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GC/MS determination of bioactive components of *Trigonella foenum grecum*

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ABSTRACT

In this study, the bioactive components of Trigonella foenum grecum seed have been evaluated using GC/MS. The chemical compositions of the hydroalcholic extract of Trigonella foenum grecum seed were investigated using Perkin-Elmer Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. GC/MS analysis of ethanol extract of Trigonella foenum grecum seed revealed the existence of á-D-Glucopyranoside, methyl (74.54.00%),3-O-Methyl-d-glucose (16.11%)2-Propen-1-amine, N-ethyl- (3.43%), Aziridine, 1,2,3-trimethyl-, trans- (2.41%),. The results of this study offer a platform of using Trigonella foenum graecum ***seed*** *as herbal alternative for the current synthetic antimicrobial agents.*

Key words: *Trigonella foenum grecum*, GC/MS, Bioactive components.

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]. Some of the plants are used as food or medicine. These plants exhibit a wide range of biological and pharmacological activities such as anti-cancer, anti inflammatory, diuretic, oxytocic, laxative, antispasmodic, antihypertensive, anti-diabetic, and anti-microbial functions. The secondary metabolites of plants provides humans with numerous biological active products which has been used extensively as drugs, foods, additives, flavors, insecticides, colorants, fragrances and chemicals[2].

Fenugreek is commonly used as a spice in cooking and in small quantities is categorized as "Generally Recognized as Safe" by the U.S. Food and Drug Administration [3] Fenugreek is a member of the Leguminosae (Fabaceae) family and is commonly cultivated in India, Egypt, the Middle East and North Africa. The seeds of the plant have been used as a traditional remedy for numerous conditions including gastrointestinal disorders, gout, wound healing and inflammation, hyperlipidemia and diabetes [3].

The antihyperglycemic effects of fenugreek seeds and its subfractions are demonstrated in diabetic rats [4] dogs [5] and humans .The seeds also show beneficial effects in hypolipidemic subjects [6] and in cancer [7]. Supplementation of seeds in the diet enhances the antioxidant potential in control and diabetic rats [8.]. It has been reported to have restorative and nutritive properties and to stimulate digestive processes, useful in healing of ulcers in digestive tract [9]. Fenugreek has also been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant.

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 [10, 3]. 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 [11].Flavonoids form a class of benzo-gamma pyrone derivatives that have high pharmacological potency. A great interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activity of these polyphenolic compounds [12]. Much of the hypoglycemic effect of fenugreek seeds in clinical studies is likely due to the inhibitory effects of mucilaginous fibers on glucose absorption [13, 14]

Hence it was planned to study the phytochemicals present in *Trigonella foenum grecum to validate its use in traditional medicine.*

EXPERIMENTAL SECTION

Plant material and extraction procedure

Trigonella foenum grecum seed were bought fresh from local market, Thanjavur. 10gm of the powered seed material was soaked in 80% alcohol for overnight and then filtered through Whatmann filter paper No.41 along with 2g sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering the filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate was then concentrated by bubbling nitrogen gas into the solution and concentrated to 1 ml. The extract contains both polar and non-polarphytocomponents. [15]

Gas Chromatography–Mass Spectrometry (GC/MS) Analysis

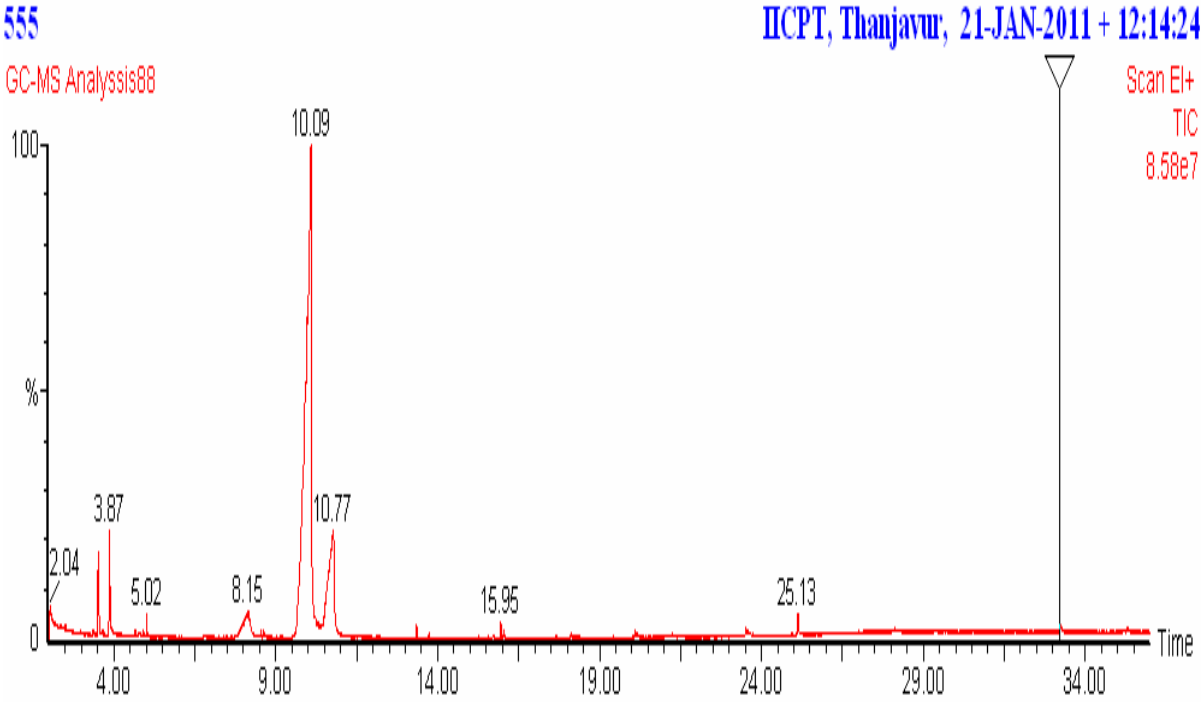
GC/MS analysis of this extract was performed using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) equipped with a Elite-1 fused silica capillary column (30 m × 0.25 mm ID. ×1 ìMdf, composed of 100% Dimethyl poly siloxane). For GC/MS detection, an electron ionization system with ionization energy of70 eV

was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min. and an injection volume of 2 ìl was employed (split ratio of 10:1). Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. 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

RESULTS AND DISCUSSION

The seeds of *Trigonella foenum graecum was subjected* GC/MS analysis .It was observed from Figure-1 and Table -1 that the seeds of *Trigonella foenum graceum* contained following components. **á-**D-Glucopyranoside, methyl (74.54.00%),3-O-Methyl-d-glucose (16.11%)2-Propen-1-amine, N- ethyl- (3.43%), Aziridine, 1,2,3-trimethyl-, trans- (2.41%). The biological activity of these phytochemicals present in trigonella foenum graecum shown in Table-2.

Figure 1: Chromatogram obtained from the GC/MS with the extract of *Trigonella foenum graecum.*



Seventeen compounds were identified in *Trigonella foenum graecum* seed extract by GC-MS analysis .The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and Concentration (%) are presented in (Table 1 and Fig 1).The prevailing compounds were á-D-Glucopyranoside, methyl (74.54.00**%),**3-O-Methyl-d-glucose (16.11%),2-Propen-1-amine, N-ethyl- (3.43%), Aziridine, 1,2,3-trimethyl-, trans- (2.41%), .

Table 1Total ionic chromatogram (GC–MS) of ethanol extract of *Trigonella foenum graecum* obtained with 70 eV using a Elite-1 fused silica capillary column with He gas as the carrier

.No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	3.52	Aziridine, 1,2,3-trimethyl-, trans-	$C_5H_{11}N$	85	2.41
2.	3.87	2-Propen-1-amine, N-ethyl-	$C_5H_{11}N$	85	3.43
3.	5.02	1-Azabicyclo[2.2.2]octane, 4-methyl-	$C_8H_{15}N$	125	0.42
4.	10.09	á-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	194	74.54
5.	10.77	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	16.11
6.	13.35	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	0.28
7.	13.74	Heptanoic acid, 2-ethyl-	$C_9H_{18}O_2$	158	0.09
8.	15.72	Hexane, 3-bromo-	$C_6H_{13}Br$	164	0.09
9.	15.95	1-Dodecyne	$C_{12}H_{22}$	166	0.32
10.	16.04	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-	$C_{10}H_{16}O$	152	0.14
11.	18.12	Piperidine, 1,1'-methylenebis-	$C_{11}H_{22}N_2$	182	0.14
12.	18.31	1-Octanol, 2-nitro-	$C_8H_{17}NO_3$	175	0.05
13.	20.11	Pentanal, 2-methyl-	$C_6H_{12}O$	100	0.19
14.	21.26	Didodecyl phthalate	$C_{32}H_{54}O_4$	502	0.09
15.	23.53	1-Tridecyne	$C_{13}H_{24}$	180	0.28
16.	25.13	Squalene	$C_{30}H_{50}$	410	0.83
17.	33.24	9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	$C_{25}H_{38}O_2$	370	0.60

Table 2: Major Phyto-components and its biological activities obtained through the GC/MS Study of *Trigonella foenum graecum*

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compound Nature	**Activity
1.	3.52	Aziridine, 1,2,3-trimethyl-, trans-	$C_5H_{11}N$	85	2.41	Nitrogen compound	Antimicrobial
2.	3.87	2-Propen-1-amine, N-ethyl-	$C_5H_{11}N$	85	3.43	Nitrogen compound	Antimicrobial
3.	5.02	1-Azabicyclo[2.2.2]octane, 4-methyl-	$C_8H_{15}N$	125	0.42	Nitrogen compound	Antimicrobial
4.	10.09	á-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	194	74.54	Sugar moiety	Preservative
5.	10.77	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	16.11	Sugar moiety	Preservative
6.	13.35	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	0.28	Plasticizer compound	Antimicrobial Antifouling
7.	13.74	Heptanoic acid, 2-ethyl-	$C_9H_{18}O_2$	158	0.09	Fatty acid compound	No activity reported
8.	15.72	Hexane, 3-bromo-	$C_6H_{13}Br$	164	0.09	Bromo compound	Antimicrobial

9.	15.95	1-Dodecyne	$C_{12}H_{22}$	166	0.32	Alkene compound	No activity reported
10.	16.04	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-	$C_{10}H_{16}O$	152	0.14	Ketone compound	No activity reported
11.	18.12	Piperidine, 1,1'-methylenebis-	$C_{11}H_{22}N_2$	182	0.14	Alkaloid	Antimicrobial Antiinflammatory Anticancer
12.	18.31	1-Octanol, 2-nitro-	$C_8H_{17}NO_3$	175	0.05	Nitrogen compound	Antimicrobial
13.	20.11	Pentanal, 2-methyl-	$C_6H_{12}O$	100	0.19	Aldehyde compound	Antimicrobial
14.	21.26	Didodecyl phthalate	$C_{32}H_{54}O_4$	502	0.09	Plasticizer compound	Antimicrobial Antifouling
15.	23.53	1-Tridecyne	$C_{13}H_{24}$	180	0.28	Alkene compound	No activity reported
16.	25.13	Squalene	$C_{30}H_{50}$	410	0.83	Triterpene	Anticancer Antimicrobial Antioxidant Chemo preventive Pesticide Anti- tumor Sunscreen
17.	33.24	9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	$C_{25}H_{38}O_2$	370	0.60	Fatty acid ester	Hypocholesterolemic Nematicide Antiarthritic Hepatoprotective Anti androgenic Hypocholesterolemic Nematicide 5-Alpha reductase inhibitor Antihistaminic Anticoronary Insectifuge Antieczemic Antiacne

CONCLUSION

From the above study it may be concluded *Trigonella foenum graceum* contains many important phytochemical like Aziridine, 1, 2,3-trimethyl-, trans-, which may prove to be a potent antimicrobial agent. Further work can be carried out to isolate the same and study its biological activity in an invitro system. The biological activities listed are based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr.Jim Duke of the Agricultural Research Service / USDA.

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