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Chemical composition of *Trigonella foenum-graecum* through gas chromatography mass spectrometry analysis

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Abstract

Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste. The phyto components in Spices help in treating in the prevention of cardiovascular diseases, carcinogenesis, inflammation, atherosclerosis. The present investigation was carried out to possible chemical components of Fenugreek, *Trigonella foenum-graecum* L (family: Leguminosae), by GC-MS. GC-MS analysis of alcoholic extract lead to identification of 13 compounds. This analysis revealed that contains *Trigonella foenum-graecum* L mainly N-Methylhomopiperazine, Vitamin E, Cholestan-3-ol, 2-methylene-, (3 α ,5 α) and 5 α -Androstan-16-one, cyclic ethylene mercaptol compounds.

Keywords: Spices, GC-MS, fenugreek, vitamin E, *Trigonella foenum-graecum*

1. Introduction

A Spice is defined as natural compound that is extracted from the seeds, fruits, flowers or trunks (skin, roots, leaves) of several plants are add to food in order to provide taste smell or flavor. Spices are a diverse group of a wide variety of staple dietary additives consumed all over the world, significantly more tropical oriental, Hispanic and Mediterranean cuisines. The Spice is a culinary term not a botanical category it does not refer to a specific kind of plant or plant part [1]. Each spice has a unique aroma and flavor which derive from compounds known as Phytochemicals or secondary compounds. These chemicals evolved in plants to protect them against herbivorous insect vertebrates, fungi pathogens and parasites [2]. Spices are used as the substances that increase the taste and variation of food [3]. Spices are the important part of human diet; humans have been used spices from the thousands of years to enhance flavor, colour and aroma of food. Consumption of herbs and spices has been implicated in the prevention of cardiovascular diseases, carcinogenesis, inflammation, atherosclerosis, etc. [4]. Fenugreek, *Trigonella foenum-graecum* L. is an annual crop from the family Leguminosae. The seed of this plant grown in South Asia, has been known to have health potential with the ability to lower blood glucose and cholesterol levels, and hence in the prevention and treatment of diabetes and coronary heart diseases. The species name "foenum-graecum" means "Greek hay" indicating its use as a forage crop in the past. Fenugreek is believed to be native to the Mediterranean region [5] but now is grown as a spice in most parts of the world. It is reported as a cultivated crop in parts of Europe, northern Africa, west and south Asia, Argentina, Canada, United States of America (USA) and Australia [6, 7] and Farnsworth 1996) India is the leading fenugreek producing country in the world [8]. Several studies have attributed the antimicrobial, antioxidant and pharmaceutical properties of spices and herbs to their phenolic compounds [9]. Fenugreek helps in balancing Cholesterol, lowering sugar-level, curing skin inflammation (wounds, rashes, boils) treating arthritis, asthma, sore throat, in which the phytoconstituents, the derived products such as flavonoids, alkaloids, terpenoids, steroids, saponins, anthocyanin, tannin etc., were involved [10].

In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a reliable method for the analysis of non-polar components and volatile essential oil, fatty acids, lipids and alkaloids [11]. GC-MS is the best technique to identify the bioactive components of long chain hydrocarbons, alcohols, acids used in the analysis of the herbal medicines, there are more significant advantages for GC-MS [12]. Thus, GC-MS should be the most preferable tool for the analysis of the volatile chemical

compounds in herbal medicines. The medicinal importance of the plant is due to the presence of some special compounds like alkaloids, flavonoids, phenols, tannins and saponins. These active principles usually remain concentrated in the storage organs of the plants, the present investigation is designed to find out the GC-MS of Fenugreek, *Trigonella foenum-graecum* which evokes various therapeutic effects.

2. Material and Methods

2.1 Sample Preparation

Fenugreek, *Trigonella foenum-graecum* L sample was collected from local market, Thanjavur. A sample weight of 10 gms were taken and soaked overnight in ethanol. The concentration of the sample was optimized after trail running. The ethanol was found to be suitable for polar and non polar compounds and was selected for Analysis.

2.2 Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

GC-MS analysis of this extract was performed using a Perkin Elmer GC Claurus 500 system which is interfaced to a Mass Spectrometer equipped with a Elite-5 MS fused silica capillary column (30m x 0.25mm x 0.25µm df) composed of 5% Diphenyl and 95% Dimethyl poly siloxane. For GC-MS detection, an electron ionization system with ionization energy of 70 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 µl was employed (split ratio of 10:1). 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 Turbo Mass Version 5.2.0 [13].

2.3 Interpretation of mass spectrum & Relative Component Concentration calculation

In the MS Programme, NIST Version 2.0 library database of National Institute Standard and Technology (NIST) having more than 2,00,000 patterns were used for identifying the chemical components. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage of each component of three concentrations of the sample was calculated by comparing its base peak area to the total areas.

3. Results and Discussion

The compounds present in the alcoholic extracts of Fenugreek, *Trigonella foenum-graecum* L were identified by GC-MS analysis (Figure 1). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in Table 2. Thirteen compounds were identified in ethanolic extract by GC-MS. The major components present in the spice of *Trigonella foenum-graecum* L were N-Methylhomopiperazine, 8-Nonynoic acid, 1-Octanol, 2,7-dimethyl-,2,4-Pentanedione, 3-(2-propenyl), Undecanal, 2-methyl, 1-Iodo-2-methylundecane, Didodecyl phthalate, Vitamin E, 5α-Androstan-16-one, cyclic ethylene mercaptole, Cholestan-3-ol, 2-methylene-, (3á,5á), 2H-Pyran, 2-(7-heptadecyloxy)tetrahydro-, and Cholest-5-ene, 3-bromo-, (3á) compounds. These phytochemicals are responsible for various pharmacological actions like antimicrobial, antioxidant, and anti-inflammation, Anti cancer activities. By interpreting these compounds, it is found that Fenugreek, *Trigonella foenum-graecum* L possess various therapeutic applications.

Table 1: Compounds identified from ethanolic extract of Fenugreek using GC-MS analysis

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compound Nature	**Activity
1.	2.22	N-Methylhomopiperazine	C6H14N2	114	13.45	Alkaloid	Antimicrobial, Anti-inflammatory
2.	8.05	8-Nonynoic acid	C9H14O2	154	0.61	Amino compound	Antimicrobial Anti-inflammatory
3.	16.62	1-Octanol, 2,7-dimethyl-	C10H22O	158	0.37		
4.	16.96	2,4-Pentanedione, 3-(2-propenyl)-	C8H12O2	140	0.97	Ketone compound	No activity reported
5.	18.86	Undecanal, 2-methyl-	C12H24O	184	1.38	Aldehyde compound	Antimicrobial Anti-inflammatory
6.	19.40	1-Iodo-2-methylundecane	C12H25I	296	1.56	Iodine compound	Antimicrobial
7.	19.98	Didodecyl phthalate	C32H54O4	502	0.78	Plasticizer compound	Antimicrobial Antifouling
8.	28.08	Vitamin E	C29H50O2	430	4.92	Vitamin compound	Antiageing, Analgesic, Antidiabetic, Antiinflammator, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, Antibronchitic, Anticoronary
9.	29.43	5α-Androstan-16-one, cyclic ethylene mercaptole	C21H34S2	350	2.31	Steroid	Antiarthritic, Anticancer, Hepatoprotective, Antimicrobial, Antiasthma, Diuretic
10.	31.01	Cholestan-3-ol, 2-methylene-, (3á,5á)-	C28H48O	400	15.81	Steroid	Antiarthritic, Anticancer, Hepatoprotective, Antimicrobial, Antiasthma, Diuretic
11.	31.59	2H-Pyran, 2-(7-heptadecyloxy)tetrahydro-	C22H40O2	336	3.27	Pyran compound	No activity reported
12.	33.52	Cholest-5-ene, 3-bromo-, (3á)-	C27H45Br	448	5.50	Steroid	Antiarthritic, Anticancer, Hepatoprotective, Antimicrobial, Antiasthma, Diuretic

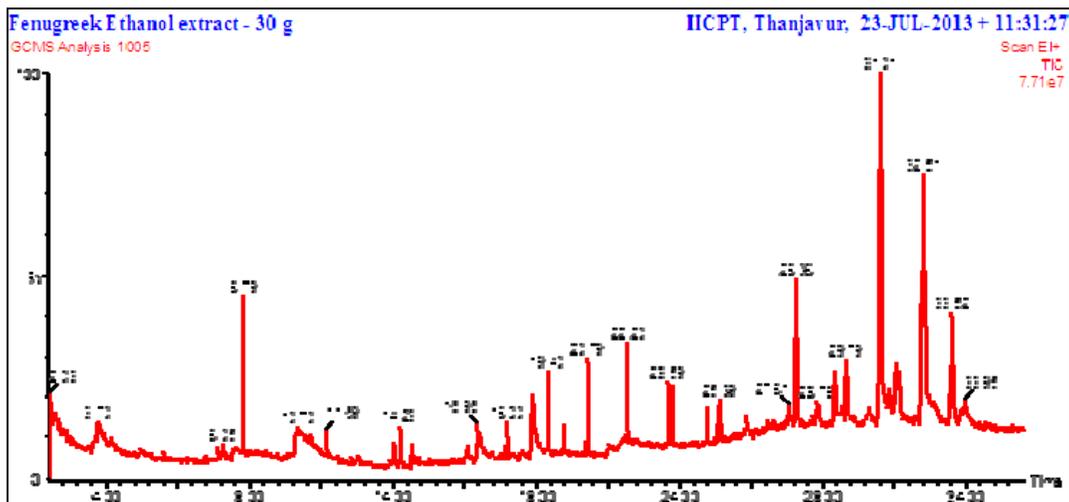


Fig 1: Shows GC-MS Chromatogram of ethanolic extract of Fenugreek

4. Conclusion

The result of the present investigation reveals that the ethanolic extract of Fenugreek, *Trigonella foenum-graecum* L possessed significant anti-inflammatory, anticancer, antioxidant, antitumor, immunostimulant and antimicrobial properties. Thus, the medicinal spice Fenugreek, is found to possess significant phytoconstituents such as Alkaloid, Amino compound, Ketone compound, Aldehyde compound, Iodine compound, Plasticizer compound, Vitamin compound, Steroid and Pyran compound. The presence of various bioactive compounds justifies the use of the spice for various ailments by traditional practitioners. So it is recommended as a plant of phytopharmaceutical importance. The importance of the study is due to the biological activity of some of these compounds. The present study, which reveals the presence of components in Fenugreek, *Trigonella foenum-graecum* L suggest that the contribution of these compounds on the pharmacological activity should be evaluated

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