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Separation and Identification of Oil from *Trigonella Foenum-Graecumn* Seeds by using Spectrophotometric and Chromatographic Methods

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The research concerned with oil of *Trigonella Foenum-Graecumn* seeds after treatment with different solvents by solvent extractor apparatus (Soxhelt). The composition of Fenugreek seeds in the form of oil were investigated through (FTIR , HPLC , GC - Mass and U.V spectrophotometry apparatus), the results indicate that Feungreek have 5-8% oil ,many compounds were identify such as Dioctyline oxid Dicyclohexyl Keton, Dihexylamine, Isopropylmyristat. Bio assay also applied.

Introduction

Trigonella foenum-graecum L. (Fenugreek) is a plant from the family Fabaceae that grows anywhere around the world , it supplemented to a dairy cattle diet significantly increased the amount of polyunsaturated fatty acids in the milk ,and decreased the level of milk cholesterol. [1] Fenugreek seed oil has a pungent odor and bitter taste and is often used as an insect repellent for grains and fabrics [2] .*Trigonella foenum graecum* (Fenugreek) was administered at 2 to 8 g/kg dose orally to normal and alloxaniduced diabetic rats. It produced a significant fall ($P < 0.05$) in blood glucose both in the normal as well as diabetic rats and the hypoglycemic effect was dose related [3] .The endosperm of the seed is rich in galactomannan; young seeds mainly contain carbohydrates and sugar. Mature seeds content amino acid, fatty acid, vitamins, and saponins. The seeds of fenugreek contain a large quantity of folic acid (84 mg/100 g). The main chemical constituents of T. foenum – graecumn are fibers, flavonoids, polysaccharides, saponins, flavonoids and polysaccharides fixed oils and some identified alkaloids viz., trigonelline and cholin [4].

The composition of Fenugreek seeds in the form of powder, ash, and oil is investigated through FTIR and FT Raman spectra measurements. The results indicate that Fenugreek seeds (powder) are rich in proteins. Fats (lipids) and starch are present in small amounts in the seeds. The FTIR absorption bands of seed powder appeared at: 3365 cm-1 assigned as N-H stretching vibrations (amide A of protein), 1657 cm-1 (C=O, amide I), 1540 cm-1 (N-H bending vibration, amide II), and 1240 cm-1(N-H bending, amide III). In the FT Raman spectra the band at 1661 cm-1 is ascribed to amide I (C=O) of proteins while the band at 1080 cm-1 indicates the starch. On the other hand, the ash of fenugreek is very rich in phosphate compounds.

The spectra showed absorption bands at frequencies 1082, 1000, 618, and 566 cm-1, and the FT Raman spectrum showed a strong absorbance band at 793 cm-1, which is due to phosphate compounds. It could be concluded that the inorganic part of fenugreek consists mainly of phosphate compounds.[5][6]. A high performance liquid chromatographic (HPLC) procedure for quantitating ketoprofen in isopropyl myristate(Propan-2-yl tetradecanoate) which is present in Fenugreek seeds, a compound widely used as a receptor medium in drug diffusion studies of topical aqueous-based formulations, is developed. Previously reported HPLC assays for ketoprofen in IPM have employed relatively complex and tedious methods for purifying the IPM prior to injection onto the HPLC column.[7]

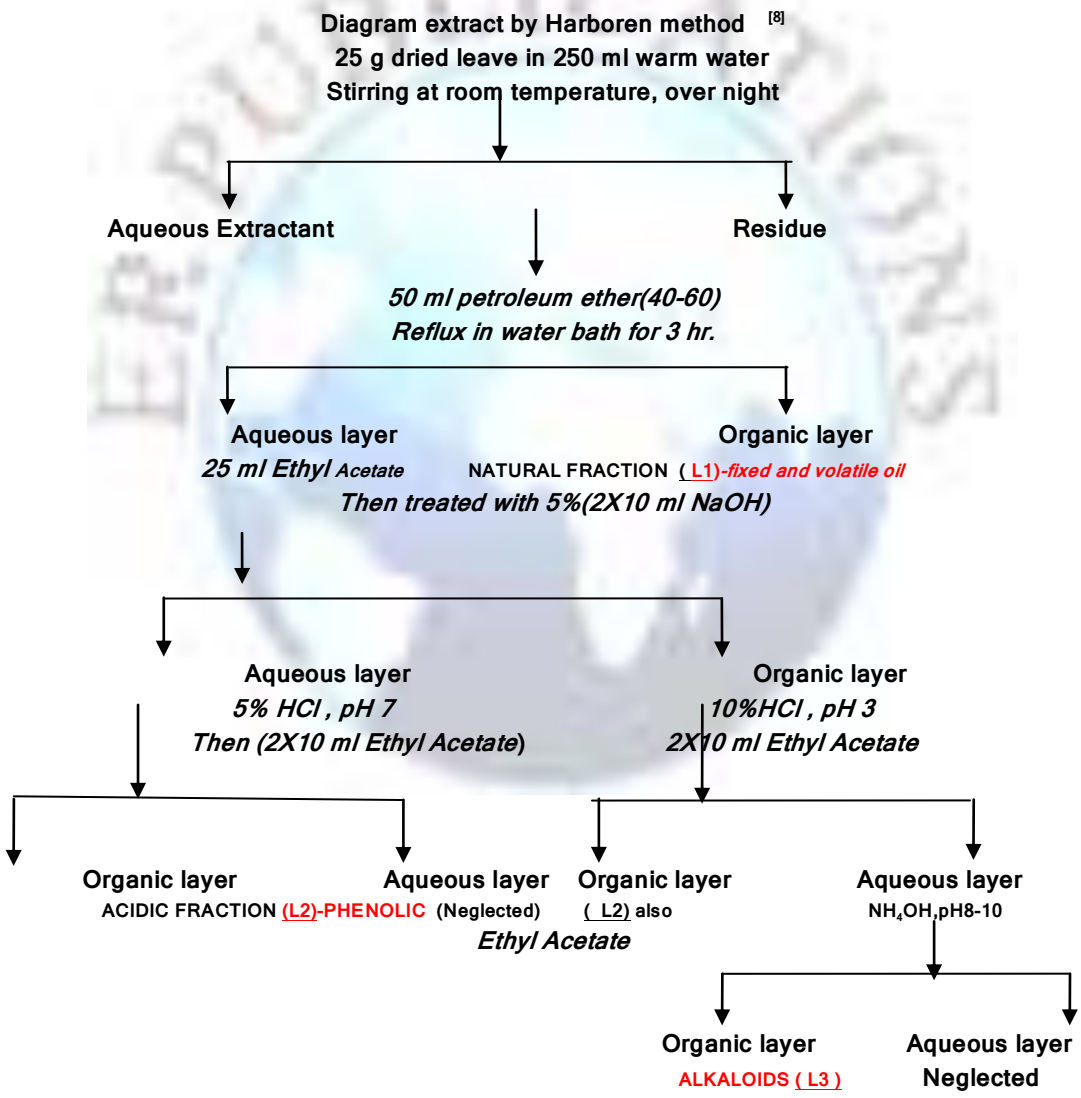
Materials and Methods

The present research work was carried in the laboratories, the chemicals used during the entire study were of analytical grade while properly washed and oven dried pyrex glass apparatus was used during the present study.

1- Solvent Extractor type "VELP SCIENTIFICA, SER 148"(Italy) used, Figure 1.

Fig. (1): Solvent Extractor apparatus

All experiments were performed with analytical reagents grade chemicals: Petroleum ether (40-60 C), n-hexane, ethanol, ethyl acetate, methanol, acetonitrile, HCl, NAOH .Another procedures for extraction of oil from seed are applied form diagram listed below:



2- A Shimadzu UV / Visible recording spectrophotometer (Model UV-160) and one cm quartz cells were used(Japan).

3- A BRUCKER OPTICS Fourier transmitting infrared (FT-IR) Model " ALPH" with Attenuated total reflection crystal diamond (ATR) had been established as a standard method for both routine and research

applications is the spectral range from 4,000 – 400 cm- were used for determination of liquid samples directly with Merck library containing 2000 standard compounds built in memory.

4- Gas chromatograph mass spectrometer(GC-MASS)- QP2010 Ultra (SHIMADZU)

5- A SHIMADZU High performance liquid chromatograph (HPLC) model L 2010A Analyzer with optimum condition:

(1) Type of column = 15 Cm X 4.6 mm,5 µM
(2) TM Sulpelcosil = LC- 8
(3) Mobile phase = 30:30:40 Methanol:H2O:Acetonitrile
(4) Flow rate = 1 ml /min
(5) Pressure = 7 mm/Hg
(6) Detector = type U.V at 320 n.m.
(7) Sample size = 15µl
(8) Temperature = 40 C◦

Procedure

Fenugreek seeds were ground by coffee grinding before oil extraction Ground seed (5g) were homogeneous with 75 ml different solvents (water, 95% Ethanol, Diethyl ether, n-Hexane, petroleum ether 40-60,and Chloroform)in Solvent extractor apparatus programming 200 ◦C at three step (60 min immerse, 30minwash 20 min recovery) [9].

1-Solvent free oil were weight to determine oil extract for each solvent , and transfer to brown glass vials and stored at 4 Co until further analysis
2-Each extract was identified by using FT-IR(ATR diamond) [10]. and HPLC using column C8 with Methanol:H2O:actonitrile as mobile phase.
3-Trogoderma granarium (Everts) was put in glass petry dish for 72 hours at 27oC.

Results and Discussion

In general ,petroleum ether is good extract ant comparing the other ,such as ethanol are polar solvent which can extract other constituent such as alkaloid and phenolic compounds; Table 1 show that ,which depend on the polarity of each solvent, show table 2 .

Table (1): The effect of solvent extract ant on seed Fenugreek

Type of solvent	Weight. of oil (gram)	Oil content (%)
Water	0.124	2.48
Ethanol	0.107	2.14
Diethyl ether	0.181	3.62
n-Hexane	0.120	2.40
Petroleum ether 40-60	0.371	7.42
Chloroform	0.232	4.64

From above table show that the petroleum ether have more ability to extract of oil, therefore it use for famous chemical organic laboratories, also see table (2).

Table (2): The polarity index of solvent [11]

Solvent	Polarity (Debby)
Petroleum ether	31.0
H_2O	9.0
Ethanol	5.2
Chloroform	4.1
Diethyl ether	2.8
n-Hexane	0.0

1-UV-VIS. identification: A sample which was extracted by petroleum ether introduce in UV-VIS spectrometry using quartz cell against blank, Three bands appear at (270, 234, 206 nm.) respectively, it mean have three functional groups compounds occur, see Figure 2.

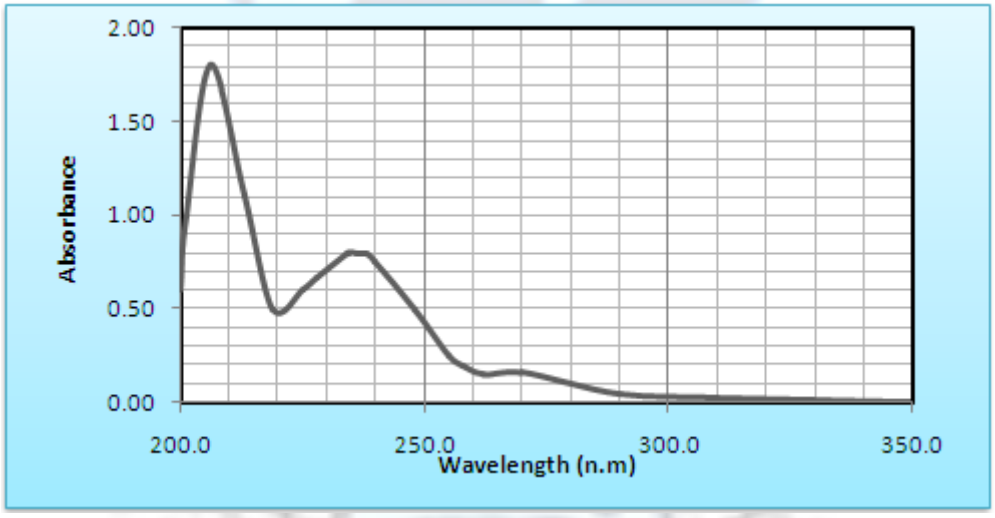


Fig. (2): UV spectrum of oil extract by petroleum ether.

2- FT-IR identification: After identification for each extract in FT-IR under optimum condition mention above and comparing with library standard compounds saved in FT-IR program, see Table 3 and Figures (3 to 8), the more probability of compound which appear firstly; therefore we select the famous compounds present in extract oil; which show that isopropyl myristate and Vaseline, diphenylamine and Dicycloexyl Keton were more constituents in seed Fenugreek .

Table (3): The suggest compounds containing in each solvent extract ant by FT-IR

Extractant	Water	Ethanol	Diethyl ether	n-Hexane	Petroleum ether	Chloroform
Compounds extract for each solvent	Thianthrene	Polystyrene	Isopropyl Myristate	Isopropyl Myristate	Isopropyl Myristate	Isopropyl Myristate
	Poly(Amide-6)	Poly(Amide-6)	Poly(Amide-6)	Dioctyltin Oxid	Dioctyltin Oxid	4-Phenylcyclohexanone
	Methadone-HCl	Indoline	Vaseline 8401	Vaseline 8401	Vaseline 8401	Vaseline 8401
	4-Amino-3-Methylphenol	Phenylbutazone	Dicycloexyl Ketone	Dicyclohexyl Ketone	Dicyclohexyl Ketone	--------
	Terephthalic Acid	Polystyrene	Dihexylamine	Dihexylamine	Dihexylamine	--------

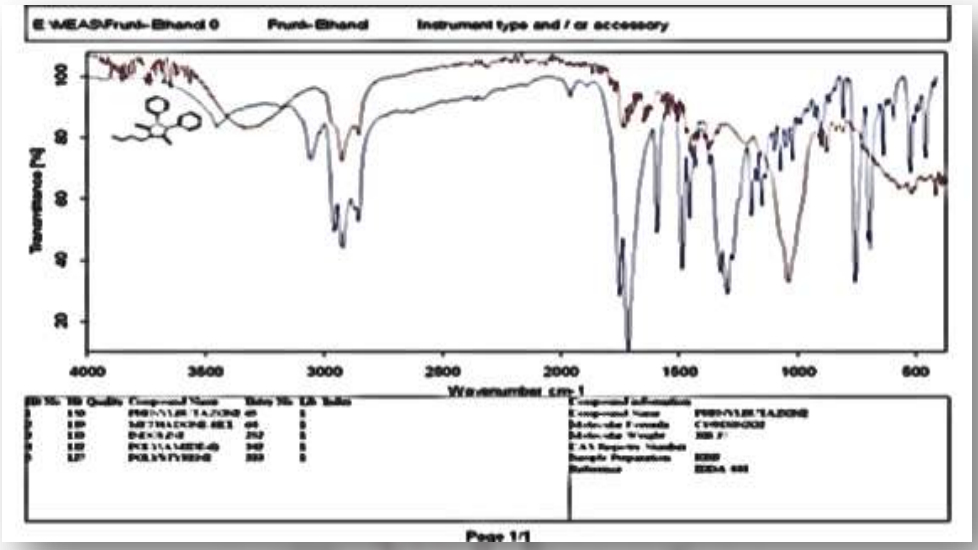


Fig. (3): FT-IR chart for seed Fenugreek extract by water

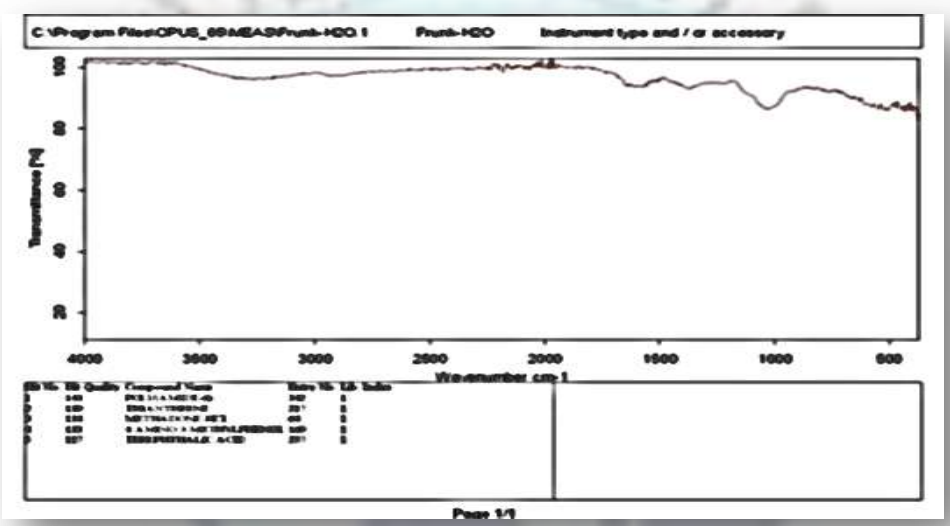


Fig. (4): FT-IR chart for seed Fenugreek extract by Ethanol

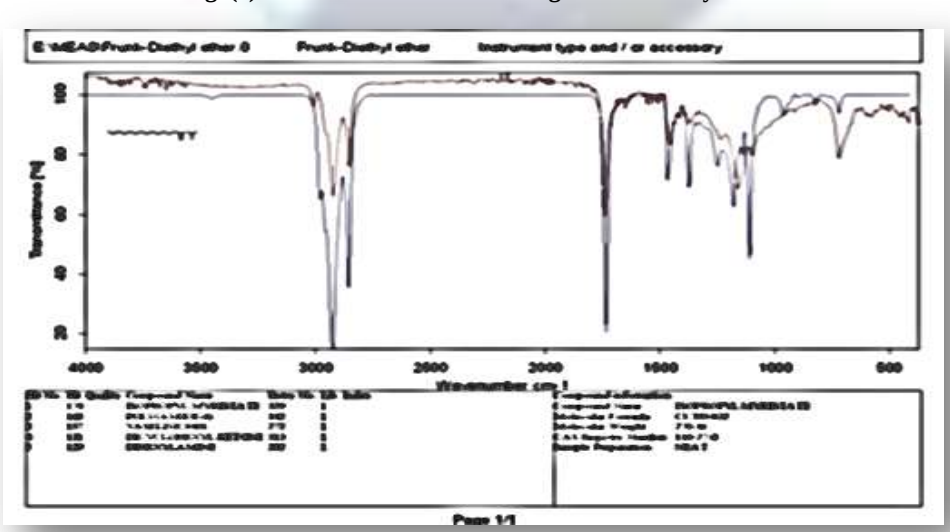


Fig (5): FT-IR chart for seed Fenugreek extract by Diethyl ether

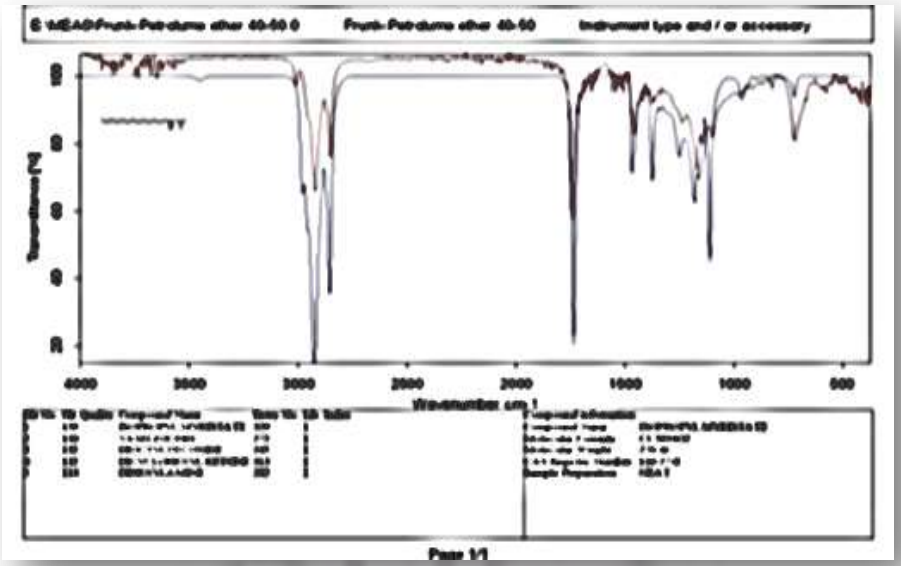


Fig. (6): FT-IR chart for seed Fenugreek extract by petroleum ether.

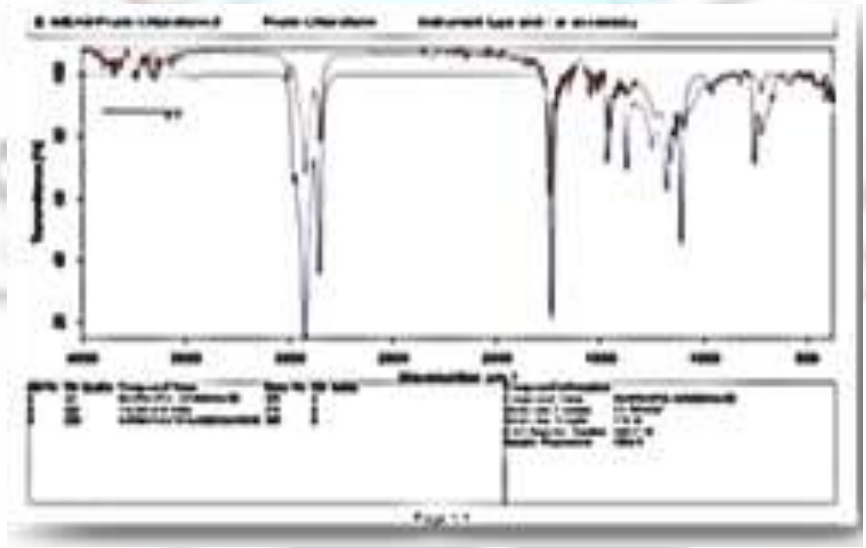


Fig. (7): FT-IR chart for seed Fenugreek extract by Chloroform

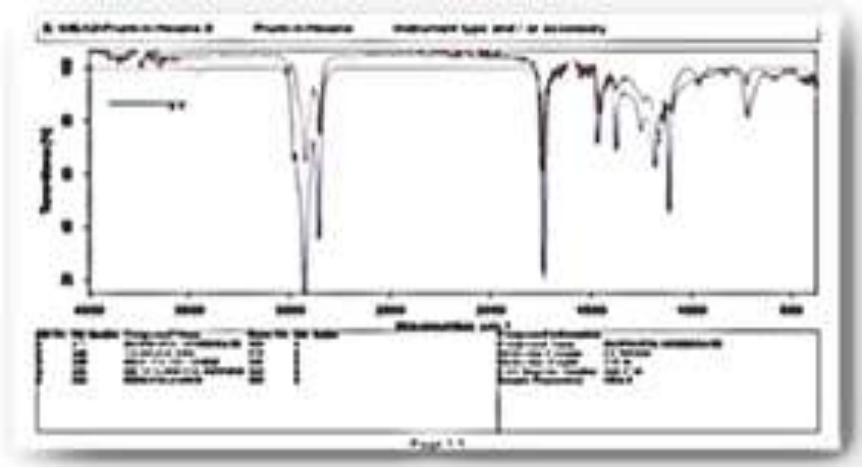


Fig. (8): FT-IR chart for seed Fenugreek extract by n-Hexane

3- GC-MASS identification: A sample was injected in the GC-Mass instrumental, after separation many peak were appears; each peak characteristic to individual compound, Fig. (9 to 12).

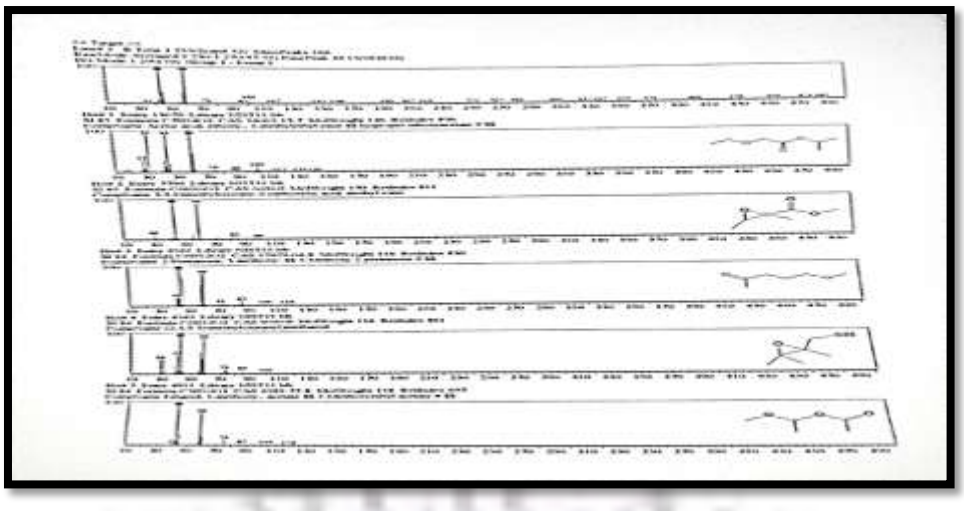


Fig (9): GC-Mass spectra for acetic acid, ethoxy

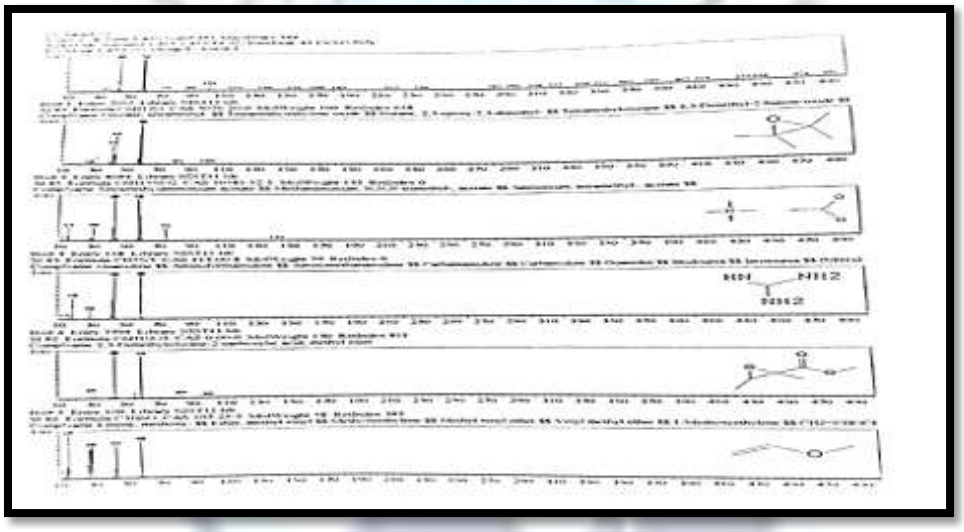


Fig (10): GC-Mass spectra for Oxirane, tetraethyl

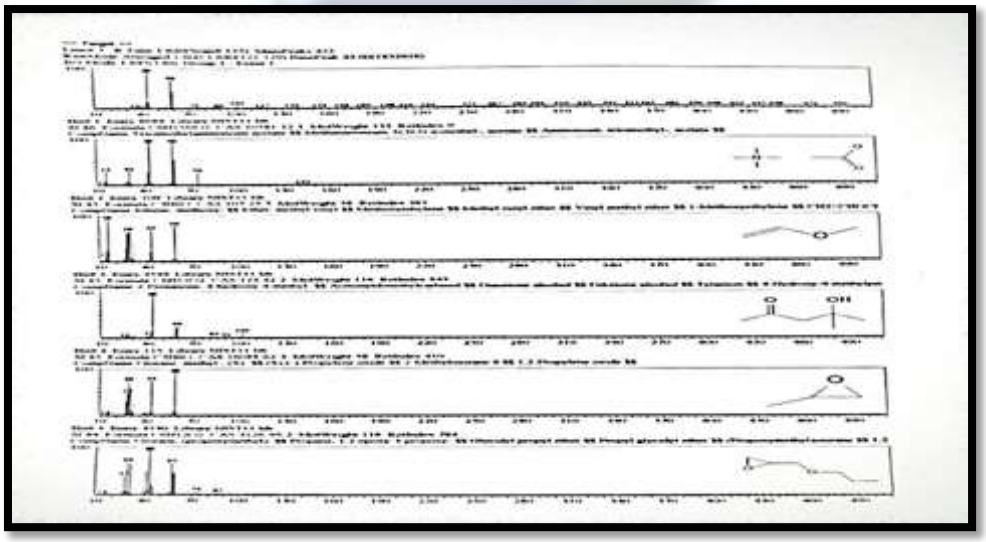


Fig (11): GC-Mass spectra for Tetramethylammonium acetate.

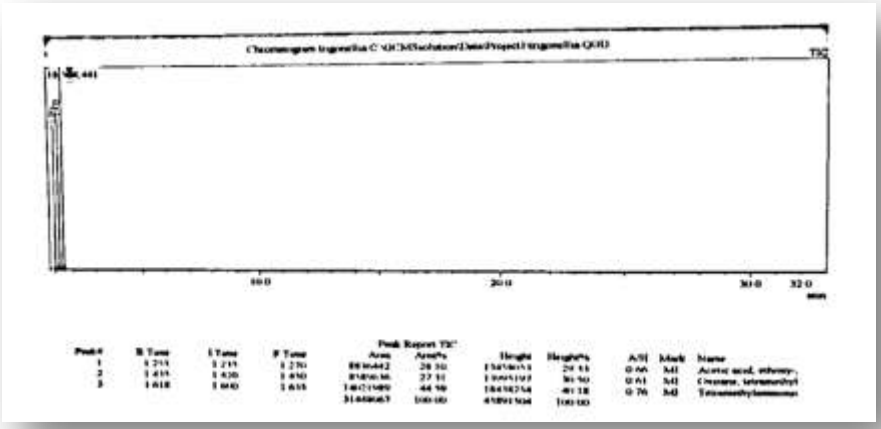


Fig (12): Peak Report in GC –Mass Analysis for Trigonella Foenum-Graecumn oil

4- HPLC identification: for HPLC spectrums containing the value of retention time for predominate material and comparing with some standards after injection in the column, Fig. (13)

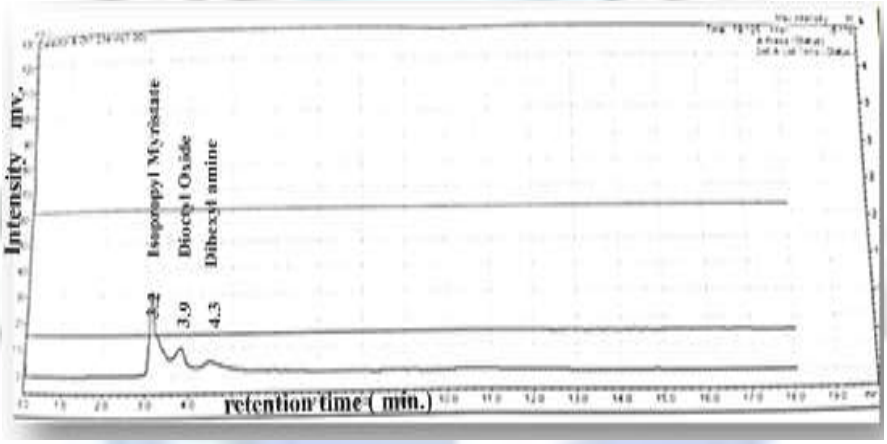
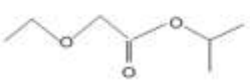
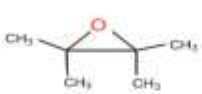
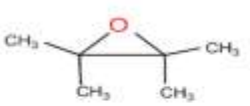
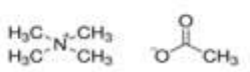


Fig (13): HPLC spectrum for Trigonella Foenum-Graecumn oil

Table (4): Comparison between four applied techniques

UV-Vis	FTIR		GC-Mass				HPLC			
FTIR λ (cm⁻¹)	Compound	Structure	Compound	Structure	M.Wt	Retention time (min.)	Compound	Retention Time (min) Found	Retention Time (min) literature	REFERENCES
206	*Isopropyl Myristate* $C_{17}H_{33}O_2$		*Acetic acid, ethoxy* $C_4H_{14}O_2$		146	1.27	*Isopropyl Myristate*	3.2	3.1	[7]
234	*Dioctyline oxide* $C_8H_{12}O$		*Oxirane, tetramethyl* $C_8H_{12}O$		100	1.45	*Dioctyline oxide*	3.9	3.8	[12]
270	*Dihexyl amine* $C_{12}H_{27}N$		*Tetrmethyl ammonium acetate* $C_6H_{15}NO_2$		133	1.62	*Dihexyl amine*	4.3	4.3	[13]

5 -Bioassay of *Trigonella Foenum-Graecumn* oil extract:

Many concentration *Trigonella Foenum-Graecumn* oil was applied on live Trogoderma granarium (Everts) insect in petry dish for 72 hours ,table 4 show that .

Table (4): Bioassay on *Trogoderma granarium* (Evert's) by exposure on surface of petry dish

Notice	Concentration ppm	Insect killer %			General mean affected %
		After 24hr.	After 48hr.	After 72hr.	
No Moulting and no development in other insect (live)	(0.001 1000ppm)	0	0	20	6.6
	(0.003 3000ppm)	0	10	20	10.0
	0.007 (7000ppm)	0	20	40	20.0
	0.01(10000ppm)	0	0	50	26.6
Moulting and development in insect happen	Comparing insect(control)	0	----	----	----

The concentration of oil increase, the killer of insect increase, in addition to the exposure time increase which are more effect.

References

[1]. Shah M., Mir RS (2004) ."Effect of dietary fenugreek seed on dairy cow performance and milk Characteristics". Can J Anim Sci 84:725–729.
[2]. Duke J. (1985) "Handbook of medicinal herbs". CRC Press, Boca Raton, FL P. Khosla, D. D. Gupta and R. K. Nagpal(1995)." Effect of Trigonella Foenum Graecum (Fenugreek) on Blood Glucose in Normal and Diabetic Rats". Indian J. Physiol Pharmacol . 39(2): 173-174.
[3]. Khosla P., D. D. Gupta and R. K. Nagpal(1995)." Effect of *Trigonella Foenum Graecum* (Fenugreek) on Blood Glucose in Normal and Diabetic Rats". Indian J. Physiol Pharmacol . 39(2): 173-174.
[4]. Harpreet Sharma, Leena Parihar and Pradeep Parihar (2011),"Review on cancer and anticancerous properties of some medicinal plants" . Journal of Medicinal Plants Research Vol. 5(10), pp. 1818-1835 .
[5]. El-Bahy, G. Source. (2005)."FTIR and Raman spectroscopic study of Fenugreek (Trigonella foenum graecum L.)seeds".Journal of Applied Spectroscopy .Jan,Vol.72 Issue 1, p111-116.
[6]. Ozan Nazim Ciftci and Magdalena Rudzinska.(2011),"Characterization of Fenugreek Seed Lipids", J.Am.oil Chem.soc.88:1603-1610.
[7]. Proniuk S, Lerkpulsawad S, Blanchard J.(1998)., A simplified and rapid high- performance liquid chromatographic assay for ketoprofen in isopropyl myristate. J Chromatogr Sci. Oct;36(10):495-8.
[8]. Harborne,J.B.(1973)"Phyto chemical methods".Halsted Press.John Wiely and sons ,New York.Folch J, Lees M, Stanley GHS (1957)" A simple method for the isolation and purification of total lipids from animal tissues ". J Biol Chem 266:497–509.
[9]. Folch J, Lees M, Stanley GHS (1957)" A simple method for the isolation and purification of total lipids from animal tissues ". J Biol Chem 266:497–509.
[10]. Moussa, A. M., Emam, A. M., Mohamed, M. A. and Diab, Y. M(2010). "International Food Research Journal" 17, pp: 287-294 .
[11]. John A. Byers. ".(2003)" Solvent polarity chart, Phenomenex catalog.
[12]. Haralad pasch,Adele Brull and Karin Cabrera.,(2005),Fast HPLC analysis of poly (ethylene oxide) for highroughput experimentation.,e-Polymers vol.5,Issue 1.p 206-220.
[13]. Atsuro N., Taketoshi O.,Toshihito K.,Shigeyuki C.,Kazuyuki T.,Masashi N.,Makoto G., Kuniaki K.,Masanori Y.,(2001)., Nitrogen containing tetracylic compounds.,United state patent .No. US 6, 281, 355 B1.