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PHYTOCHEMICAL AND GC-MS ANALYSIS OF LEAF EXTRACTS OF *TRIGONELLA FOENUM-GRAECUM* (L.)

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ABSTRACT

The bioactive components found in medicinal plants have been utilized for centuries, making them a valuable therapeutic alternative. The secondary metabolites found in medicinal plants are significant for the treatment of various diseases and serve as essential raw materials for the manufacture of both traditional and modern medications. The annual herb fenugreek (*Trigonella foenum-graecum*), belongs to family Fabaceae, found all over the world. It is cultivated for human consumption in the various developed countries as Europe, North Africa, Yemen, India, Mediterranean and France. In the present investigation for phytochemical and GC-MS analysis of *Trigonella foenum- graecum*, the extracts were prepared in aqueous, alcohol and acetone solvents through successive Soxhlet extraction. Among the tested extracts, the acetone extracts of *T. foenum -graecum*, showed highest number of phytochemicals as compared to other extracts. These phytochemical compounds present in the extracts may be useful in future pharmaceutical formulations.

KEYWORDS: *Trigonella foenum-graecum*, Soxhlet apparatus, Phytochemicals, GC-MS.

INTRODUCTION

In many parts of the world, medicinal herbs were used for more than 2,000 years, and it is occasionally cited as the oldest medicinal plant in human history.[1] Medicinal values include secondary metabolites that are essential for curing a number of disorders and are essential

raw materials for the creation of both conventional and modern medications.[2] Traditional uses of fenugreek include lactation stimulation, laxative, and demulcent effects. It also has hypocholesterolemic, hypolipidemic, and hypoglycemic effects in both healthy and diabetic patients and various animals.[3] Vitamins, iron, β-carotene, and other vital minerals are rich in fenugreek. Numerous pharmacological characteristics, such as antibacterial, antiviral, anticancer, anti-inflammatory, and antioxidant activity, have also been reported. Fenugreek has long been a part of the standard diet because of its hematinic properties.[4] The bioactive substances in fenugreek and their potential medical benefits have been the subject of numerous investigations. The largest amounts of flavonoids and phenolic chemicals are found in fenugreek leaves, which greatly enhance its therapeutic qualities.[5] While GC-MS research has shown itself to be a dependable methodology for the study of non-polar components and volatile essential oils, fatty acids, lipids, and alkaloids, it has been used in recent years for the analysis of medicinal plants. The GC-MS analysis of *Trigonella foenum-graecum* has identified a varied range of bioactive compounds that are responsible for its diverse therapeutic effects.[6]

MATERIAL AND METHODS

Collection and Authentication of Plant

The (Fenugreek) *T. foenum-graecum* plant collected from a farm in Satara District, Maharashtra, India. The identification and authentication of plant was done at Department of Botany, Yashwantrao Chavan Institute of Science, Satara. (See fig.1)

Fig.1 Fenugreek leaves.

Preparation of Extract (Soxhlet method)

The leaves were removed, cleaned, and air-dried under a canopy at room temperature for a 8 days. The shade dried leaves were grinded into a fine powder and stored in glass vials at

room temperature. Using Soxhlet apparatus 20 gm of leaf powder in 200 ml of solvent aqueous, alcohol and acetone extract were prepared.[7] Then plant extracts were concentrated using a high- speed evaporator and preserved in the refrigerator.

Phytochemical Analysis

Preliminary screening of Aqueous, Alcohol and Acetone leaves extract were carried out by standard method.[8]

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

GC-MS analysis of alcohol and Acetone Leaf extracts of *Trigonella foenum-graecum* were done from the Common Facility Centre, Shivaji University, Kolhapur. Compound identification was achieved by comparing the fragmentation pattern and retention time with the GC-MS mass spectra.[9]

OBSERVATIONS AND RESULTS

Phytochemical analysis

Table No. 1: Preliminary phytochemical analysis of Aqueous, Alcohol and Acetone Leaves extracts of *Trigonella foenum-graecum*.

Part used	Extract	Steroids	Triterpenoids	Glycosides	Saponins	Carbohydrates	Alkaloids	Flavonoids	Phenols	Proteins	Tannins	Starch	Flavonol	Anthocynin
Leaf	Aqueous	+	+	+	-	+	-	+	+	+	-	-	+	-
	Alcohol	+	+	+	-	+	-	+	+	+	+	-	+	-
	Acetone	+	+	+	-	+	-	+	+	+	+	-	+	-

(+ Present - Absent)

Preliminary phytochemical investigation of Aqueous leaves extract of *Trigonella foenum-graecum* showed the presence of Steroids, Triterpenoids, Glycosides, Carbohydrates, Flavonoids, Phenols, Proteins and Flavanol while in Alcohol leaves extract of *Trigonella foenum-graecum* showed the presence of Steroids, Triterpenoids, Glycosides, Carbohydrates, Flavonoids, Proteins, Tannins and Flavanol and Phytochemical analysis of Acetone leaves extract of *Trigonella foenum-graecum* showed the presence of Steroids, Triterpenoids, Glycosides, Carbohydrates, Flavonoids, Phenols, Proteins, Tannins and Flavanol. Among

these three solvents, Acetone extract showed the presence of more compounds as compared to Aqueous and Alcohol extracts.

Identification of phytoconstituents by Gas Chromatography- Mass Spectrometry (GC-MS)

A. GC-MS analysis of alcohol leaves extract

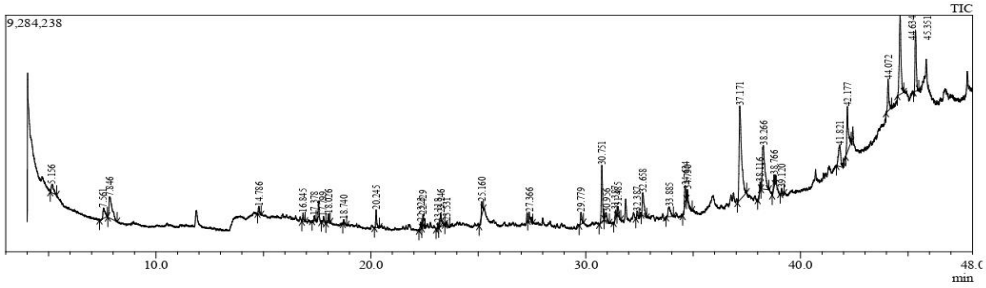

Fig. 2: Chromatograph alcohol leaves extracts.

Bioactive compounds of alcohol leaf extracts of fenugreek, analysed by chromatography are shown in Table No.2

Table No. 2: Bioactive compounds of alcohol leaf extracts of fenugreek by Gas Chromatography- Mass Spectrometry (GC-MS).

Peak	Name	R. T	Area	Area%
1	Propane, 1,1-diethoxy-2-methyl-	5.156	2448434	1.60
2	Butane,1,1-diethoxy-3-methyl-	7.561	2619336	1.71
3	Ethane,1,1,1-triethoxy	7.846	8697183	5.69
4	Dodecane	14.786	1273664	0.83
5	Dodecane,2,6,11-trimethyl-	16.845	966239	0.63
6	3-Decane,2,2-dimethyl-(E)-	17.378	264902	0.17
7	3-Octadecene,(E)-	17.799	951932	0.62
8	2-Isopropyl-5-methyl-1-1-heptanol	18.7026	872058	0.57
9	3-Heptadecenal	18.740	477284	0.31
10	Tetradecane	20.245	2253709	1.47
11	5,5-Diethylheptadecane	22.323	739617	0.48
12	Dodecane,2,6,11-trimethyl-	22.429	1757187	1.15
13	Methyl tetratriacontyl ether	23.118	541562	0.35
14	2,4-Di-tert butylphenol	23.246	2652495	1.73
15	Tridecanol,2-ethyl-2-methyl-	23.531	387063	0.25
16	Heptadecane	25.160	1374553	0.90
17	Dotriacontane	27.366	1651715	1.08
18	Octadecane	29.779	1716416	1.12
19	Neophytadiene	30.751	8524394	5.57
20	2-Pentadecanone,6,10,14-trimethyl-	30.956	1809985	1.18
21	3.7.11.15-Tetramethyl-2-hexadecen-1-ol	31.387	679069	0.44
22	Phthalic acid,hex-3-yl isobutyl ester	31.485	1303574	0.85
23	Docosane	32.387	1157844	0.76

24	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-dien	32.658	5362050	3.51
25	Dibutyl phthalate	33.885	2861407	1.87
26	Ethyl 13-methyl-tetradecanoate	34.634	2641960	1.73
27	Eicosane	34.730	354297	0.23
28	Phytol	37.171	28916654	18.91
29	11,14-Eicosadienoic acid,methyl ester	38.116	789847	0.52
30	9,12,15-Octadecatrienoic acid, ethyl ester	38.266	14179892	9.27
31	Ethyl 14-methyl-hexadecanote	38.766	1451685	0.95
32	Phytol,acetate	39.120	325664	0.21
33	Dotriacontane	41.821	6206744	4.06
34	3,7,11,15-Teramethyhexadec-2en-1-yl acetate	42.177	8981315	5.87
35	Tetracontane	44.072	5304891	3.47
36	Diisooctyl phthalate	44.634	16961460	11.09
37	Phytol,acetate	45.351	9693051	6.34
38	1,3-Benzenedicarboxylic acid,bis (2-ethylhexy	48.593	1897487	1.24
39	Tetracosane	49.709	1863589	1.22

B. GC-MS analysis of acetone leaves extract

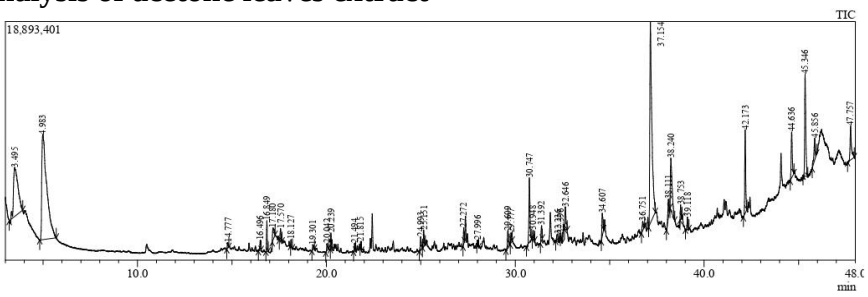

Fig. 3: Chromatograph of acetone leaves extract.

Bioactive compounds of acetone leaves extract of fenugreek analysed by chromatograph are shown in Table No.3

Table No. 3: Bioactive compounds of Acetone leaf extracts of fenugreek by Gas Chromatography- Mass Spectrometry (GC-MS).

Peak	Name	R. T	Area	Area%
1	Toluene	3.495	63835442	10.91
2	2-Pentanone, 4-hydroxy-4-methyl-	4.983	152586702	26.08
3	Undecane, 4,7-dimethyl-	14.777	1414145	0.24
4	Tetradecane,5-methyl-	16.496	2437559	0.42
5	Dodecane, 4,6-dimethyl-	16.849	6503932	1.11
6	1,3,5-Trizine-2-carboxylic acid,4-amino-6-m	17.180	5450669	0.93
7	1-Octanol, 2-butyl-	17.570	3102257	0.53
8	Hexadecane	18.127	1095341	0.19
9	Undecane, 6-cyclohexyl-	19.301	1456628	0.25
10	N-Tridecan-1-ol	20.042	1548300	0.27
11	Tetradecane	20.239	3290850	0.56
12	Eicosane, 9-cyclohexyl	21.494	1744346	0.30
13	2,4-Dimethyldodecane	21.815	1043217	0.18

14	1-Hepatadecene	24.993	4281466	0.73
15	Hepatadecane	25.151	3733485	0.64
16	Eicosane	27.272	3049914	0.52
17	Octatriacontyl pentafluoropropionate	27.996	857169	0.15
18	1- Nonadecene	29.609	4559802	0.78
19	Heneicosane	29.777	2441028	0.42
20	Neophytadiene	30.747	18982521	3.25
21	2-Pentadecanone,6,10,14-trimethyl-	30.948	2306446	0.39
22	3,7,11,15- Tetramethyl-2-hexadecen-1-ol	31.392	3668204	0.63
23	Pentatriacontane	32.236	2558224	0.44
24	Dotriacontane	32.396	1003246	0.17
25	7,9- Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-dien	32.646	7525361	1.29
26	1-Nonadecene	34.607	6690305	1.14
27	Terapentacontane, 1, 54-dibromo-	36.751	2773458	0.47
28	Phytol	37.154	117012906	20.00
29	11,14-Eicosadienoic acid, methyl ester	38.111	4478738	0.77
30	9,12,15-Octadecatrienoic acid	38.240	26672072	4.56
31	N-Nonadecanol-1	38.753	2711599	0.46
32	Phytol, acetate	39.118	2658933	0.45
33	3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate	42.173	22637725	3.87
34	Di-n-octyl phythalate	44.636	14991663	2.56
35	Phytyl palmitate	45.346	32625441	5.58
36	Tetraconatane	45.856	8142358	1.39
37	Hexatriaconatane	47.757	13624118	2.33
38	Phytyl,2-methlbutanoate	48.962	18976564	3.24
39	Hexatriaconate	49.696	8404647	1.44
40	Supraene	49.940	2054435	0.35

In the present study GC-MS spectrum of the leaves confirms the presence of diverse phytoconstituents at different retention time. Among these 39 phytoconstituents were found in alcohol leaves extracts (Table 2) and 40 in acetone leaves extract (Table 3). The similar Phytochemicals constituents like Tetradecane, Heptadecane, 2-Pentadecanone,6,10,14-trimethyl-,3.7.11.15-Tetramethyl-2-hexadecen-1-ol, 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-dien, Eicosane, Phytol, 11,14-Eicosadienoic acid, methyl ester, 9,12,15-Octadecatrienoic acid, ethyl ester, Dotriacontane, Tetracontane, Phytol, acetate were found in the alcohol as well as acetone leaves extracts of *T.foenum-graecum*. From the above result it has been noticed that the Preliminary phytochemical evaluation of Aqueous, Alcohol and Acetone leaves extract of *T. foenum-graecum* constitutes Steroids, Triterpenoids, Glycosides, Carbohydrates, Flavonoids, Phenols, Proteins, Tannins and Flavanol. A similar results were also observed by[10] that the methanolic extract of the fenugreek leaves was sequentially fractioned in petroleum ether, chloroform, ethyl acetate, butanol and aqueous fractions also contain alkaloids, tannins, flavonoids, terpenoids, saponins, steroids and glycosides.

Similar phytochemical observations were also noted by.[11] In two different areas at Gujarat in methanol extract of fenugreek leaves possesses Tannin, phenol, carbohydrates and diterpenes. Similar observations were also noticed by[12] aqueous, methanol, chloroform, toluene, petroleum ether extract of fenugreek leaves contain of acid compounds, aleurone grains, alkaloids, proteins, carbohydrates, fats, fixed oils, glycosides, flavonoids, starch, steroids, tannins, resins, essential oils and saponins.

CONCLUSION

In present work, it has been concluded that the constituents observed in aqueous, alcohol and acetone extracts of *T. foenum-graecum* are Steroids, Triterpenoids, Glycosides, Carbohydrates, Flavonoids, Tannins, Phenols, Proteins and Flavanol. Among these three solvents, the acetone extract showed the presence of more compounds as compared to aqueous and alcohol extracts. Gas Chromatography-Mass Spectrometry showed a total of seventy-nine bioactive compounds in alcohol and acetone extract of the leaves of *T.foenum-graecum*. Both phytochemical and GC-MS compounds are necessary to evaluate its potential activity regarding vasculogenesis. Research in this line is in progress in our laboratory.

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