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ISOLATION OF KAEMPFEROL DERIVATIVES FROM METHANOLIC EXTRACT OF *TRIGONELLA FOENUM-GRAECUM*

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ABSTRACT

Trigonella foenum-graecum is an annual plant in the family Fabaceae. In the present study, methanolic extract of the leaves of the Trigonella foenum-graecum was partitioned using water, butanol and ethyl acetate. Then ethyl acetate soluble fraction was subjected to column chromatography to isolate and purify the phytoconstituents. One compound was isolated, purified and characterized as (Kaempferol 3-o-D-glucosyl (1 2)- (6^{11}-O-acetyl)-β-D-galactoside 7-O-β-D-glucoside by using IR, Mass and NMR spectroscopy.

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INTRODUCTION

Trigonella foenum-graecum L. is an annual herb from the family Fabaceae, indigenous to the Mediterranean area. The producers of fenugreek are India, Iran, Nepal, Bangladesh, Pakistan, Argentina, Egypt, France, Spain, Turkey, Morocco and China. However, nowadays it grows anywhere around the world. Its height goes up to 60cm, branching off with trefoil leaves and small white flowers. If it grows more, can develop sickle shaped pods including 10-20 brownish seeds 3x4 mm in dimensions [1]. Its seeds are known as Trigonella seeds or as Fenugreek. Moreover, its popularity refers to the pungent aromatic properties [2]. Most applicable part of T. foenum-graecum (as spice and medicinal purpose) is the seed [3- 9]. In the present study, methanolic extract of the leaves of the *Trigonella foenum-graecum* was partitioned using water, butanol and ethyl acetate. Then ethyl acetate soluble fraction was subjected to column chromatography to isolate and purify the phytoconstituents.

Chemical compound of T.foenum-graecum L.: The leaves contain 7 saponins, known as graecunins. These compounds are glycosides of diosgenin. Leaves contain moisture 86.1%, protein 4.4%, fat 0.9%, minerals 1.5%, fiber 1.1%, and carbohydrates 6%. The mineral and vitamin contents are calcium, iron, phosphorous, carotene, thiamine, riboflavin, niacin and vitamin C.[10]

Uses:-

The plant has been scientifically used for wounds, inflammation, gastrointestinal ailments, cholesterol lowering agent [11], diabetes [12], bronchitis, inflammation, chronic cough, liver disorders [13]

Pharmacology:-

Antidiabetic activity [14], antimicrobial activity [15], antifungal activity [16], antioxidant activity[17], immunomodulatory activity [18], anti-inflammatory and antipyretic activity[19], antibacterial activity[20], diuretic activity[21], prophylaxis effect[22], anti-tumor and anti-cancer activity [23], antiulcer activity[24], antifertility activity [25] and hypocholesterolaemic avtivity [26]

MATERIAL AND METHODS

Plant collection:

Leaves of *Trigonella foenum-graecum* were authenticated and obtained from Natural Remedies Pvt. Ltd., Bangalore.

Chemicals:

Dried leaves of Trigonella foenum-graecum, methanol, ethyl acetate, pet ether, water, and acetone were collected From Natural Remedies Pvt. Ltd., Bangalore. The leaves were authentified and subjected to methanolic extraction

Plant extraction

23kg of leaves were extracted with methanol by refluxation; three washes were carried out initial wash with 150lts for 3hrs followed by two washes with 150lts of methanol for 2hrs at 55- 65°C.Each wash was filtered using fine 5µm cloth (muslin cloth). Soluble and insoluble portions were taken separately. Combined soluble portions of the 3 washes were concentrated under the vaccum55-60°C.Final drying was done using VTD drier. The final yield was found to be 2.5kg.

Procedure for partition of methanol extract

2.5kg of extract was dissolved in 23lts of water.23lts ethyl acetate was added into the separating funnel and then the extract was added and partitioned for 3 times. All 3 washes was collected, combined and concentrated, Then the extract was further partitioned with butanol as similar procedure as that of ethyl acetate. Final drying was done using rotary evaporator. Ethyl acetate soluble fraction was selected for further isolation of compounds [27]

OPTIMIZATION OF TLC SYSTEM

Butanol : Water (20:25)

COLUMN CHROMATOGRAPHY OF METHANOLIC EXTRACT OF *TRIGONELLA FOENUM-GRAECUM*

Requirements:

Stationary phase : Silica gel 60/120
Mobile phase : Pet ether, ethyl acetate, methanol
Charged material : 1.06kg Ethyl acetate soluble extract
Adsorption ratio : 1:1(charged material: Silica gel)
Volume of each fraction collected: 1-5lts

COLUMN:

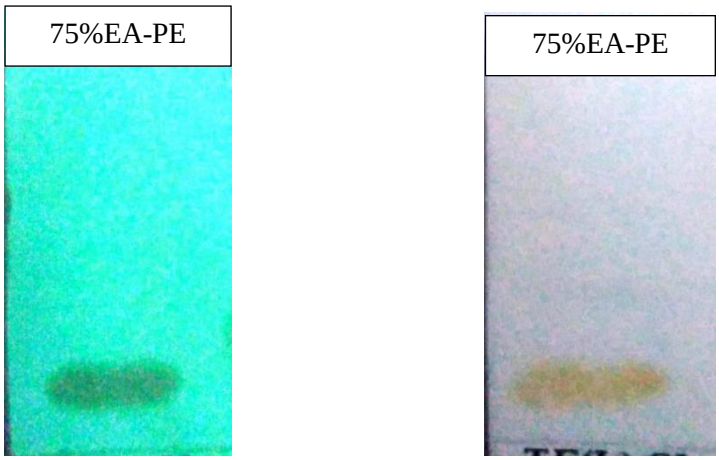
1.06kg of Ethyl acetate soluble fraction was dissolved in minimum quantity of methanol and adsorbed, in the ratio of 1:1 and dried at room temperature, it is used as a charged material. A column was first loaded with 4.6kg of silica gel as bed, with pet-ether as solvent (dry packing) the column was first eluted with 100% PE then polarity of mobile phase was varied with, ethyl acetate, methanol. The fractions collected were dried using rotary evaporator and weighed.

TLC of fractions collected from column , based on the TLC pattern 75% ethyl acetate/pet ether of column was further purified with the help of preparative HPLC

Preparative HPLC of 75% ethyl acetate/pet ether

Column :- Kromasil C-8
Mobile phase :- 20%Methanol/Water
Elution :- Isocratic
Flow rate :- 20ml/min
Injection volume:- 200mg/ml
Detector :- SPD-M10 AVP Photodiode array detector at 205nm

RESULT AND DISCUSSION



Visualization by UV at 254 nm **Visualization after ANS spray**

Figure 1:Photograph of TLC of ethyl acetate fraction.

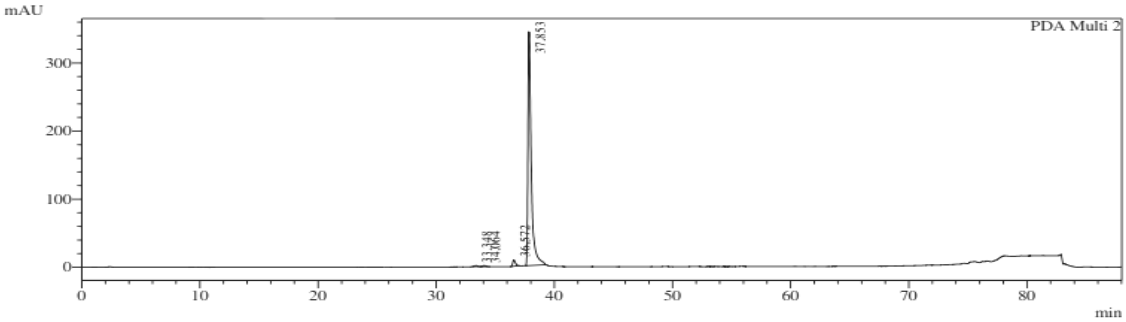


Figure 2: HPLC Chromatogram of isolated compound.

Table No. 1.

Retention	Area	% Area
37.853	6706421	97.079

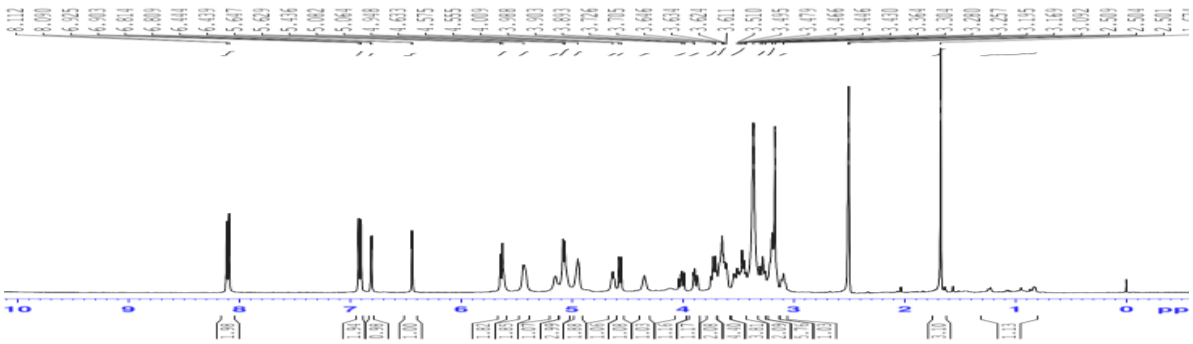


Figure 3: ^{1}H NMR spectrum of isolated compound.

Table no 2: Integral values of ^{1}H NMR.

Main	Positions of hydrogen's
8.112	2H,d,J=8.8Hz, H-2', 6'
6.925	2H,d,J=8.8Hz, H-3', 5'
6.814	1H,d,J=2 Hz, H-8
6.444	1H,d,J=2, H-6
5.647	1H,d,J=7.2, H-1''
5.082	1H,d,J=7.1 H-1''''
4.575	1H,d,J=8.8, H-1'''
1.674	3H, s, CH_3CO

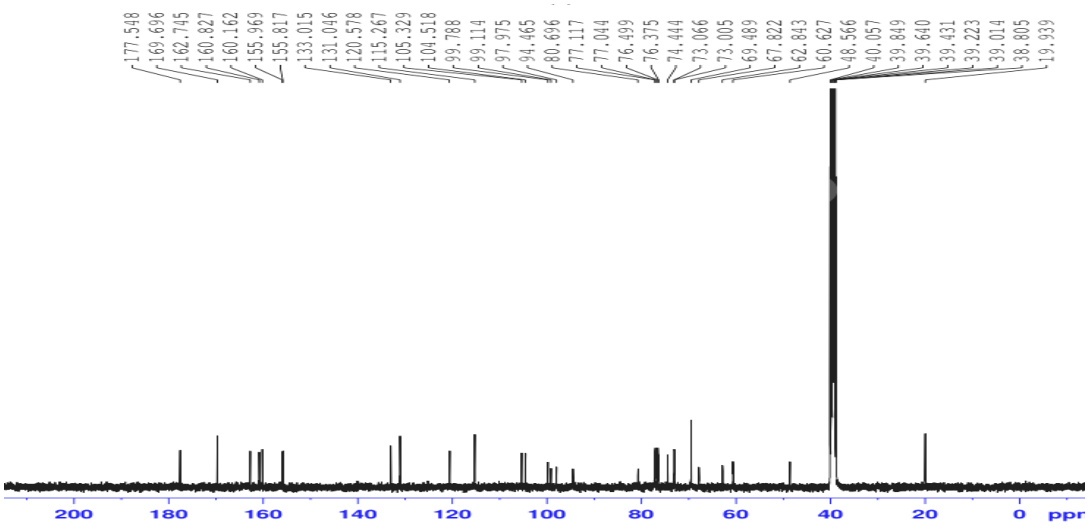


Figure 4: ^{13}C NMR spectrum of isolated compound.

Table no 3: Integral values of ^{13}C NMR.

Position	Shift	Position	Shift
2	159.969	3-Gal-1"	97.9
3	133.015	2"	80.6
4	177.548	3,,	73.06
5	160.827	4,,	67.82
6	99.788	5,,	73.005
7	162.745	6,,	62.843
8	94.465	2,,-Gle 1,,	104.51
9	155.817	2,,,	74.4
10	105.329	3,,,	76.499
1,	120.578	4,,,	69.48
2,	131.046	5,,,	77.1
3,	115.267	6,,,	60.6
4,	160.162	7-Gle 1,,,,	99.7
5,	115.2	2,,,,	73.06
6,	131.046	3,,,,	76.375
		4,,,,	69.4
		5,,,,	77.044
		6,,,,	60.627
		Ac	19.939
			169.696

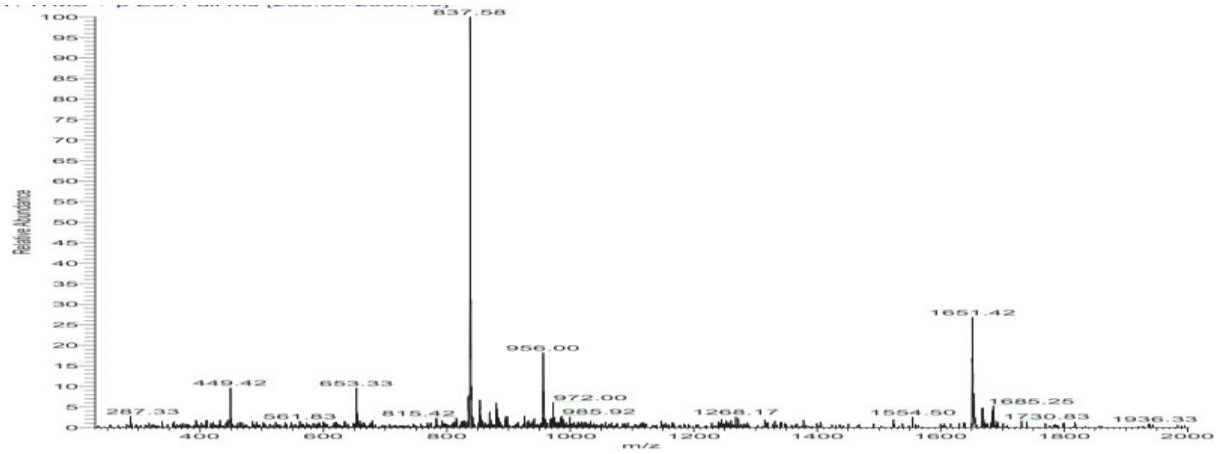


Figure 5: LC-MS spectrum of isolated compound.

The molecular ion peak obtained at 837.58 m/z showed the interference of sodium ion used during spectral analysis

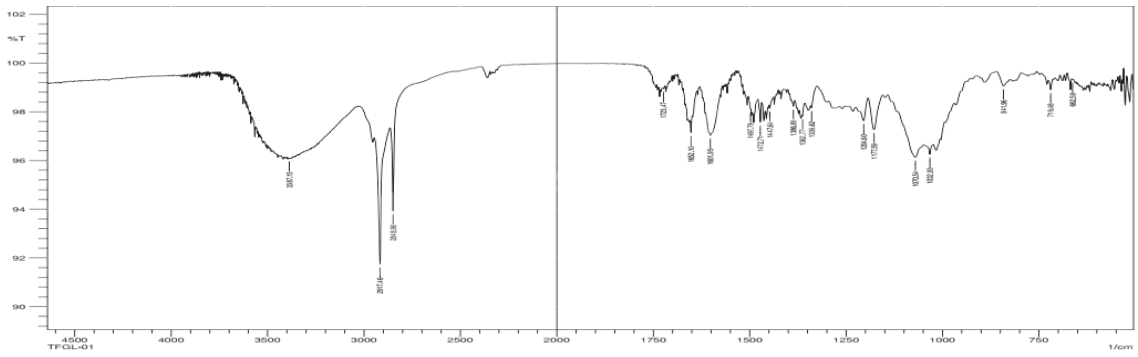


Figure 6: IR spectrum of isolated compound.

Supporting IR values

3387.15cm^{-1}-OH stretching
2917.46cm^{-1}-CH stretching
2848.98cm^{-1}-CH aliphatic stretching
1723.47cm^{-1}- C-O ester
1652.10cm^{-1}-C=O Carbonyl

Structure

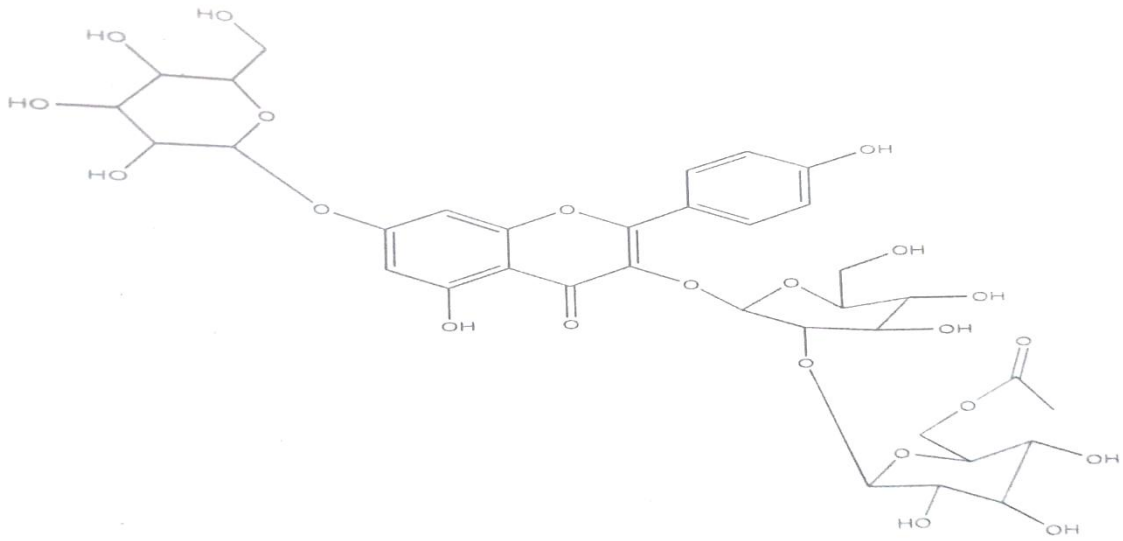


Figure 7: structure of isolated compound.

In the present study an attempt has been made to isolate and characterize the constituents from *Trigonella foenum-graecum* leaves, and one compound were isolated from the Ethyl acetate soluble fraction. *Trigonella foenum-graecum* leaves were taken extracted with methanol and dried in distillation unit. The methanolic extract was subjected to partition with Water, Butanol and Ethyl acetate. Ethyl acetate soluble extract from *Trigonella foenum-graecum* leaves was subjected to column chromatography for the separation of constituents. The column chromatography was made with different stationary as well as mobile phases which resulted in the isolation of one constituents. HPLC method was used to analyse the purity of compounds. The HPLC method indicated compound is 97% pure. FTIR, Mass spectroscopy, ^{1}H NMR, ^{13}C NMR spectral values of isolated compound was compared with the literature, clearly suggested that the compounds were characterized as Kaempferol 3-o-β-D-glucosyl(1 2)-(6^{11}-O-acetyl)-β-D-galactoside 7-O-β-D-glucoside. This compound can be used for the standardization of the extracts and preparations containing *Trigonella foenum-graecum.*

CONCLUSION

The methanolic extract of *Trigonella foenum-graecum* leaves was found to contain maximum yield of 10.86%. Butanol:Water (20:25) was the optimized solvent system used for all the TLC solvent system. One compound was isolated, The isolated compound was characterized and confirmed as kaempferol 3-o-β-D-glucosyl (1→2)- (6^{11}-O-acetyl)-β-D-galactoside 7-O-β-D-glucoside. In this work, we conclude the column chromatography for the isolated compound was found to be suitable for commercial scale. The isolated compound was also used to standardize the methanolic extract of leaves of *Trigonella foenum-graecum*. Recommend future Research.

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Authors' Statements

Competing Interests

The authors declare no conflict of interest.

Glossary of abbreviations

UV	:	Ultra violet
P.HPLC	:	Preparative HPLC
HPLC	:	High performance liquid chromatography
TLC	:	Thin layer chromatography
FTIR	:	Fourier transform infrared spectroscopy
IR	:	Infrared spectroscopy
LC-MS	:	Liquid chromatography- Mass spectroscopy
1H NMR	:	Proton nuclear magnetic resonance spectroscopy
13C NMR	:	Carbon 13 nuclear magnetic resonance spectroscopy
α	:	Alpha
β	:	Beta
γ	:	Gama
C18	:	Carbon 18

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