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Phytochemical screening and gas chromatography– mass spectrometry and analysis of seed extract of *trigonella foenum-graecum*, linn (fenugreek /methi)

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Fenugreek (Methi) is an important spice; its dried seeds have wide application in food and beverages as flavoring additive as well as in medicines. The present study was carried out to identify chemical present in extract the methanolic seed extract of the plant by phytochemical screening methods and GC-MS analysis. The Phytochemical studies test shown the presence of carotenoids, polyphenols alkaloids, fatty acids, essential oils, proteins with essential aminoacids, iron, ascorbate, folate content. The gas chromatography -mass spectrometry (GC-MS) analysis also identified the presence of phytochemical components like 2-isocyanato – 2- methyl propane (RT:6.555), (5R,6R)-5,6-dihydroxy-5-methyl dihydro-2, 4 (1H,3H) – pyrimidinedione (RT: 6.878), (5R) – 2 – iso propylidine-5-methylcyclohexanone (RT: 12.217).

Keyword: *Trigonella foenum- graecum;* Gas chromatography; Mass spectrometry; Phytochemical screening.

1. Introduction

From ancient time spices are used to flavor and improve the taste of food recipes. Besides this they are used in cosmetics and medicinal preparation of Indians and Nepali systems such as Ayurveda and Unani. *Trigonella foenum-graecum* (fenugreek/Methi) have great medicinal value and proved to serve as good hypoglycemic, hypocholesterolemic, galactogouge, laxative stimulant, carminative, stomachic, antacid, antiulcerative, antibacterial, antihypertensive, antithrombotic, anticarcinogenic, antioxidant and diuretic [2, 4, 6, 7, 8, 14].

The previous pharmacological studies of various seed extracts of Fenugreek have also shown that it contains mucilage, volatile oils and alkaloids such as choline and trigonelline, sotolone and pyrazines,. Bitterness of fenugreek seeds is mainly due to the oil, steroidal saponins and alkaloids which are all non-toxic on consumption [9]. Various ethnobotanical surveys observed fenugreek seeds can be used in simple remedies for treating a variety of aliments [5, 15]. A reference cited wrote, one teaspoon of fenugreek seeds cooked with rice and eaten regularly for 10 to 15 days shows remarkable rise in hemoglobin [3].

Native to North Africa and countries bordering the eastern Mediterranean, fenugreek grows in open areas and is widely cultivated in India, Nepal, and Pakistan. It requires well drained, good soil of medium texture having pH range is 5.3 to 8.2. Seeds are sown directly in the garden in spring. The plant reaches a height of 0.3 to 0.8 meters and has trifoliate leaves, while flowers appear in early summer and develop into long, slender, yellow brown pods containing the brown seeds, which are hard, yellowish brown and angular or oblong or rhombic with a size of about 3 mm (1/8").

Several human intervention trials and animals experiments demonstrated that antidiabetic effect of fenugreek seeds ameliorate most metabolic symptoms associated with type 1 and type II diabetes [11, 12, 13]. Xue WL et al in their study concluded that *T. foenum-graecum* extract can lower kidney or body weight ratio, blood glucose, blood lipids and improve hematological properties in experimental diabetes rats [16]. In recent research fenugreek seeds were shown to protect against experimental cancers of breast [1] and colon [10]. In this study, further investigation was carried out to identify other active constituents present in the methanolic seed extract of the plant by phytochemical screening and gas chromatography-mass spectrometry (GC-MS) analytical methods.

2. Methodology

A Fenugreek seed sample was collected from the Ason chawk (Kathmandu) and identified by botanist.

A. Preparation of fenugreek seed extract

Dry fenugreek seed (10 gm) was cleaned and ground into small pieces by a warring blender and passed through a 1mm sieve 150 ml, methanol was used for extraction by sexhlet extraction method for six hours. The extract was filtered. The residue was re-extracted twice under the same condition to ensure complete extraction. The extract was combined, filtered and evaporated to dryness under reduced pressure at 60 °C by a rotary evaporator. Extract was placed in dark bottle and stored at -8 °C until further analysis.

2.1 Phytochemical screening

The seed extract was subjected to preliminary phytochemical screening to test for the presence or absence of phytochemical constituents using the methods described below by (Ajayi *et al*. 2011).

A. Alkaloids

1.0 ml extract was stirred with 5 ml dil hydrochloric acid on a steam bath, filtered and 1.0 ml of each Dragendoff's /Mayer's/Wagner's reagents was added to 1.0ml separate portions of filtrate. A cloudy orange red/slightly yellow/turbid brown colour indicates the presence of alkaloids

B. Tannins

1.0 ml extract was stirred with 1.0 ml of ferric chloride solution. A greenish black precipitate indicates the presence of tannins; 1.0 ml extract was also stirred with 1.0 ml bromine water. A reddish brown turbid colour indicates the presence of tannins.

C. Steroids (Liebermann – Burchard's test)

0.5 g extract dissolved in 2.0 ml acetic anhydride, cooled in ice and 1.0 ml Conc H_2SO_4 was carefully added, a blue green ring indicates the presence of steroids.

D. Saponins (Frothing Test)

0.2 g extract was mixed with 5.0 ml distilled water, shaken for 20 min. persistence of foam indicates presence of saponins.

E. Flavonoids (ferric chloride test)

0.2 ml extract was added to 10% ferric chloride and mixture was shaken. a wooly brownish precipitate indicates the presence of flavonoids.

F. Carbohydrate

a) 1.0 ml extract was added to 2.0 ml Fehling's solution and boiled for 5 min, a red precipitate indicates the presence of reducing sugars
b) 1.0 ml extract was added to 2.0 ml Barford's reagent and boiled for 1 min. A red precipitate indicates the presence of reducing monosaccharides.
c) Molisch's test: 10 ml extract was added to 1.0 ml molisch's reagent and 1.0 ml Conc H_2SO_4 was carefully added. A reddish violet ring indicates the presence of carbohydrates.

2.2 Gas Chromatography-Mass Spectrometry Analysis

The GC-MS analysis was carried out using a Hewlett packed gas chromatography (model 6890

series) equipped with a flame ionization detector and Hewlett Packard 7633 series indicator, MS transfer line temperature of 250 °C. The GC was equipped with a fused silica capillary column HP -5MS (30x0.25 mm), film thickness 1.0 µm. The oven temperature was held at 50 °C for 5 min holding time and raised from 50 to 250 °C at a rate of 2 °C/min, employing helium gas (99.999%) as a carrier gas at a constant flow rate of 22 cm/s. 1.0 micron of extract (1 mg dissolved in 1ml absolute alcohol), at a split ratio of 1:30 was injected.

MS analysis was carried out on Agilent. Technology network Mass spectrometer (model 5973 series) coupled to a Hewlett Packard Gas chromatography Model 6890 series) equipped with NIST08 Library software database. Mass spectra were taken at 70 ev/200 °C scanning rate of 1 scan/s.

Identification of compounders was conducted using the database of NIST08 Library. Mass spectrum of individual unknown compound was compared with the known compounds stored in the software database library.

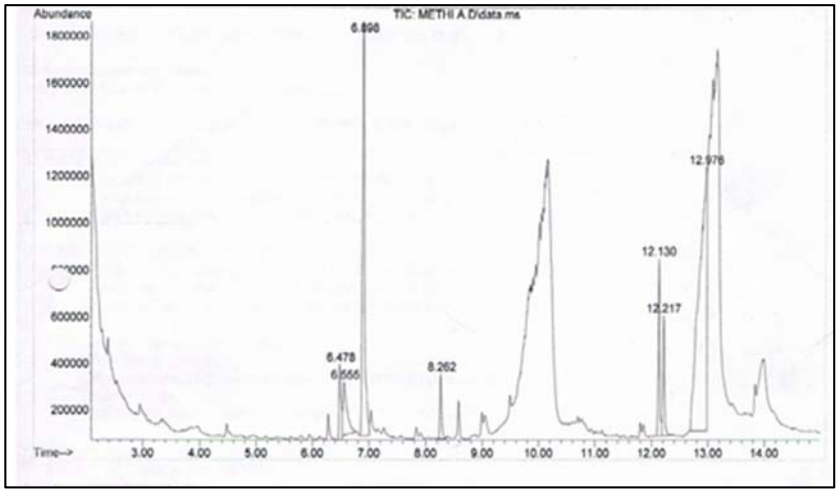


Fig 1: GC-MS Chromatogram of Methanolic seed extract of T.foenum-graecum

3. Results and discussion

Table 1 shows the results of the phytochemical screening of the methanolic seed extract of *Trigonella foenum–graecum*. This reveals the presence of flavonglycosides (high), carotenoids (high), polyphenols (medium), alkaloid (medium), fatty acids (medium), essential oil (medium).

4. Gas Chromatography-Mass Determination (GC-MS) Analysis

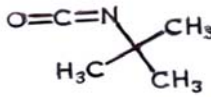
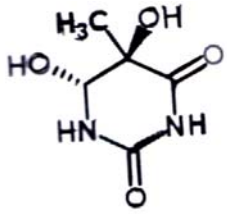
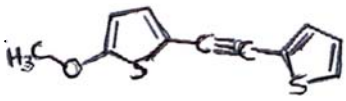
The results of the GC–MS analysis identified the various compounds present in the seed extract (figure – 1 and table2). Figure 1 shows the gas chromatogram of the seed extract which shows seven distinct peaks identified by GC-MS while the compounds identified through the NIST08 Library database are listed in table 2. The major compounds present in the methanolic seed extract of fenugreek as identified by GC-MS was 2-isocyanato-2-methyl propane (RT6.555), (5R,6R)-5,6- dihydroxy -5 methyldihydro-2,4 (1H,3H) pyromidinedione (RT:6.898), (5R)-2- iso propylidene -5 – methylcyclohexanonone (RT:8.262), 2-methoxy(2-thienylethynyl) thiophene(RT: 6.898), 2,4 – dimethyl-1H-pyrrole (RT:12.130), Diethylchloro-malonate(RT:12.130), (2E)-4-Oxo-2-pentenoic acid (RT:12.130), Methyl α-D-glucopyranoside (RT:12.127).(figure 1)

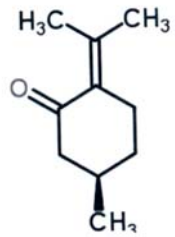
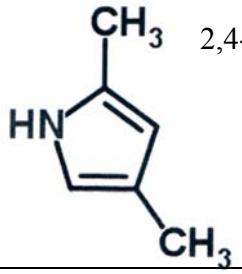
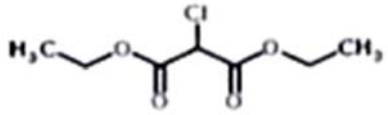
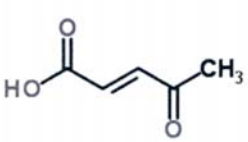
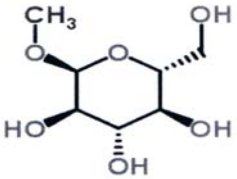
Table 1: Results of phytochemical screening of Methanolic seed extract of *T. foenum-graecum* (fenugreek)

Phytochemicals	Results
Flavonic glycosides	+++
Carotenoids	+++
Polyphenols	++
Alkaloid	++
Fatty acids	++
Essentials oil	++
Reducing sugar	-
Courmarin	-
Anthocyanosides	-
Anthracenosides	-

Remarks: +++ = Shows (high); ++ = Shows (Medium); + = Shows (Low); - = shows not Detected

Table 2: Phytochemical components identified in the Methanolic seed extract of *T. foenum-graecum*, Linn, showing their Systematic name, Average mass, Retention Value and Percentage area.

Compounds	Systematic Names	Molecular Formula	Average Mass	Retention Value	Area%
1. 	2-Isocyanato-2-methyl propane	C_5H_9NO	99.131	6.555	5.31
2. 	(5R, 6R)- 5-6-Dihydroxy-5-methyl dihydro-2, 4(1H, 3H)-pyrimidinedione	$C_5H_8N_2O_4$	160.128	6.898	23.34
3. 	2-Methoxy-5-(2-thineylethynyl)	$C_{11}H_8OS_2$	220.310	6.898	23.34

4.  (5R)-2-Isopropylidene-5methylcyclohexanone	$C_{10}H_{16}0$	152.233	8.262	3.10
5.  2,4-Dimethyl-1H-pyrrole	C_6H_9N	95.142	12.130	6.51
6. Diethyl 	$C_7H_{11}Cl0_4$	194.612	12.130	6.51
7.  (2E)-4-Oxo-2-pentenoic acid	$C_5H_6O_3$	114.031	12.130	6.51
8.  Methyl α-D-glucopyranoside	$C_7H_{14}O_6$	194.182	12.217	4.74

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