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COMPARATIVE GC–MS ANALYSIS OF SECONDARY METABOLITES FROM LEAF, STEM AND CALLUS OF GLYCYRRHIZA GLABRA

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ABSTRACT

The aim of this study is identification and comparative analysis of secondary metabolites present in ethanol extracts of leaf, stem and callus of *Glycyrrhiza glara* through Gas Chromatography–Mass Spectrometry analysis. Leaf, stem and callus cultures were harvested, shade dried, powdered in a mechanical grinder and extracted in 70% ethanol at 85°C for 4 hours with constant agitation. The extract was filtered and re-extracted two times under same conditions. Each time the filtrate was collected in the same flask and partitioned using the following solvents: petroleum ether, diethyl ether and ethyl acetate and the analysis was through GC-MS. The GC–MS analysis of the extracts revealed the presence of 31, 23 and 44 different types of sceondary metabolites in leaf, stem and callus respectively. The callus was detected with the presence of some valuable compounds including the flavonoids licoisoflavone B, licochalcone A and liquirtigenin which were absent in stem and leaves. These chemical compounds are considered biologically active and pharmacologically important. This study not only gives a detailed comparison of detection and identification of various bioactive phytochemicals from different plant parts (leaves, stem) and callus of *Glycyrrhiza glabra* but also provides a scope for the further study and enhancement in the production of the valuable compounds specially in callus.

KEYWORDS: Callus, Glycyrrhiza glabra, Leaf, Stem, GC-MS.

INTRODUCTION

Glycyrrhiza glabra is a traditional medicinal plant and found to be a rich source of bioactive phytochemicals possessing various biological activities.[1] Pharmacological activities of *Glycyrrhiza glabra* are attributed mainly to its phytochemical constituents that are 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.[2] Phytochemical research has been greatly facilitated by use of modern analytical techniques. It has become easier to detect different classes of compounds such as alkaloids, flavonoids and terpenoids using modern techniques like GC-MS.[3] Reports on detailed identification and characterization of biologically active compounds of in leaf, stem and callus of *Glycyrrhiza glabra* are scarce and despite its well documented medicinal uses, a complete profile of its active phytochemicals has not been determined yet.[4] Hence, the present study was carried out to identify and do a comparative analysis of all the possible phytochemicals present in ethanolic extracts of *Glycyrrhiza glabra* leaf, stem and callus using gas chromatography mass spectrometric (GC-MS) analysis.

MATERIALS AND METHODS

Collection of plant sample: Leaf and stem were collected from authentic *Glycyrrhiza glabra* plants were obtained from forest nursery, Faridabad in the month of August.

Callus induction: Young leaf explants were inoculated into MS medium supplemented with 1 mg/L NAA and 0.5 mg/L BAP and antioxidant concentration and combination of 20 mg/L ascorbic acid and 10 mg/L citric acid. Callus cultures were maintained on this medium and were used for further studies. All the inoculations were carried out in a laminar air flow chamber and the cultures were incubated for 16 hours light and 8 hours dark photoperiod at 25±2ºC under controlled light intensity of 2000 Lux.[5]

Preparation of extracts and GC-MS analysis: The dried plant parts and dried callus were pulverized to powder in a mechanical grinder. One gram of the powder was transferred into a flask and extracted with 70% 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 analytical grade methanol for GC-MS analysis. The samples were derivatized with trimethylsilyl (TMS) prior to injection in chromatography column and separation was done using helium as a carrier gas at a flow of 1.21 ml/min, 85.4 kpa inlet pressure and 250°C temperature. Mass spectra were recorded at 70 eV with scan interval of 0.5 seconds.[6]

RESULTS AND DISCUSSION

Phytochemical investigation of *Glycyrrhiza glabra* leaves

The GC-MS chromatogram (Figure 1) of ethanolic extracts of *Glycyrrhiza glabra* leaves revealed only 31 compounds which mostly belonged to the alkaloids and related compounds and fatty acids and their esters, alcohols and ethers. Out of the 31 compounds, about 15 compounds such as isonicotinic acid (RT = 18.822), 1-(4-Amino-furazan-3-yl)-5-pyrrolidin-1-ylmethyl-1H-[1,2,3] triazole-4-carboxylic acid ethyl ester (RT = 19.43), Beta methyl 4-methoxy cinnamic acid (RT=20.406), pyridine carboxylic acid (RT = 21.6), tetradeconoic acid (RT = 22.4), Benzoic acid (RT = 22.6), Linalool (RT = 23.56), eicosanoic acid (RT = 24.428), 5-Formyl-2-methoxy phenyl 4-morpholine (RT = 25.595), ascorbic acid (RT = 26.522), nonadecene (RT = 27.057), phenanthrene carboxylic acid (RT = 33.045), and tetracosan-1-ol are the commonly reported secondary metabolite that are also reported to be found in *Glycyrrhiza glabra* roots (Table 1). The most predominant compounds in the leaves of *Glycyrrhiza glabra* were hexadecanoic acid, 1-methylethyl ester (RT = 27.626) and eicosanoic acid (RT = 24.428). Surprisingly the major flavonoids of *Glycyrrhiza glabra* and some other compounds of commercial value such as furfural, herniarin, hymechromone, glabridin, and stigmasterol were not found in the GC-MS profile of leaves.

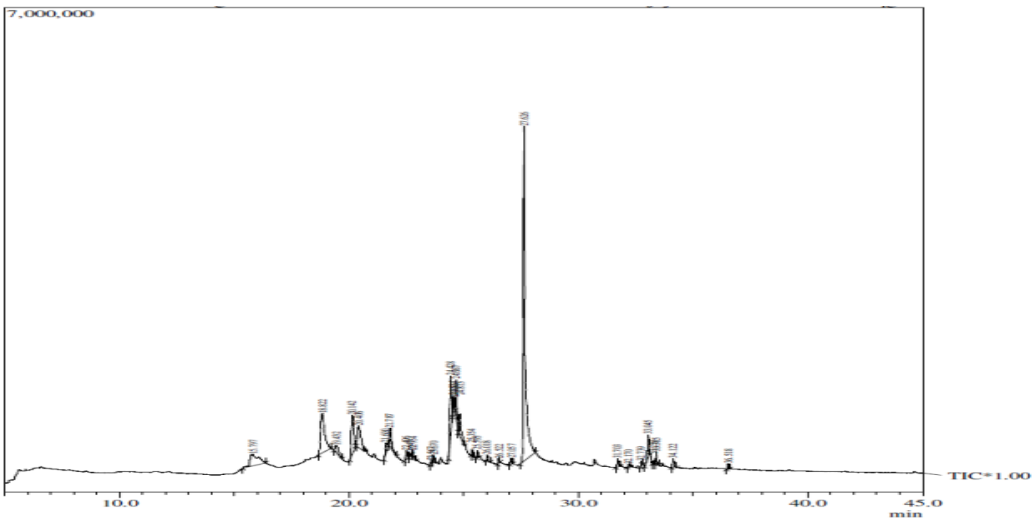

Figure. 1: The GC-MS chromatogram of ethanolic extract of *Glycyrrhiza glabra* leaves.

Table. 1: Compounds identified in the ethanolic extract of *Glycyrrhiza glabra* leaves.

Peak No.	Retension Time	Area%	Name of the compound	Molecular formula	M.W
1	15.797	6.5	Benzaldehyde, 4-ethoxy-3-hydroxy	$C_9H_{10}O_3$	166
2	18.822	11.14	Isonicotinic acid, 2-tetrahydro furylmethyl ester	$C_{11}H_{13}NO_3$	207
3	19.432	1.51	1-(4-Amino-furazan-3-yl)-5-pyrrolidin-1-ylmethyl-1H-[1,2,3]triazole-4-carboxylic acid ethyl ester	$C_{12}H_{17}N_7O_3$	307
4	20.142	6.05	Methyl (3-oxo-2-pentylcyclopentyL) Acetate	C13H22O3	226
5	20.406	4.97	beta.-Methyl-4-methoxycinnamic acid	C11H12O3	192
6	21.6	1.33	2,4-Dihydroxy-3,6-dimethyl benzoic acid	C10H12O4	196
7	21.787	2.87	6-Ethyl-2-methyl-6-hepten-2-ol	C10H20O	156
8	22.496	1.06	Tetra decanoic acid	C14H28O2	228
9	22.642	0.18	Benzoic acid	C14H12O2	212
10	22.754	0.42	(1-Methyl-1 propylpentyl)benzene	C15H24	204
11	23.567	0.16	6,7-Diethyl-1,2,3,4-tetrahydro-1,1,4,4-tetramethyl naphthalene	C18H28	244
12	23.67	0.56	Linalool	C10H18O	154
13	24.428	6.23	Eicosanoic acid	C20H40O2	312
14	24.524	0.64	Cycloprop[e]indene-1a,2(1H)-dicarboxaldehyde	C15H20O2	232
15	24.586	0.38	2,3,3-Trimethyl-2-(3-methylbuta-1,3- dienyl)-6-methylenecyclohexanone	C15H22O	232
16	24.667	2.62	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	C18H26O	258
17	24.813	2.65	Salicylic acid	C14H12O3	228
18	25.354	0.59	4,6,6,7,8,8-Hexamethyl-1,3,4,6,7,8 hexahydrocyclopenta[g] isochromene	C18H26O	258
19	25.595	0.58	5-Formyl-2-methoxyphenyl 4-morpholine carboxylate	C13H15NO5	265
20	26.018	0.5	Oxacyclohexadecan-2-one	C15H28O2	240
21	26.522	0.36	Ascorbic acid 2,6-dihexadecanoate	C38H68O8	652
22	27.057	0.51	1-Nonadecene	C19H38	266
23	27.626	42.56	Hexadecanoic acid, 1-methylethyl ester	C19H38O2	298
24	31.703	0.73	3(2H)-Phenanthrenone	C18H26O2	274
25	32.17	0.27	1-Naphthalenepentanoic acid	C22H36O4	364
26	32.739	0.27	(5.alpha.)-Androst-7-ene	C19H30	258
27	33.045	2.1	1-Phenanthrenecarboxylic acid	C21H34O2	318
28	33.259	0.2	Hydroxydehydrostevic acid	C20H30O3	318
29	33.385	0.9	Dehydroabietylamine	C20H31N	285
30	34.122	0.77	Tetracosan-1-ol	C24H50O	354
31	36.518	0.39	1,2-Benzenedicarboxylic acid	C24H38O4	390

Phytochemical Investigation of *Glycyrrhiza glabra* Stem: The GC-MS profile of the stem revealed only 23 compounds (Figure 2), among which 21 compounds were common to that of the leaves. As in the case of leaves, compounds detected mostly belonged to the fatty

acids, their esters, alcohols and ethers. Hexadecanoic acid, 1-methylethyl ester (RT = 27.6) was the predominant compound found in *Glycyrrhiza glabra* stem with a peak area % of 37.2 (Table 2).

The phytochemical profile of leaf and stem were almost similar to each other except for the compounds 22-tricosanoic acid (RT = 29.81) and cyclotetracosane (RT = 30.72) which were not found in leaves. The major secondary metabolites of *Glycyrrhiza* which belonged to the group of phenolic compounds including flavonoids and the terpenoid group such as glabridin, licochalcone A, liquirtigenin, Licoisoflavone, herniarin, hymechromone, glycyrrhetinic acid etc which are commony found in roots were not detected in leaves and stem. This is in agreement with the fact that most of the vital secondary metabolites of *Glycyrrhiza glabra* are root borne and are mainly accumulated in the roots.

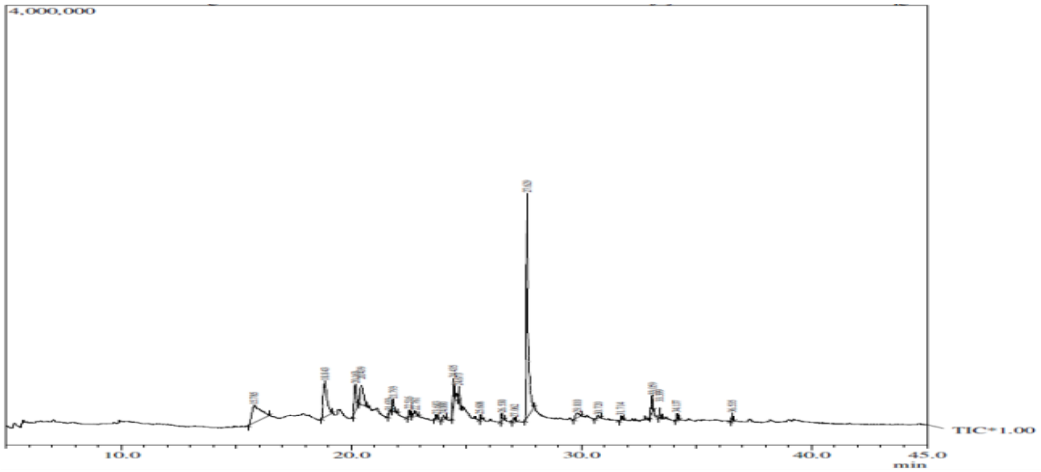

Figure. 5.3: The GC-MS chromatogram of ethanolic extract of *Glycyrrhiza glabra* Stem.

Table. 2: Compounds identified in the ethanolic extract of *Glycyrrhiza glabra* stem.

Peak No.	Retension Time	Area %	Name of the compound	Molecular formula	M.W
1	15.785	14.75	Benzaldehyde, 4-ethoxy-3-hydroxy	$C_9H_{10}O_3$	166
2	18.843	13.22	Isonicotinic acid, 2-tetrahydro furylmethyl ester	$C_{11}H_{13}NO_3$	207
3	20.163	5.92	methyl (3-oxo-2-pentylcyclopentyl)acetate	C13H22O3	226
4	20.439	6.44	beta.-Methyl-4-methoxycinnamic acid	C11H12O3	192
5	21.633	0.56	2,4-Dihydroxy-3,6-dimethyl benzoic acid	C10H12O4	196
6	21.793	2.25	6-Ethyl-2-methyl-6-hepten-2-ol	C10H20O	156
7	22.516	1.2	3a,6,6,9a-Tetramethyldodecahydronaphtho[2,1-b]furan	C16H28O	236
8	22.761	1.27	(1-Methyl-1-propylpentyl)benzene	C15H24	204
9	23.683	0.57	Linalool	C10H18O	154
10	24	0.75	9-Hexadecenoic acid	C16H30O2	254
11	24.435	4.14	Eicosanoic acid	C20H40O2	312

12	24.673	1.63	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	C18H26O	258
13	25.608	0.5	5-Formyl-2-methoxyphenyl 4-morpholine carboxylate	C13H15NO5	265
14	26.53	1.18	Ascorbic acid	C38H68O8	652
15	27.062	0.64	1-Nonadecene	C19H38	266
16	27.629	37.2	Hexadecanoic acid, 1-methylethyl ester	C19H38O2	298
17	29.81	1.79	22-Tricosanoic acid	C23H44O2	352
18	30.72	0.56	Cyclotetracosane	C24H48	336
19	31.714	0.48	3(2H)-Phenanthrenone	C18H26O2	274
20	33.059	2.35	1-Phenanthrenecarboxylic acid	C21H34O2	318
21	33.399	0.99	5.alpha.-Androst-7-ene	C19H30	258
22	34.137	0.76	Tetracosan-1-ol	C24H50O	354
23	36.535	0.82	1,2-Benzenedicarboxylic acid	C24H38O4	390

Phytochemical Investigation of *Glycyrrhiza glabra* Callus

GC-MS profile of crude ethanolic extract of callus showed the presence of 44 compounds (Figure 3) of different categories like hydrocarbons, coumarines, flavonoids, fatty acid esters, sterols and some alkaloid related compounds such as cis- dimethyl morphine (RT = 12.27). Among the 44 compounds were the three important flavonoids licoisoflavone B (RT=38.8, Peak area% =0.10), licochalcone A (RT = 48.2, peak area % = 0.11) and Liquirtigenin (RT = 47.5, peak area % = 0.03). However they appeared in lesser amounts.

5-Ethyl-furan-2-carboxyllic acid (RT = 15.22, peak area % = 16.79) and 6-Methoxy-8 methyl-8-azabicyclo [3.2.1] octan-3-ol (RT = 15.0, peak area % = 13.47) were the predominent compounds that appeared in *Glycyrrhiza glabra* callus (Table 3). When compared with the profiles of stem and leaf, only 5 compounds similar to that of leaves such as 4-ethoxy-3 hydroxy benzaldehyde (RT = 15.79), linalool (RT = 23.67), 5-Formyl-2-methoxy phenyl-4-morphine carboxylate (RT = 24.8), Nonadecene (RT = 27.1), Phenanthrenone (RT = 31.9) and 3 compounds similar to both leaf and stem (4-ethoxy-3 hydroxy Benzaldehyde, and linalool) could be found.

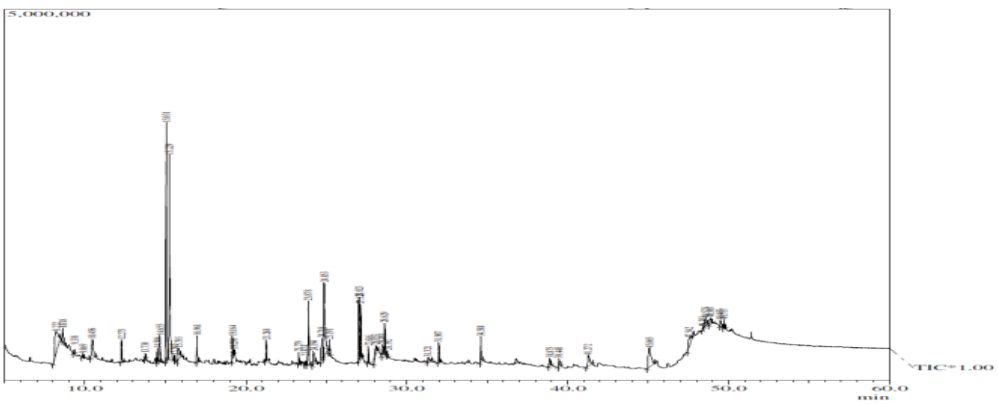

Figure. 3: The GC-MS chromatogram of ethanolic extract of *Glycyrrhiza glabra* callus.

Table 3: Compounds identified in the ethanolic extract of *Glycyrrhiza glabra* callus.

Peak No.	Retension Time	Area %	Name of the compound	Molecular formula	M.W
1	8.222	6.17	Benzoic acid	C7H6O2	122
2	8.618	1.16	Cycloserine	$C_3H_6N_2O_2$	102
3	9.338	0.22	Nonane, 5-butyl-	C13H28	184
4	9.869	0.28	Pentadecene	C15H30	210
5	10.456	3.10	Glycerine monoacetate	$C_5H_{10}O_4$	134
6	12.275	1.40	Cis- Dimethyl morphine	$C_{13}H_{14}ClNO_5$	299
7	13.730	0.32	1,2,4 Trimethyl piperazine	$C_7H_{16}N_2$	128
8	14.506	0.71	N-Methyl-3 hyroxymethyl pyrolidine 2-one	$C_6H_{11}NO_2$	129
9	14.655	1.62	Heptadecane	C17H36	240
10	15.031	13.47	6-Methoxy-8 methyl-8-azabicyclo[3.2.1] octan-3-ol	$C_9H_{17}NO_2$	171
11	15.229	16.79	5-Ethyl-furan-2-carboxyllic acid	$C_7H_8O_3$	140
12	15.565	0.34	Hexadecane	C16H34	226
13	15.793	2.01	Benzaldehyde 4-ethoxy-3 hydroxy	$C_9H_{10}O_3$	166
14	16.961	1.75	3,7,Dimethylimidazol[1,2-a]pyrimidine 2,5(1H, 3H dione)	$C_8H_9N_3O_2$	179
15	19.164	1.24	3(z)-3-ethyl-2-methyl-1,3, hexadiene	C_9H_{16}	124
16	19.250	0.22	Pentadecane, 2,6,10,14-tetramethyl-	C19H40	268
17	21.268	1.15	Beta-methyl-4-methoxy cinnamic acid	$C_{11}H_{12}O_3$	192
18	23.279	0.69	4-(7-methoxy-3,3,7-trimethyl oxepan -2-pyridene-butan-2-one)	$C_{14}H_{24}O_3$	240
19	23.674	0.28	Linalool	$C_{10}H_{18}O$	154
20	23.878	4.61	Hexadecanoic acid, methyl ester	C17H34O2	270
21	24.194	1.65	1-Tert butyl 2-methoxy-4-methyl-3,5-dinitro benzene	$C_{12}H_{16}N_2O_5$	268
22	24.710	1.58	Salicylic acid	C14H12O3	228
23	24.853	7.87	5-Formyl-2-methoxy phenyl-4-morphine carboxylate	$C_{13}H_{15}NO_5$	265
24	25.191	0.77	Tetratriacontane	C34H70	478
25	27.023	3.48	Methyl linoleate	C19H34O2	294
26	27.128	3.37	1-Nonadecene	$C_{19}H_{38}$	266
27	27.618	2.99	Octadecanoic acid, methyl ester	C19H38O2	298
29	28.481	0.98	cis-9-Hexadecenal	C16H30O	238
30	28.629	1.63	Stearic acid	CH3(CH2)16 COOH	284
31	28.792	0.36	Hexadecanoic acid, butyl ester	$C_{20}H_{40}O_2$	312
32	31.321	1.78	Undecanoic acid-11-amino	$C_{11}H_{23}NO_2$	201
33	31.987	1.29	Phenanthrenone	C18H26O2	274
34	34.588	2.15	Tetrahydro pyran-4-carboxyllic acid	$C_{20}H_{21}NO_3$	323
35	38.875	0.10	Licoisoflavone B	$C_{20}H_{16}O_6$	352
36	39.448	0.97	7-acetoxy-4-methy-coumarin	$C_{12}H_{10}O_4$	218
37	41.272	2.09	Acetyl phenyl ether	C16H14O3	254
38	45.065	4.76	4-([(propylamino)carbonyl]amino)benzoic acid	C11H14N2O3	222
39	47.542	0.03	Liquirtigenin	$C_{15}H_{12}O_4$	256
40	48.383	0.11	Licochalcone A	$C_{21}H_{22}O_4$	338
41	48.575	1.14	Cholest-5-ene -3-ol	$C_{27}H_{46}$	370
42	48.867	0.69	Stigmast-5-en-3-ol, (3.beta.)-	$C_{29}H_{50}O$	414
43	49.483	0.71	2h,8h-benzo(1, 2b: 4-b) dipyran-2-one	$C_{22}H_{20}O_6$	380
44	49.700	0.50	Cycloisolongifolene, 9,10-dehydro-	$C_{15}H_{22}$	202

It can be clearly seen from the results that root has an extensive phytochemical profile with large number of flavonoids followed by callus with 44 compounds including some flavonoids, where as in leaf and stem only limited numbers of phytochemicals appear that too in low amounts and the flavonoids were not detected in stem and leaf. The roots and the callus were identified with the presence of pharmaceutically valuable secondary metabolites including flavonoids of high value such as licoisoflavone B, licochalcone and liquirtigenin. These bioactive flavonoids liquiritigenin, licoisoflavone B and licochalcone A are of high pharmaceutical value. These flavonoids exhibit many pharmaceutical activities such as anti-inflammatory, anti-oxidant, anti-tumor and anti-microbial properties and are used in medicinal formulations.[7-12]

CONCLUSION

From the GC-MS profile of different plant parts and callus of *Glycyrrhiza glabra*, it is evident that when compared to that of Leaf and stem, the callus was enriched with variety of phytochemical constituents and was detected with the presence of some valuable compounds including the flavonoids licoisoflavone B, licochalcone A and liquirtigenin which were Absent in stem and leaves. Presence of such phytochemicals in callus provides a scope for the further study and enhancement in the production of these compounds for various pharmacological properties and their use as potent drugs in near future.

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