzene, 1,3,5-trimethyl-2-(2-phenylethenyl)-, (z)-	C <sub>17</sub> H <sub>18</sub>	222
89	40.346	0.62	4-(3,4-Dimethoxyphenyl)-6-phenyl-2-pyrimidinol	C <sub>18</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub>	308
90	40.933	0.7	5,5-Dimethyl-4-phenylcarbonyl-1,3,4-oxadoazoline	C <sub>11</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub>	204
91	41.055	0.73	3-Quinoline carboxylic acid	C <sub>10</sub> H <sub>7</sub> NO <sub>4</sub>	205
92	41.681	0.48	1-Hexadecanesulfonyl chloride	C <sub>16</sub> H <sub>33</sub> ClO <sub>2</sub> S	324
93	41.763	0.64	7-(Ethylamino)-4,6-Dimethyl-2h-chromenone	C <sub>13</sub> H <sub>15</sub> NO <sub>2</sub>	217
94	42.714	0.5	Squalene, 2,6,10,14,18,22-Tetracosahexaene	C <sub>30</sub> H <sub>50</sub>	410
95	43.345	2.02	Glabridin	C <sub>20</sub> H <sub>20</sub> O <sub>4</sub>	324
96	43.93	1.82	1,2,3,4,5,6,6a,6b,7,12,12a,12b-dodecahydrodicyclopent(a,c)-anthracene-7,	C <sub>20</sub> H <sub>20</sub> O <sub>2</sub>	292
97	45.288	7.18	Benzamide, N-methyl-4-(4-methyl-1-phthalazinylamino)-	C <sub>17</sub> H <sub>16</sub> N <sub>4</sub> O	292
98	45.505	0.16	Isocordoin	C <sub>20</sub> H <sub>20</sub> O <sub>3</sub>	308
99	45.843	0.68	Pyrimidine-2,4(1H,3H)-dione, 6-amino-1-(2-methylphenyl)-3-(2-phenylethyl)-	C <sub>19</sub> H <sub>19</sub> N <sub>3</sub> O <sub>2</sub>	321
100	46.573	0.13	4h,8h-Benzo(1,2-b:3,4-b')dipyran-4-one	C <sub>21</sub> H <sub>20</sub> O <sub>4</sub>	336
101	46.8	0.81	1-{4-[6-(4-Acetylphenyl)hexyl]phenyl}ethanone	C <sub>22</sub> H <sub>26</sub> O <sub>2</sub>	322
102	47.024	0.42	2-(5-Oxo-1,5-diphenyl-3-p-tolyl-pent-2-enylidene)-indan-1-one	C <sub>33</sub> H <sub>26</sub> O <sub>2</sub>	454
103	47.314	0.34	1-Triacontanol	C <sub>30</sub> H <sub>62</sub> O	438
104	47.426	0.27	Ethanone, 1-(4-cyclohexylphenyl)-, 4-Cyclohexylacetophenone	C <sub>14</sub> H <sub>18</sub> O	202
105	47.566	0.09	Liquirtigenin	C <sub>15</sub> H <sub>12</sub> O <sub>4</sub>	256
106	47.673	0.12	Coumarine, 8-allyl-7-hydroxy-6-ethyl-4-methyl-	C <sub>15</sub> H <sub>16</sub> O <sub>3</sub>	244



107	47.885	0.88	1,3-Dioxolo[4,5-g]isoquinolin-5-ol, 5,6,7,8-tetrahydro-6-methyl-	C <sub>11</sub> H <sub>13</sub> NO	207
108	47.9	0.56	Glycyrrhiza chalcone (Licochalcone A)	C <sub>21</sub> H <sub>22</sub> O <sub>4</sub>	338
109	48.136	0.44	Quinazolin-4(1H)-one,2,3-dihydro-2-methyl-3-(4-dimethylaminobenzylidenamino)-	C <sub>18</sub> H <sub>18</sub> N <sub>4</sub> O	306
110	48.2	0.32	Chalcone,3'-(3,7-dimethyl-2,6-octadienyl)-2,2',4',5-tetrahydroxy-5'-methoxy.	C <sub>26</sub> H <sub>30</sub> O <sub>6</sub>	438
111	48.392	2.7	Dicyclocloctanopyridazine	C <sub>16</sub> H <sub>24</sub> N <sub>2</sub>	244
112	48.553	0.08	Cholest-5-en-3-ol (3.beta.)-	C <sub>27</sub> H <sub>46</sub> O	386
113	48.9	0.25	Olean-12 ene-28 al	C <sub>30</sub> H <sub>48</sub> O	424
114	49.155	0.3	Dihydrocoumarin, 5,7,8-trimethyl	C <sub>12</sub> H <sub>14</sub> O <sub>2</sub>	190
115	49.299	0.17	2h,8h-benzo[1,2-b:5,4-b']dipyran-2-one	C <sub>22</sub> H <sub>20</sub> O <sub>6</sub>	380
116	49.623	1.08	Cycloisolongifolene, 9,10-dehydro-	C <sub>15</sub> H <sub>22</sub>	202
117	49.901	0.06	Beta- Sitosterol	C <sub>29</sub> H <sub>50</sub> O	412
118	50.084	0.18	3,4-Heptadien-2-one, 3,5-dicyclopentyl-6-methyl-	C <sub>18</sub> H <sub>28</sub> O	260
119	50.345	0.08	2,2-Dimethyl-7-hydroxy-6-(2,4-dimethoxycinnamoyl)chromene	C <sub>22</sub> H <sub>22</sub> O <sub>5</sub>	366
120	50.5	0.07	Stigmasterol	C <sub>29</sub> H <sub>48</sub> O	414
121	50.977	0.11	3-[2-(2-Chloro-benzyloxy)-ethyl]-1h quinazolin 2h-dione	C <sub>17</sub> H <sub>15</sub> ClN <sub>2</sub> O <sub>3</sub>	330
122	51.185	0.08	2h,8h-Benzo[1,2-b:5,4-b']dipyran-2-one, 4-hydroxy-5-methoxy-3-(4-methoxyphenyl)-8,8-dimethyl-	C <sub>22</sub> H <sub>20</sub> O <sub>6</sub>	380
123	51.919	0.15	Octadecamethyl-cyclononasiloxane	C <sub>18</sub> H <sub>54</sub> O <sub>9</sub> Si <sub>9</sub>	666
124	52.36	0.13	4-(7,8-Dihydro-tetrazolo[1,5-b][1,2,4]triazin-7-yl)-2,6-dimethyl-phenol	C <sub>11</sub> H <sub>12</sub> N <sub>6</sub> O	244
125	53.845	0.12	2-Tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol	C <sub>40</sub> H <sub>58</sub> O <sub>3</sub>	586
126	55.161	0.15	7-Methyl-1,2,3,4,4a,9a-hexahydro-9h-fluoren-9-one	C <sub>14</sub> H <sub>16</sub> O	200

There were approximately 12 phenolic compounds including flavonoids and coumarins, 14 terpenoids including sterols and saponins, 14 alkaloids and related compounds and 17 fatty acids and their esters. Rest of the compounds were long chain unsaturated hydrocarbons, alcohols, ethers and amino acids. The most abundant compounds found in *Glycyrrhiza glabra* root extracts in this study

were 5-(Hydroxymethyl)-2-furaldehyde (Rt= 10.11), and N-methyl-4-(4-methyl-1-phthalazinylamino)-Benzamide (Rt= 45.28), 2-phenyl-furo[b]benzopyran-4(4H)-one, a flavone (Rt= 37.88) and 1,2-benzenedi carboxylic acid (Rt= 36.68) which accounted for approximately 23.15% , 7.18 % , 5.69 % and 5.31 % peak area respectively. Among organic acids and their esters, the dominant components were

Dodecanoic acid (Rt= 18.0), Eicosanoic acid (Rt= 24.4), 2,6-nonadecanoic acid (Rt= 23.9), Octadecanoic acid methyl ester (Rt=29.5) and Linoleic acid methyl ester (Rt=29.6). Within the flavonoids group, the major constituents were characterized as Glabridin (Rt = 43.34 and peak area % = 2.02), Liquiritigenin (Rt= 47.56 and peak area % = 0.09), Licoisoflavone B (Rt= 38.70 and peak area % = 0.25), followed by Licochalcone A with retention time 47.9 and peak area % 0.56. Among terpenoids were  $\beta$ -sitosterol (Rt= 49.90 and peak area % = 0.06), Stigmasterol (Rt= 50.5 and peak area% = 0.07), Olean-12ene-28 al (Rt =48.9 and peak area %=0.25), and Linalool (Rt = 23.63 and peak area % = 0.10). Amino acid like 2-Allyl-4-hydroxy proline (Rt= 16.10), cycloserine (Rt= 8.58), mome inositol (Rt= 26.60) and 3-isopropoxy alanine (Rt=11.57) were also

identified. Other compounds of economic importance were Spathulenol (Rt= 24.2), Herniarin (Rt= 38.0), 4-methyl umbelliferone (Rt= 39.1), Isocordoin (Rt= 45.5) and cyclononasiloxane (Rt= 51.9). Alkaloid Isonicotinic acid (Rt=18.8) along with some pyridine and quinolone derivatives like 3,7-Dimethylimidazo[1,2-a]pyrimidine-2,5 (1H,3H)-dione (Rt=16.8), 1-(4-Amino-furazan-3-yl)-5-pyrrolidin-1-ylmethyl-1H-[1,2,3] triazole-4-carboxylic acid ethyl ester (Rt=19.5), 2-pyridine carboxylic acid (Rt=21.4), 4-(4-Methoxyphenyl)-6-phenyl-2-pyrimidinol (Rt = 36.8), 1,2,3,4-Tetrahydroisoquinolin (Rt=37.1) and, 3-Quinoline carboxylic acid (RT=37.12) were also found in this plant. The mass spectra of few pharmaceutically valuable compounds found in *G.glabra* are given in Figure-2.

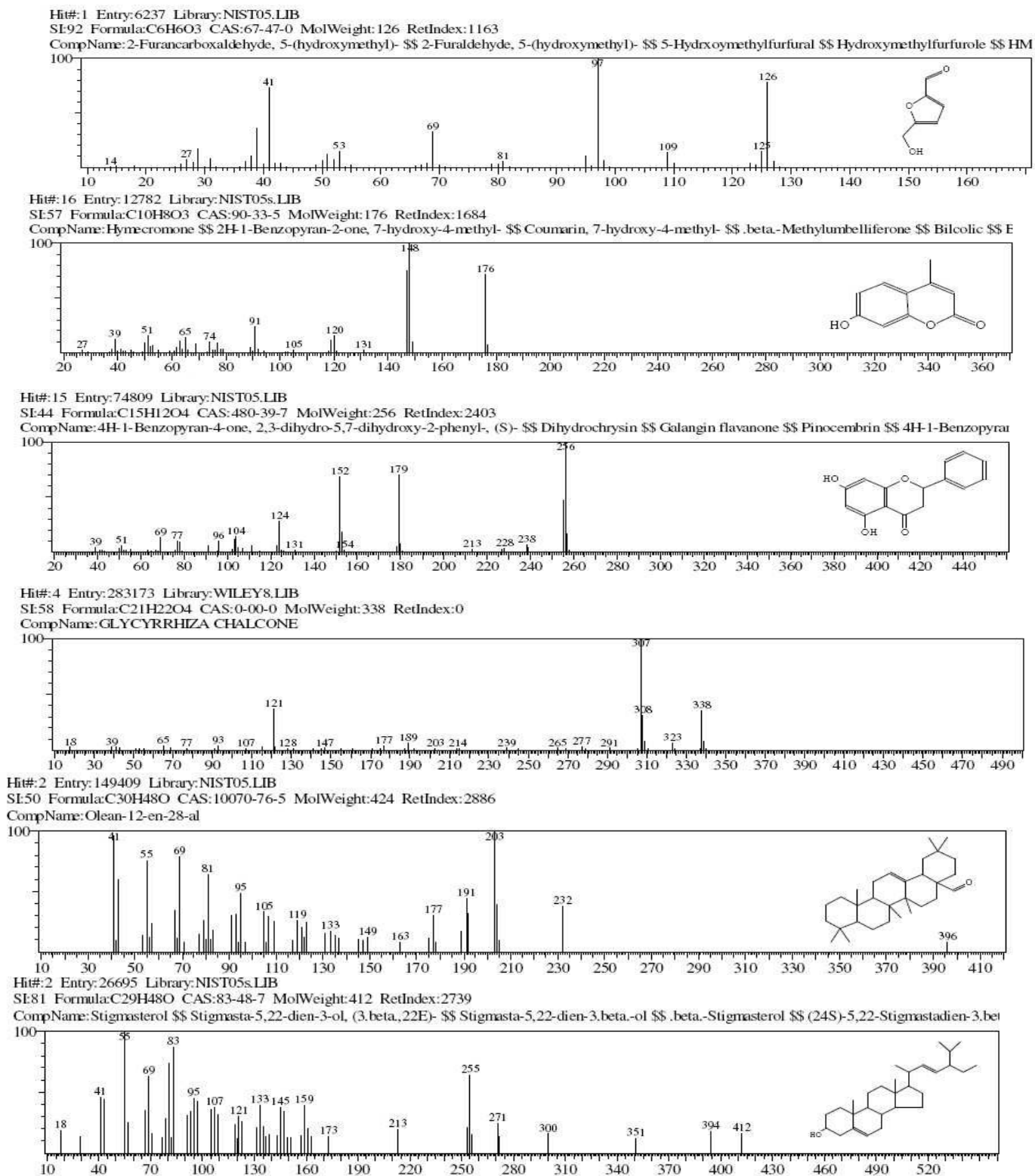


Figure 2

Mass Spectrum of some pharmaceutically valuable compounds found in ethanolic extract of Glycyrrhiza as obtained from NIST05 and WILEY 8 mass spectral libraries.

## DISCUSSION

Earlier reports on the metabolic profile of *Glycyrrhiza glabra* are mostly confined to the identification and characterization of few specific saponins, phenolics or fatty acids using a combined approach of GC-MS, LC-MS and 1D-NMR<sup>9</sup> or LC-ESI/MS/MS<sup>10</sup>. In this study, an extensive list of one hundred and twenty six phytochemicals were revealed through the GC-MS analysis of ethanolic extract of *Glycyrrhiza glabra* roots and most of the compounds identified in this report seem to possess distinguished medicinal properties which complement the age long medicinal uses of licorice extracts. Of these, a few pharmaceutically relevant compounds are discussed further. The most abundant compound identified as furan-2-carboxaldehyde (Furfural) is an important chemical solvent and a useful chemical intermediate which may be further hydrogenated to tetrahydrofurfuryl alcohol (THFA) used as a non-hazardous solvent in agricultural formulations. It is also used to make other furan chemicals, such as furoic acid via oxidation<sup>11</sup>. Furan-2-carboxaldehyde acts as the precursor for the synthesis of some new furan-imine derivatives which show potent CNS depressant and analgesic activities in vivo<sup>12</sup>. The flavonoids Glabridin, Liquiritigenin, Licoisoflavone and Licochalcone A are said to have anti-inflammatory<sup>13-15</sup>, antioxidant, antitumor<sup>16-18</sup> and antimicrobial properties<sup>[19-21]</sup> and are used in several cosmetics and medicinal formulations. Isochordoin, a chalcone is shown to have antiprotozoal and cytotoxic effects<sup>22</sup> and hence can be a potent drug in future. Triterpenoid Olean-12en-28al shows structural similarity with Glycyrrhetic acid and other oleanane derivatives having medicinal properties<sup>23</sup> are reported by other studies to be present in *Glycyrrhiza glabra*<sup>24</sup>. Another triterpene stigmasterol, acts as a precursor in the synthesis of progesterone and acts as an intermediate in the biosynthesis of androgens, estrogens, and corticoids<sup>25</sup> and in the synthesis of vitamin D3<sup>26</sup>. Stigmasterol has been investigated for its pharmacological prospects

such as antiosteoarthritic<sup>27</sup>, antihypercholesterolemic, cytotoxicity, antitumor, hypoglycemic, antimutagenic, antioxidant, anti-inflammatory<sup>28</sup>, anti fungal and CNS effects. It is also proposed to be used for the treatment of Alzheimer's disease- for which there is currently no cure<sup>29</sup>.  $\beta$ -sitosterol found in *G. glabra* roots is reported to inhibit a number of cancer lines, including ovarian, prostate, breast and colon cancers, and have been promising even on estrogen-dependent cancer lines. It is also effective in the treatment of culture proven pulmonary tuberculosis<sup>30</sup>. Another compound found in this extract is a terpene alcohol Linalool, which is a flavoring agent and scent used in consumer goods such as cosmetics, lotions, creams, shampoos, soaps, perfumes etc. Linalool is used in aromatherapy as it help to soothe stress and provide relief from a wide range of ailments, from allergies to headaches<sup>31</sup> and also exhibits inflammatory properties<sup>32</sup>. Another medicinally valuable metabolite found in GC-MS profile of *Glycyrrhiza glabra* is 4-methyl umbelliferone or Hymecromone, which is an anti choleric and antispasmodic drug has been used for more than 20 years for the treatment of functional and obstructive spasms of the biliary tract<sup>33</sup>. It is also used as a standard for the fluorometric determination of enzyme activity. Recently, it is reported to have antitumor effects in-vivo<sup>34</sup>. Herniarin, another coumarin, has shown a range of biological activities including haemostatic and anthelmintic properties. Among essential oils were found 9,10-dihydrocycloisolingofolene and spathulenol which are used as scent and flavoring agents. Spathulenols are also reported to have immunomodulatory effects<sup>35</sup>. Pyridine Derivatives are value added intermediates and are used for the manufacture of active ingredients in the pharmaceutical, agricultural and nutritional industries as well as for corrosion inhibition in the oilfield industry. Quinolines and their derivatives have amazing intrinsic pharmacological and biological activities such as antimalaria, anti inflammatory, antiasthmatic, antibacterial and antihypersensitive activities<sup>36</sup>.

## CONCLUSION

From the GC-MS profile of this plant, it is evident that a large number and range of phytochemicals like terpenoids, saponins, flavonoids, phenols, chalcones, coumarines, fatty acids, sterols, amino acids, hydrocarbons and nitrogen containing compounds are present each of which have its own importance. Presence of such a wide range of phytochemicals provides a scope for the further investigation of these compounds for various pharmacological properties and their use as

potent drugs in near future. Therefore, this report is a step towards exploration of novel compounds in extracts of *Glycyrrhiza glabra* which can further be isolated and studied for their important pharmacological properties.

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## REFERENCES

1. Bhakti C, Rajagopala M, Shah AK, Bavalatti N, A Clinical evaluation of Haridra Khanda and Pippalyadi Taila Nasya on Pratishtyaya (Allergic Rhinitis). *Ayu*, 30(2): 188-193, (2009).
2. Meena AK, Singh A, Sharma K, Kumari S, Rao MM, Physicochemical and Preliminary Phytochemical Studies on The Rhizomes of *Glycyrrhiza glabra* Linn. *Int J Pharmacy Pharm Sci*, 2(2): 48-50, (2010).
3. Rathee P, Rathee S, Ahuja D, Simultaneous Quantification of Glycyrrhetic acid and Apigenin using HPTLC from *Glycyrrhiza glabra* Linn. *Eurasian J. Anal. Chem*, 5(1): 95-103, (2010).
4. Vispute S, Khopade A, *Glycyrrhiza Glabra* Linn. - "Klitaka": A review. *IJPBS*, 2(3): 42-51, (2011).
5. Kaur P, Sharma N, Singh B, Kumar S, Kaur S, Modulation of genotoxicity of oxidative mutagens by glycyrrhizic acid from *Glycyrrhiza glabra* L. *Pharmacognosy Res*, 4(4): 189-195, (2012).
6. Wang QE, Lee FS, Wang X, Isolation and purification of inflacoumarinA and licochalcone A from licorice by high-speed counter-current chromatography. *J. Chromatogr*, 1048(1):51-57, (2004).
7. Kaur P, Kumar M, Singh B, Kumar S, Kaur S, Amelioration of oxidative stress induced by oxidative mutagens and COX-2 inhibitory activity of umbelliferone isolated from *Glycyrrhiza glabra* L. *Asian Pac J Trop Biomed*, 2(1): 120–126, (2012).
8. Elgamal MHA, Abdel-Hady FK, Hanna AG, Mahran GH, Duddeck H. A further contribution to the triterpenoid constituents of *Glycyrrhiza glabra* L. *J Biosci*, 45(9-10): 937-941, (1990).
9. Farag MA, Porzel, A, Wessjohann, LA, Comparative metabolite profiling and fingerprinting of medicinal licorice roots using a multiplex approach of GC–MS, LC–MS and 1D NMR techniques. *Phytochemistry*, 76: 60–72, (2012).
10. Montoro P, Maldini M, Russo M., Postorino S, Piacente S, Pizza CJ, Metabolic profiling of roots of liquorice (*Glycyrrhiza glabra*) from different geographical areas by ESI/MS/MS/ and determination of major metabolites by LC-ESI/MS and LC-ESI/MS/MS. *Pharm.Biomed. Anal*, 54(3): 535-544, (2010).
11. Tin Win D, Furfural – Gold from Garbage. *AU J.T*, 8(4): 185-190, (2005).
12. Sen S, De B. Panday L, Easwari TS, Nagarajan AS, Physicochemical and pharmacological study of some newly synthesized furan imine Derivatives. *J. Chem. Pharm. Res*, 2(3): 469-477, (2010).
13. Yokota T, Nishio H, Kubota, Y, Mizoguchi M, The inhibitory effect of glabridin from licorice extracts on melanogenesis and

- inflammation. *Pigment Cell Res*, 11(6): 355-361, (1998).
14. Kim YW, Zhao RJ, Park SJ, Lee JR, Cho IJ, Yang CH, Kim SJ, Kim SC, Anti inflammatory effects of liquiritigenin as a consequence of the inhibition of NF-kappaB dependent iNOS and pro inflammatory cytokines production. *J Pharmacol*, 154(1): 165–173, (2008).
  15. Kolbe L, Immeyer J, Batzer J, Wensorra U, tom Dieck K, Mundt C, Wolber R, Stäß F, Schönrock U, Ceillely RI, Wenck H, Anti-inflammatory efficacy of Licochalcone A: correlation of clinical potency and in vitro effects. *Arch Dermatol Res*, 298(1): 23-30, (2006).
  16. Mourboul A, Rena K, Shu-yan M, Vincent I, Gorard S, Isolation and Antioxidant Property of Glabridin. *Nat Prod Res. Dev*, 19(4): 675-682, (2007).
  17. Liu Y, Sirou X, Wang Y, Luo K, Wang Y, Cai Y. Liquiritigenin Inhibits Tumor Growth and Vascularization in a Mouse Model of Hela Cells. *Molecules*, 17(6): 7206-7216, (2012).
  18. Fu Y, Hsieh TC, Guo J, Kunicki J, Lee MY, Darzynkiewicz Z, Wu JM. Licochalcone A, a novel flavonoid isolated from licorice root (*Glycyrrhiza glabra*), causes G2 and late G1 arrests in androgen-independent PC-3 prostate cancer cells. *Biochem Biophys Res Commun*, 322(1): 263-270 (2004).
  19. Fatima A, Gupta VK, Luqman S, Negi AS, Kumar JK, Shanker K, Saikia D, Srivastava S, Darokar MP, Khanuja SP, Antifungal activity of *Glycyrrhiza glabra* Extract and its active constituent glabridin. *Phytother Res*, 23(8): 1190-1193, (2009).
  20. Tsukiyama RI, Katsura H, Tokuriki N, Kobayashi M, Antibacterial Activity of Licochalcone A against Spore-Forming Bacteria. *Chemother*, 46(5): 1226–1230, (2002).
  21. Fukai T, Marumo A, Kaitou K, Kanda T, Terada S, Nomura T, Anti *Helicobacter pylori* flavonoids from licorice extract. *Life Sci*, 71(12): 1449-1463, (2002).
  22. Argáez RB, Catzín TV, Puc AY, Bacab MJC, Puc, REM, Farfan MC, Antiprotozoal and Cytotoxic Studies on Some Isocordoin Derivatives. *Planta Med*, 75(12): 1336-1338, (2009).
  23. Sultana N, Ata A. Oleanolic acid and related derivatives as medicinally important compounds. *J Enzyme Inhib Med Chem*, 23(6): 739-756, (2008).
  24. Claude B, Morin P, Lafosse M, Belmont AS, Haupt, K, Selective solid-phase extraction of a triterpene acid from a plant extract by molecularly imprinted polymer. *Talanta*. 75(2): 344–350, (2008).
  25. Sundararaman P, Djerassi C, A convenient synthesis of progesterone from stigmaterol. *J Org Chem*, 42(22): 3633–3634, (1977).
  26. Kametani T, Furuyama H, Synthesis of vitamin D3 and related compounds. *Med Res Rev*, 7(2): 147–171, (1987).
  27. Gabayy O, Sanchezyz C, Salvaty C, Chevy F, Breton M, Nourissat G, Wolf C, Jacques C, Berenbaum F, Stigmaterol: a phytosterol with potential anti-osteoarthritic properties. *Osteoarthr. Cartil*, 18(1): 106-116, (2010).
  28. Githinji CG, Mbugua PM, Kanui TI, Kariuki DK, Analgesic and anti-inflammatory activities of 9-Hexacosene and Stigmaterol isolated from *Mondia whytei*. *Phytopharmacology*, 2(1): 212-223, 2012.
  29. Grimm, Hartmann, javorkova F, Laufs , Weingartner, Stigmaterol for the treatment of Alzheimer's disease WIPO Patent WO 104375, 2010.
  30. Donald PR, Lamprecht JH, Freestone M, Albrecht CF, Bouic PJ, Kotze D, van Jaarsveld PP, A randomised placebo-controlled trial of the efficacy of beta-sitosterol and its glucoside as adjuvants in the treatment of pulmonary tuberculosis. *Int J Tuberc Lung Dis*, 1(6): 518-522, (1997).
  31. Linck VM., da Silva AL., Figueiro A, Piato AL, Herrmann AP, Dupont Birck F, Caramão EB, Nunes DS, Moreno PR, Elisabetsky E. Inhaled linalool-induced sedation in mice. *Phytomedicine*, 16(4): 303–307, (2009).

32. Peana AT, D'Aquila PS, Panin F, Serra G., Pippia P, Moretti MD, Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. *Phytomedicine*, 9(8): 721-726, (2002).
33. Abate A, Dimartino V, Spina P, Costa PL, Lombardo C, Santini A, Del Piano M, Alimonti P. Hymecromone in the treatment of motor disorders of the bile ducts: a multicenter, double-blind, placebo-controlled clinical study. *Drugs Exp Clin Res*, 27(5-6): 223-231, (2001).
34. Piccioni F, Malvicini M, Garcia MG, Rodriguez A, Atorrasagasi C, Kippes N, Piedra Buena IT, Rizzo MM, Bayo J, Aquino J, Viola M, Passi A, Alaniz L, Mazzolini G, Antitumor effects of hyaluronic acid inhibitor 4-methylumbelliferone in an orthotopic hepatocellular carcinoma model in mice. *Glycobiology*, 22(3): 400–410, (2012).
35. Ziaei M, Ramezani L, Wright C, Paetz B, Schneider Z, Amirghofran, Identification of Spathulenol in *Salvia mirzayanii* and the immunomodulatory effects. *Phytother Res*, 25(4): 557-562. (2011).
36. Heravi MRP, An efficient synthesis of quinolines derivatives promoted by a room temperature ionic liquid at ambient conditions under ultrasound irradiation via the tandem addition/annulation reaction of o-aminoaryl ketones with  $\alpha$ -methylene ketones. *Ultrason Sonochem*, 16(3): 361–366, (2009).