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GAS CHROMATOGRAPHY-MASS SPECTROMETRY DETERMINATION OF BIO-ACTIVE COMPONENTS FROM *TRIGONELLA FOENUM GRECUM* L.

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ABSTRACT

Background: Herbs have high medicinal value in Indian homes and proved to arrest, reduce and terminate many diseases by the use of active constituents prepared out of them. *Trigonella foenum-graecum* L. plant is widely distributed throughout the world and which belongs to the family Fabacecae. **Aim:** In this present study determination of the bioactive components from seeds of *Trigonella foenum grecum* L has been evaluated using standard GC/MS method. **Result:** Result of this GC/MS analysis reveals that, methanolic extract of *Trigonella foenum grecum* L. consists of 3,5-Decadiyne, 2,2-dimethyl, 12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one, 4H-1Benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl), 16-Octadecenoic acid, methyl ester, [1,1'Bicyclopropyl]-2-octanoic acid,2'-hexyl-, methyl ester, Curan-17-oic acid, 19, 20-dihydroxy-, methyl ester, (19S), 9-Cycloheptadecen-1-one. **Conclusion:** This study offer a platform of using *Trigonella foenum graecum* L. seed as raw drug alternative for the current synthetic agents.

KEYWORDS: *Trigonella foenum grecum*, GC/MS, Bioactive components. Apigenin, Civetone.

1. INTRODUCTION

In developing countries, communities rely heavily on traditional herbal medicines in order to meet their primary health care needs. In many industrialized countries herbal medicines are gaining popularity as alternative and complimentary therapies.[1] *Trigonella foenum graecum* L. plant is widely distributed throughout the world and which belongs to the family Fabacecae have been used as a traditional remedy for numerous conditions including gastrointestinal disorders, gout, wound healing and inflammation, hyperlipidemia and diabetes.[2] Bioactive compounds isolated from fenugreek seeds include saponins (ie: fenugreekine, diosgenin), alkaloids (ie: trigonelline, gentianine, carpaine), amino acids, flavinoids, some of which act as insulin secretogogues (ie: 4-hydroxyisoleucine, arginine), coumarins, mucilaginous fibers (galactomannan), nicotinic acid and other vitamins and minerals.[3,2] Flavonoids have remarkable biological activities, including inhibitory effects on enzymes, modulatory effect on some cell types, protection against allergies, antibacterial, antifungal, antiviral, anti-malarial, antioxidant, anti-inflammatory and anticarcinogenic properties.[4] Recent research has identified fenugreek as a valuable medicinal plant with potential for curing diseases and also as a source for preparing raw materials of pharmaceutical industry, like in steroidal hormones. The present study was mainly focused on, GC-MS determination of bioactive components from seed of *Trigonella foenum grecum* L.

2. MATERIALS AND METHODS

2.1. Collection of plant materials

Fresh dried seeds of *Trigonella foenumgraecum* L. were commercially purchased from Nilgiris market Tambaram, Kancheepuram district, Tamil Nadu. Sample was authenticated based on organoleptic, macroscopic examination and certified by department staffs.

Figure 1: *Seeds of Trigonella foenum grecum* L.

2.2. Preparation of plant extract

The extracts were prepared as described by the standard method.[5] 500gm of dried clean *Trigonella foenumgraecum* L. seeds were coarsely powdered and weighed. The dried powder 250gm was soaked with methanol for 72 hours with intermediate shaking separately in two beakers. At the end of the extraction, it was passed through Whatman No.1filter paper (Whatman Ltd., England). Then the filtrate was extracted with soxhlet apparatus, filtered and concentrated to dryness.[6] The last traces of solvent were removed by transferring them into a china dish and allow heating the china dish using a sand bath at normal temperature, carefully in order to prevent charring and denaturation of compounds due to overheating. The yield of the methanolic extract (5gm) was noted. Dried crude extract is stored in sterile amber colored storage vials in refrigerator until used for my further studies.

2.3. Gas Chromatography–Mass Spectrometry (GC/MS) Analysis

GC/MS-a combination of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS), is used to analyze complex organic and biochemical mixtures.[7] GC/MS analysis of this extract was performed using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) equipped with a Elite-1 fused silica capillary column (30m × 0.25mm ID. ×1 ìMdf, composed of 100% Dimethyl poly siloxane with 0.25µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium as carrier gas (99.999%) was used as the carrier gas at a constant flow rate of 1ml/min and an injection volume of 2 ìl was employed (split ratio of 10:1). Injector was operated at temperature 200 to 250°C; Ion-source temperature 250 to 280°C. The column oven temperature was programmed from 110°C (isothermal for 2min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450Da.; interface temperature of 250°C; mass range of 50-600 mass units. Total GC running time was 36minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was TurboMass Ver5.2.0.

2.4. Identification of components

The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for the interpretation on mass spectrum of GC-MS. The mass spectrum of

the unknown component was compared with the spectrum of the known components stored in the NIST library.

3. RESULT AND DISCUSSION

3.1. Isolation of bioactive components by GC/MS

The methanolic extracts from seeds of *Trigonella foenum graecum* L. were subjected to GC/MS analysis and it was observed that seven compounds were identified based on active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and Concentration (%). The Figure 1 and Table 1 and 2 shows that the prevailing compounds were 3,5-Decadiyne, 2,2-dimethyl, 12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one, 4H-1Benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl), 16-Octadecenoic acid, methyl ester, [1,1'Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester, Curan- 17-oic acid, 19, 20-dihydroxy-methyl ester (19S), 9-Cycloheptadecen-1-one[Z]. Early report says that methanol, extract of seeds of *Trigonella foenum graecum* L. was subjected GC/MS analysis contained D-Glucopyranoside, methyl,3-O-Methyl-d-glucose, 2-Propen-1-amine, N- ethyl-, Aziridine, 1,2,3-trimethyl-, trans.[8]

In this present study the most abundant compounds were 9-Cycloheptadecen-1-one, [Z], followed by [1,1'Bicyclopropyl]-2-octanoic acid, 2'-hexyl-methyl ester, Curan-17-oicacid, 19,20-dihydroxy, methyl ester (19S), 4H-1Benzopyran-4-one, 5,7di-hydroxy-2-(4-hydroxyphenyl), and 16-Octadecenoic acid-methyl ester respectively. Each and every compound has a unique property and is uses and natures were collected from PubMed, as curative agents for many diseases.[9,10] Hence this result reveals that some compounds like apigenin, civetone, 16-Octadecenoic acid, methyl ester obtained through GC/MS of *Trigonella foenumgraecum* L. are well known for their medicinal properties**.**

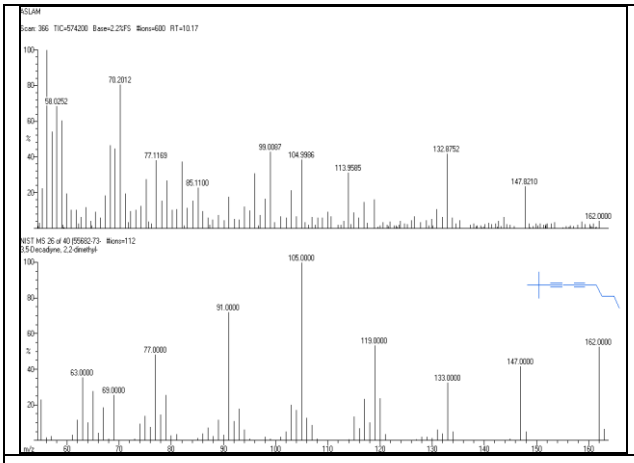


Figure 2(a): 3,5-Decadiyne, 2,2-dimethyl

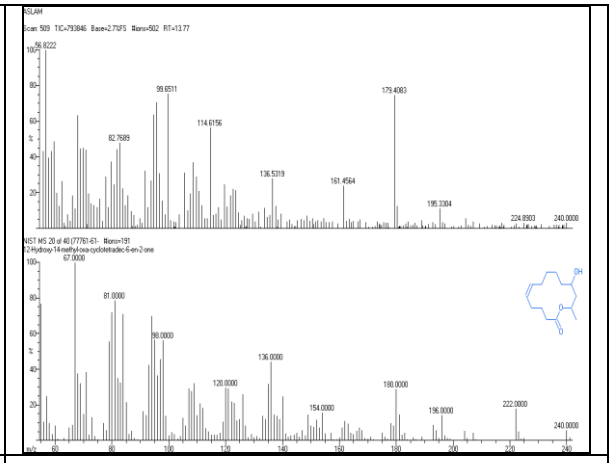


Figure 2(b): 12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one

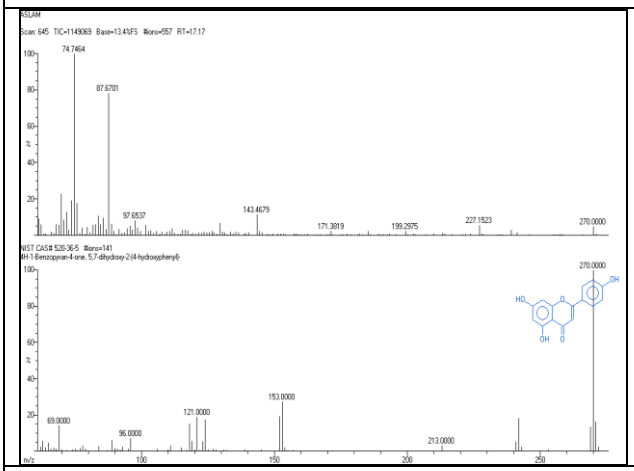


Figure 2(c): 4H-1Benzopyran-4-one,5,7di-hydroxy-2-(4-hydroxyphenyl)

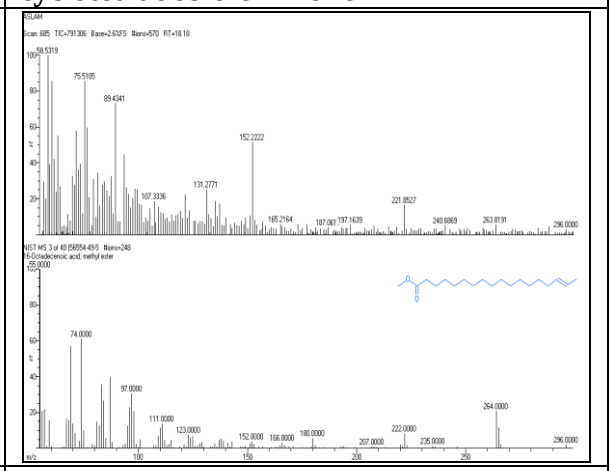


Figure 2(d): 16-Octadecenoic acid, methyl ester

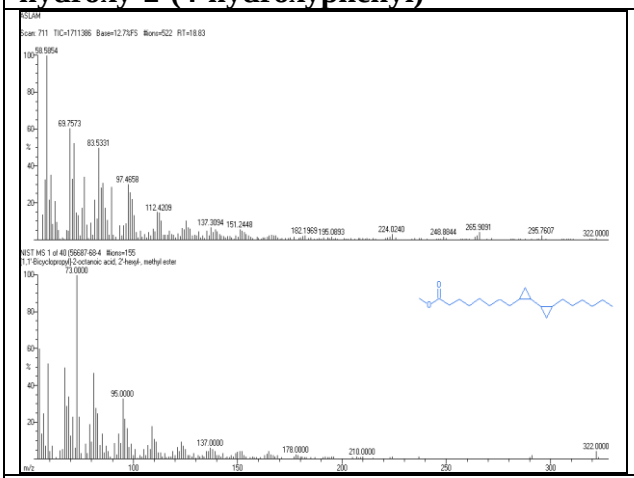


Figure 2(e): [1,1'Bicyclopropyl]-2-octanoic acid,2'-hexyl-, methyl ester

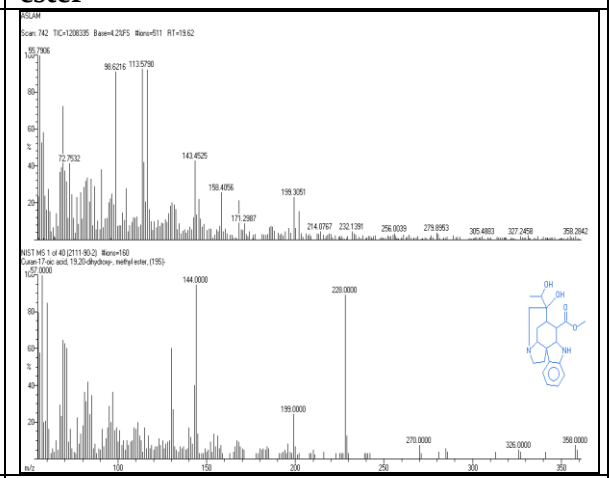


Figure 2(f): Curan-17-oicacid,19,20-dihydroxy, methyl ester, (19S)

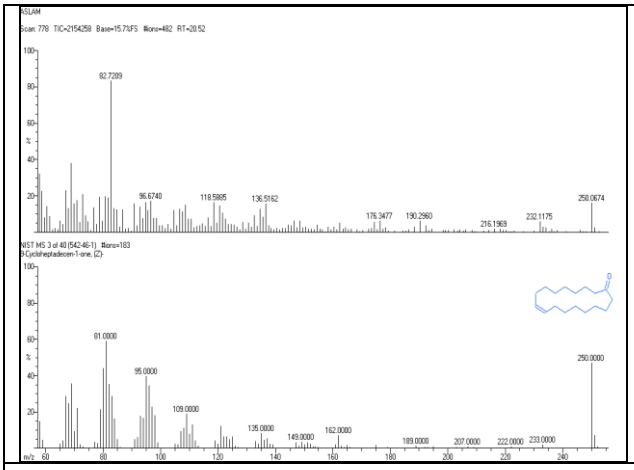


Figure 2(g): 9-Cycloheptadecen-1-one, [Z]

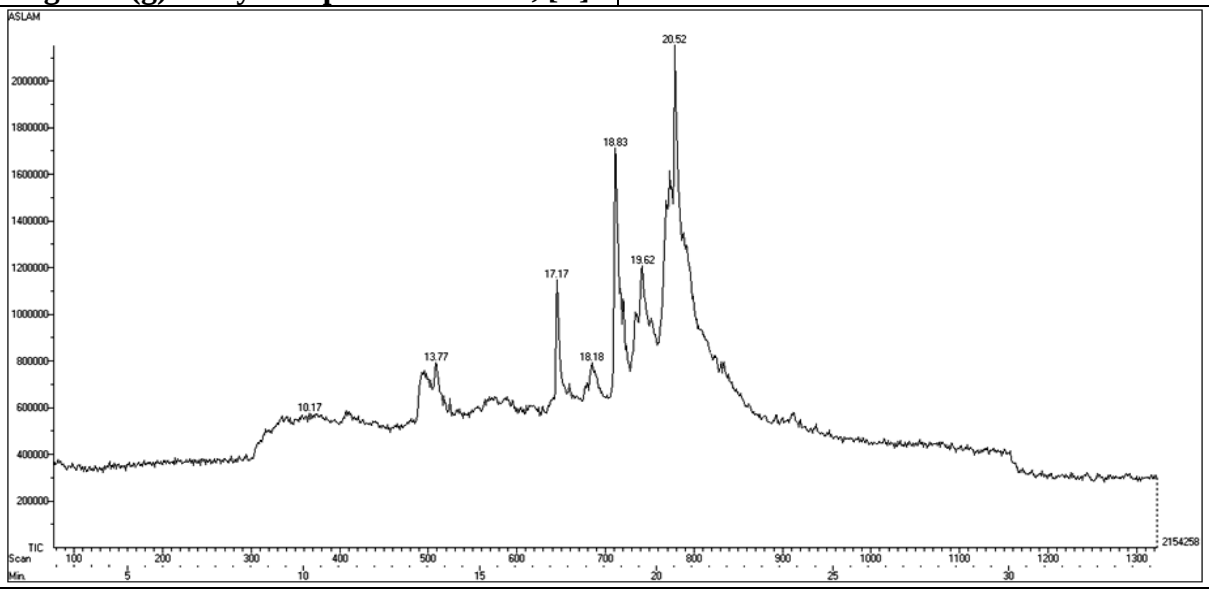


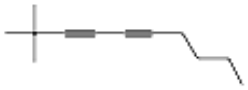
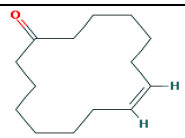
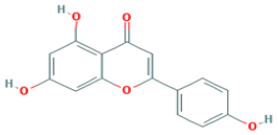
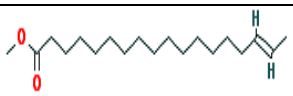
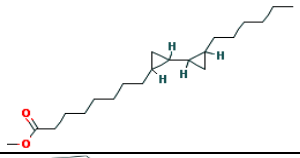
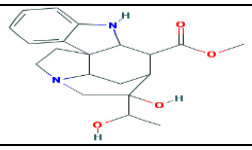
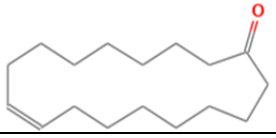
Figure 2(h): Chromatogram (GC/MS) of methanol extract from seeds of *Trigonella foenum graecum* L.

Figure 1(a, b, c, d, e, f, g and h): Total ionic chromatogram (GC/MS) of methanol extract from seeds of *Trigonella foenum graecum* L obtained with 70eV using a Elite-1 fused silica capillary column with He gas as the carrier.

Table 1: Isolation of active principle components of methanol extract from seeds of *Trigonella foenum graecum* L. based on Retention time, Molecular formula, Molecular weight by using (GC/MS).

Name of the compound	RT	Molecular formula	MW g/mol
3,5-Decadiyne, 2,2-dimethyl	10.17	$C_{12}H_{18}$	162.27
12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one	13.77	$C_{17}H_{30}O$	250.42
4H-1Benzopyran-4-one,5,7di-hydroxy-2-(4-hydroxyphenyl)	17.17	$C_{15}H_{10}O_5$	270.24
16-Octadecenoic acid, methyl ester	18.18	$C_{19}H_{36}O_2$	296.50
[1,1'Bicyclopropyl]-2-octanoic acid,2'-hexyl-, methyl ester	18.83	$C_{21}H_{38}O_2$	322.53
Curan-17-oicacid,19,20-dihydroxy, methyl ester, (19S)	19.62	$C_{20}H_{26}N_2O_4$	358.44
9-Cycloheptadecen-1-one, [Z]	20.52	$C_{17}H_{30}O$	250.42

Table 2: GC/MS Isolated active principle components their IUPAC name, structure and their nature of methanol extract from seeds of *Trigonella foenum graecum* L.

Name of the compound	IUPAC Name	Structure	Nature of the Compounds
3,5-Decadiyne, 2,2-dimethyl	2,2-dimethyldeca-3,5-diyne		Antioxidant, anti-inflammatory, anti-tumor and anti-diabetic activity.
12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one	(7Z)-cyclotetradec-7-en-1-one		No specific activity had been activity reported
4H-1Benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl)	Apigenin		No specific activity had been activity reported
16-Octadecenoic acid, methyl ester	Methyl (E)-octadec-16-enoate		Flavour, Cancer preventive, Anti-inflammatory
[1,1'Bicyclopropyl]-2-octanoic acid,2'-hexyl-, methyl ester	Methyl 8-[2-(2-hexylcyclopropyl)cyclopropyl]octanoate		No specific activity had been activity reported
Curan- 17-oic acid, 19,20-dihydroxy-, methyl ester, (19S)	Methyl 19,20-dihydroxycuran-17-oate		No specific activity had been activity reported
9-Cycloheptadecen-1-one, [Z]	Civetone		Perfume fixative, Flavor

4. CONCLUSION

From the above study it may be concluded *Trigonella foenum graceum* L contains many important phytochemicals compounds like 2,2-dimethyldeca-3,5-diyne, (7Z)-cyclotetradec-7-en-1-one, Apigenin, Methyl (E)-octadec-16-enoate, Methyl 8-[2-(2-hexylcyclopropyl) cyclopropyl]octanoate, Methyl 19,20-dihydroxycuran-17-oate, Civetone, which may prove to be a potent antimicrobial, anti-inflammatory, anti-tumor, cancer preventive, anti-diabetic activity, perfume fixative and flavouring agent, in nature. The results of this study offer a platform for using *Trigonella foenum grecum* L seed as herbal drug alternative for the current synthetic antimicrobial antioxidant, anti-inflammatory, anti-tumor, cancer preventive, anti-diabetic agents. Further investigations on health promoting aspects in *in-vitro* as well as *in-vivo* were under progress.

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