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Isolation, Characterization and Antibacterial Studies of *Trigonella foenum-graecum* Seed Extract

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Abstract

Pharmaceuticals are available in managing bacteria and other health related diseases. This research focuses on the extraction using Methanol, metabolite screening, fractionation, antibacterial activities, isolation and characterization of the most potent fraction. The phytochemical screening showed Flavonoids, Alkaloids, Tannin, Glycoside, Phenol, Fatty acid, Terpenoids and saponins. The antibacterial sensitivity test carried out on the Hexane fraction using Agar well method showed inhibition range from 0-18 mm (concentration of 12.50-100 mg/cm^3) for *Staphylococcus aureus*, 12-20 mm (concentration of 12.50-100 mg/cm^3) for *Escherichia coli*, 13-22.50 mm (concentration of 12.50-100 mg/cm^3) for *Salmonella typhi* and 0-12 mm (concentration of 12.50-100 mg/cm^3) for *Bacillus subtillis* with an average inhibition range of 0-22.50 mm. For the Chloroform fraction, the antibacterial sensitivity test carried out showed zone of inhibition range from 0-16 mm (concentration of 12.50-100 mg/cm^3) for *Staphylococcus aureus*, 0-12 mm (concentration of 12.50-100 mg/cm^3) for *Escherichia coli*, 0-17 mm (concentration of 12.50-100 mg/cm^3) for *Salmonella typhi* and 0 mm (concentration of 12.50-100 mg/cm^3) for *Bacillus subtillis* with an average inhibition range of 0-17 mm. The antibacterial sensitivity test carried out on the Ethyl acetate fraction showed zone of inhibition range of 0 mm (concentration of 12.50-100 mg/cm^3) for *Staphylococcus aureus*, 0-13 mm (concentration of 12.50-100 mg/cm^3) for *Escherichia coli*, 0-12 mm (concentration of 12.50-100 mg/cm^3) for *Salmonella typhi* and 0 mm (concentration of 12.50-100 mg/cm^3) for *Bacillus subtillis* with an average inhibition range of 0-13 mm. From the above results, the n-Hexane fraction was subjected to the glass column separation technique and an ester of Methylhexadecanoic acid was gotten from the n-hexane fraction of Methanol extract of the seed of *Trigonella foenum-graecum*.

Keywords: Fenugreek; Methanol; Anti-bacterial; Chromatography; Methylhexadecanoic acid.

INTRODUCTION

Medicinal plants are priceless gifts from nature for food and medicine. In the scientific world, the diverse medicinal potentials of these plants have aroused the interests of researchers across disciplines worldwide. Medicinally, isolated natural products after been subjected to clinical trials can be used as drugs for the treatment of cancer (Crow, 2008), antimicrobial agents (Woldemichae *et al*., 2003; Jagessar *et al*., 2008; Prasad *et al*., 2008), antitumor (Flores *et al*., 2010), antioxidant agents (Manga *et al*., 2006; Kukic *et al*., 2008; Nile and Khobragade, 2010).

The seed commonly known as Fenugreek (English), Hulba (Arabic), Alholva, Feno-greco (Spanish), Eru (Yoruba), Kimba (Hausa) which belong to the Family Fabaceae has been used as a flavoring agent and as a medicinal plant for a long time. Fenugreek is a leguminous plant. The seeds are brownish yellow in colour but the colour may change to dark yellow with difference in environmental conditions e.g. temperature. It is also used in post-natal care by nursing mothers. Nowadays, fenugreek is widely cultivated as a drug plant.

The seed has been widely noticed to possess multipotent antioxidant, antimicrobial, anticancer, gastroprotective and antidiabetic properties by Zameer *et al*., (2017) and Basch *et al*., (2003). It was indicated that the seed extract might help in alleviating hyperglycemia (Pundarikakshudu *et al*., 2016). The fenugreek seed possess antidiabetic effect (Renuka, 2009). *Trigonella foenum-graecum* showed strong antioxidant activity (Pawar and Hagar, 2012).

Antioxidants have been identified as major compounds found in various medicinal plants (Szymanska *et al*., 2016).

The compounds derived from secondary metabolism of Fenugreek are phenolic compounds (Pang *et al*., 2018). The quantitative analysis of the aqueous extract showed 73.54 mg of gallic acid and 70.30 mg of quercetin per gram of extract (Kumar *et al*., 2021). Fenugreek showed a positive effect on physiological aspects of libido and testosterone levels (Megha *et al*.,

2012). The seed extract inhibited the DMBA-induced mammary hyperplasia (Amr Amin *et al*., 2005). Cholesterol level was increased from reactions between bile acid and saponin in the seed extract (Yadav *et al*., 2011).

The GCMS analysis of Fenugreek extract showed 14 different compounds, including Hexadecanoic acid, a methyl ester ($C_{18}H_{36}O_2$) at 18.81% in area (Moniruzzaman *et al*., 2015). The main constituents were found to possess antioxidant properties (Akbari *et al*., 2019). Palmitic acid is used as a food additive (Benzidia *et al*., 2018). Results showed that the major compounds in fenugreek seed oil were linoleic acid, palmitic acid, pinene, 4-Pentyl-1-(4-propylcyclohexyl)-1-cyclohexene, and linoleic acid methyl ester (Akbari *et al*., 2019).

There is also an increased interest in the pharmaceutical properties of this plant. There was only limited literature information that was found about the bioactive compounds in the seed of this plant.

Plate A. (Fenugreek leaves)

Plate B. (Fenugreek seeds)

MATERIALS AND MAETHODS

Materials

Apparatus includes Round bottomed flask, Water-bath, Glass wool, Heating mantle, Anti-bumping chips, pre-coated silica gel plate, rotary evaporator and Weighing balance, beakers, separating funnel and thin-layer chromatographic plate.

Reagents

The solvent used were of analytical grades with no further purification obtained from GH Tech chemicals, China which include; Methanol (m.mass = 34.04, m.p=-98^{0}c, b.p=64.5^{0}c, Assay= 99.50%, Density= 0.791-0.793g/cm^3), Ethyl ethanoate(Assay=99%), Density=0.899-0.902g/cm^3, Chloroform (m.mass = 119.38g/mol, b.p=61^{0}c, Assay= 99.50%, Density= 1.49g/cm^3), n-hexane (m.mass = 86.18, m.p=94.3^{0}c, b.p=69^{0}c, Assay= 97.0%, Density= 0.059-0.663g/cm^3), distilled water, Sodium Hydroxide, Meyer's reagent, Fehling Solution, Molisch's reagents, Wagner's reagents, Hydrochloric acid etc

Microorganism

The microorganisms used were *S. aureus*, *E. coli*, *B. subtillis*, and *S. typhi*.

Sampling

Fenugreek seeds were bought at Kasuwar-Bacchi, a market in Kaduna State. It was identified by Mr. U. S Gallah, Biology Department, Kaduna State University, Kaduna.

Preparation of extract

The seed of Fenugreek was first rinsed with warm water to remove any adsorbed particles and dried at (26 °C) for a month. It was ground to a fine powder with mortar and pestle, and also with a mechanical grinder and sieved. The seed was extracted using cold maceration by soaking 1200 g of dried powdered sample in 1500 cm^3 of Hexane to defat. The filtrate was collected, followed by continuous extraction until there was no observable colour inference from the sample. The Hexane filterate was collected, concentrated and weighed for further analysis. The dried residue was soaked in Methanol for 3days. At the end of extraction, the plant extracts was filtered (whatman no 10). The filtrate was then concentrated at 40°C. The yield of *Trigonella foenum-graecum* methanol crude was also calculated.

Phytochemical Screening of *Trigonella fornum-graecum* seed

The presence of the bioactive compounds from the seeds of Fenugreek forms a platform for further pharmacological studies (Colombo and Bosiosio, 2018; Okwute, 1989).

Methanolic extract was phytochemically analysed for secondary metabolites such as Alkaloids, Saponin

Glycosides, Flavonoids, Polyphenolic compounds and Tannins. This was carried out using standard protocols (Harbourne, 1988).

Fractionation of the methanol extracts

A portion (200 g) of pulverized seed was percolated in methanol for 3 days. It was concentrated using a rotary evaporator to obtain a dried extract, yielding 61 g of methanolic extract after weighing. The 61 g of dried methanol extract was transferred into a bama bottle and about 200 cm^3 of hexane was added and the mixture was shaken for 2 minutes before standing for about 3 minutes. It was then filtered and the filtrate was collected using Bama bottles and was concentrated at 30 ^0C to prevent denaturation. The process was repeated many times until no colour of the solvent from the extract. The marsh was dried and same procedure was carried out using chloroform and then followed by Ethyl acetate leaving behind the residual fraction. Bottle A represents n-Hexane fraction, bottle B represents the Chloroform fraction and bottle C represents Ethyl acetate fraction. Solvent was evaporated from each fraction, the fraction weighed and its percentage yield calculated (Nwauzoma *et al*., 2013). During the TLC profiling between bottle A, B and C, bottle A gave a lot of development which can be seen on plate D under solvent system hexane and ethyl acetate (9:1) with a slow development on the Chloroform extract(B) on plate D and no development at all on ethyl acetate extract(C) on plate D. Plate E show no development on 100% Hexane solvent.

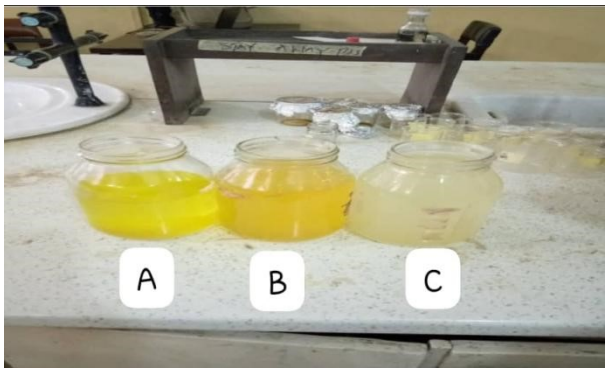


Plate C. Filterate of the extracts.

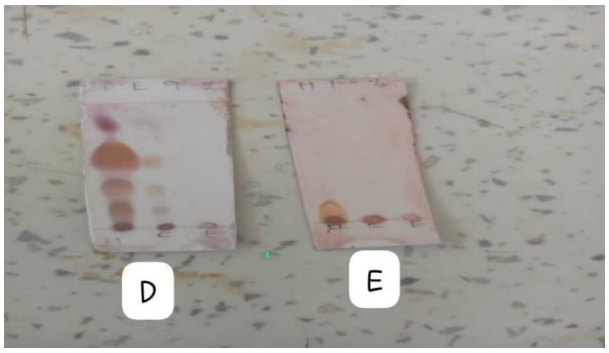


Plate D. Development of extract on TLC plate.

Antibacterial Assay of the Fractions

The antibacterial studies were carried out in the Department of Pharmaceutical Microbiology, Ahmadu Bello University against four bacterial isolates.

Antibacterial assay

A total of four bacterial cultures were used on the fractions.

Agar well diffusion method

Agar well diffusion method was used to evaluate the antibacterial activity of the fractions.

Chromatographic Separation

Simple column chromatography was used in which silica gel was used and mobile phase was passed from highly non-polar solvent to mixture of solvents. TLC grade silica gel (mesh 60-200) was used. Slurry of the silica gel was made with n-hexane. The system was preconditioned by passing the solvent continuously for 5 minutes. The sample was prepared by mixing 7.50 g of n-hexane fraction onto silica gel (mesh 60-200), dried after 24 hours and applied on top of the adsorbent. The elution started with 100 % n-hexane, n-hexane and ethyl acetate (90:10), (85:15) solvent system respectively, and was stopped at (80:20). Fractions were collected each in a 15 cm^3 container. As a result, mother solutions were obtained. The mixed fraction was concentrated for Thin-layer finger print check for compounds.

Plate E. Column chromatography of n-Hexane extract.

Characterization

The spectroscopic method used for elucidating the structure of the isolated compound was FT-IR and GC-MS.

RESULTS AND DISCUSSIONS

Results

Table 1. Phyto-screening of the Methanol crude.

Phytocompounds	Inference
Alkaloids	+ve
Flavonoid	+ve
Glycosides	+ve
Saponins	+ve
Steroids	+ve
Tanins	+ve
Phenols	+ve
Carbohydrate	+ve

Table 2. Percentage yield of T. foenum-graecum seed extract in various fractions.

Extracts	Yield (g)	Percentage Yield (%)
Hexane fraction	9.3	15.25
Chloroform fraction	2.2	3.61
Ethylacetate fraction	1.9	3.11

Antibacterial Activities of *Trigonella foenum-graecum* Seed

The fraction concentrations are 12.50 mg/cm^3, 25 mg/cm^3, 50 mg/cm^3 and 100 mg/cm^3. From the four bacteria for the n-hexane fraction, only *Bacillus subtilis* showed no inhibition at concentration of 50, 25 and 12.50 mg/cm^3. However, it showed moderate activity of 12 mm at the highest concentration of 100 mg/cm^3 for *Bacillus subtilis*. Other bacteria strain showed activities with *Salmonella typhi* having the highest zone of inhibition of 22.50 mm at 100 mg/cm^3, 19 mm,16 mm and 13 mm respectively at concentrations of 50,25 and 12.50 mg/cm^3.*Escherichia coli* also shows inhibition zones of 20 mm, 18 mm, 15 mm and 12 mm at concentrations of 100, 50, 25 and 12.50 mg/cm^3, respectively. *Staphylococcus aureus* also shows inhibition zones of 18 mm, 15 mm and 11 mm at concentrations of 100, 50 and 25 mg/cm^3.

In the case of Chloroform fraction, *Bacillus subtilis* was not inhibited from growing at any concentration. Zone of inhibitions was seen at higher concentrations for the remaining bacteria strain where *Salmonellae typhi* showed inhibitions of 17 mm, 14mm and 12 mm at various concentrations of 100, 50 and 25 mg/cm^3. *Staphylococcus aureus* also with zone of inhibitions of 16 mm, 14 mm and 12 mm at different concentration of 100, 50 and 25 mg/cm^3.

In Ethyl-acetate plate, only *Salmonella typhi* and *Escherichia coli* showed little inhibition of 12 mm and 13 mm at a concentration of 100 mg/cm^3. Positive results for both MIC and MBC test might be due to the very low concentrations of extract compared to the concentrations used in agar well diffusion test which was 100 mg/cm^3. However, it is suggested that a higher concentration should be used in the determination of MIC of the extract

Table 3: Result of antibacterial activity of Hexane fraction.

Organism	Conc of extract (mg/cm^3)	Inhibition zone(mm)
Staphylococcus aureus	Cp	35
	12.50	0
	25	11
	50	15
	100	18
Escherichia coli	Cp	40
	12.50	12
	25	15
	50	18
	100	20
Salmonellae typhi	Cp	0
	12.50	13
	25	16
	50	19
	100	22.50
Bacillus subtillis	Cp	34
	12.50	0
	25	0
	50	0
	100	12

Key:
CP: Ciproflaxin (positive control)

Table 4. Result of antibacterial activity of *Trigonella foecum-graecum* seed chloroform fraction.

Organism	Conc of extract (mg/cm^3)	Inhibition zone(mm)
Staphylococcus aureus	Cp	35
	12.50	0
	25	12
	50	14
	100	16
Escherichia coli	Cp	40
	12.50	0
	25	0
	50	0
	100	12
Salmonellae typhi	Cp	0
	12.50	0
	25	12
	50	14
	100	17
Bacillus subtillis	Cp	34
	12.50	0
	25	0
	50	0
	100	0

Table 5. Result of antibacterial activity of *Trigonella foecum-graecum* seed ethyl-acetate fraction.

Organism	Conc of extract (mg/cm^3)	Inhibition zone(mm)
Staphylococcus aureus	Cp	35
	12.50	0
	25	0
	50	0
	100	0
Escherichia coli	Cp	40
	12.50	0
	25	0
	50	0
	100	13
Salmonellae typhi	Cp	0
	12.50	0
	25	0
	50	0
	100	12
Bacillus subtillis	Cp	34
	12.50	0
	25	0
	50	0
	100	0

Table 6. Qualitative Result of Minimum Inhibition Concentrations of *Trigonella foenum-graecum* seed n-hexane fraction.

	MIC									
ORG	1	2	3	4	5	6	7	8	9	10
SA	-	-	-	-	+	+	+	+	+	+
EC	-	-	-	-	-					
ST	-	-	-	-	-	+	+	+	+	+
BS	-	+	+	+	+	+	+	+	+	+

Table 7. Qualitative Result of Minimum Inhibition Concentrations of *Trigonella foenum-graecum* seed chloroform fraction.

	MIC									
ORG	1	2	3	4	5	6	7	8	9	10
SA	-	-	-	+	+	+	+	+	+	+
EC	-	+	+	+	+	+	+	+	+	+
ST	-	-	-	+	+	+	+	+	+	+
BS	+	+	+	+	+	+	+	+	+	+

Table 8. Qualitative Result of Minimum Inhibition Concentrations of *Trigonella foenum-graecum* seed ethyl-acetate fraction.

	MIC									
ORG	1	2	3	4	5	6	7	8	9	10
SA	+	+	+	+	+	+	+	+	+	+
EC	-	+	+	+	+	+	+	+	+	+
ST	-	+	+	+	+	+	+	+	+	+
BS	+	+	+	+	+	+	+	+	+	+

Key: S.a – S. aureus; E.c – Escherichia coli; B.s – B. subtillis; Sal – S. typhi; -ve; No turbidity (growth)-Concentration that has no turbidity recorded; +ve; Turbidity (growth)-Concentration that showed turbidity or growth recorded.

Table 9: Result of Minimum Bactericidal Concentrations of *Trigonella foenum-graecum* seed n-hexane fraction.

	MBC									
ORG	1	2	3	4	5	6	7	8	9	10
SA	-	-	+	+	+	+	+	+	+	+
EC	-	-	+	+	+	+	+	+	+	+
ST	-	-	-	+	+	+	+	+	+	+
BS	+	+	+	+	+	+	+	+	+	+

Table 10. Result of Minimum Bactericidal Concentrations of *Trigonella foenum-graecum* seed chloroform fraction.

	MBC									
ORG	1	2	3	4	5	6	7	8	9	10
SA	-	+	+	+	+	+	+	+	+	+
EC	+	+	+	+	+	+	+	+	+	+
ST	-	+	+	+	+	+	+	+	+	+
BS	+	+	+	+	+	+	+	+	+	+

Table 11. Result of Minimum Bactericidal Concentrations of *Trigonella foenum-graecum* seed ethyl-acetate fraction.

	MBC									
ORG	1	2	3	4	5	6	7	8	9	10
SA	+	+	+	+	+	+	+	+	+	+
EC	+	+	+	+	+	+	+	+	+	+
ST	+	+	+	+	+	+	+	+	+	+
BS	+	+	+	+	+	+	+	+	+	+

Key:
S.a – S. aureus
E.c – E. coli
B.s – B. subtillis
Sal – S. typhi
-ve; No colony growth
+ve; colony growth

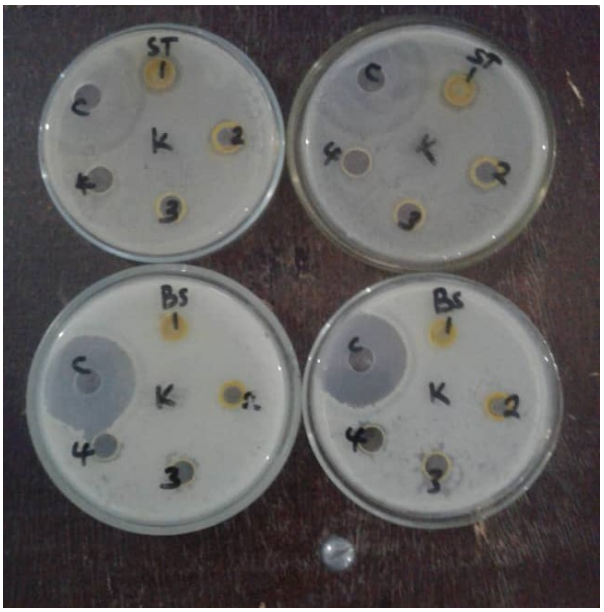


Plate F. Chloroform fraction

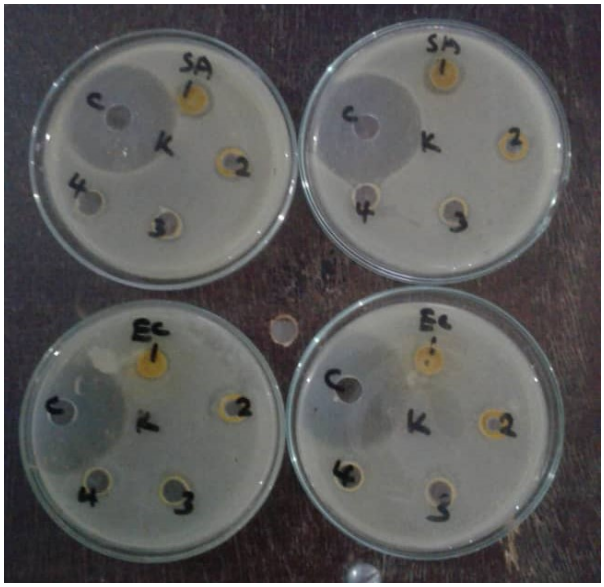


Plate G. Chloroform fraction.

Plate H. Ethylacetate fraction.

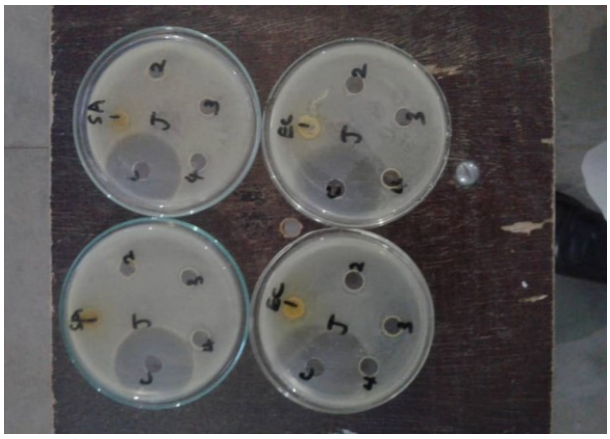


Plate I. Ethylacetate fraction.

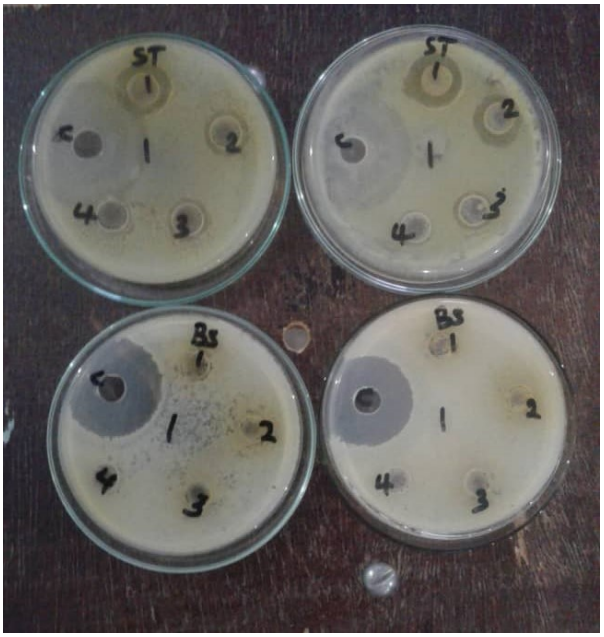


Plate J. Hexane fraction.

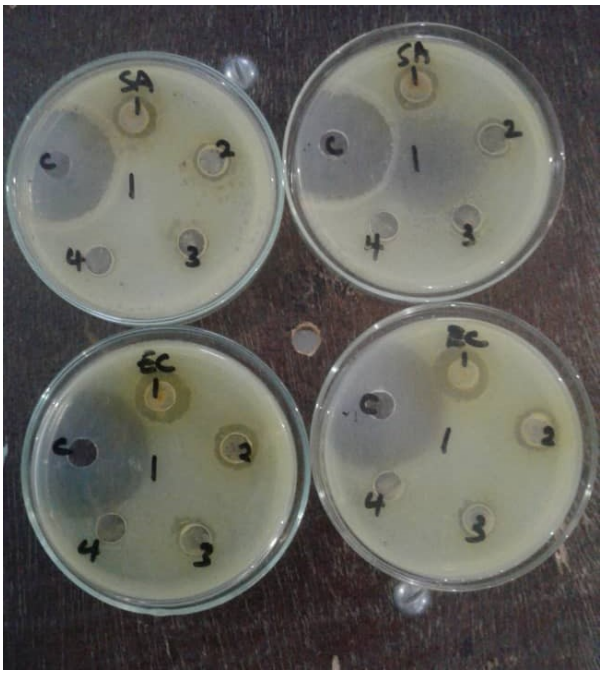


Plate K. Hexane fraction

Developing of The TLC Precoated Plate

In developing the plate, two separate solvent systems were used in running the plate. The first developing tank was Hexane: Ethyl acetate (Ratio 9:1) which shows little movement from the base, the second developing tank was Hexane: Ethyl acetate (Ratio 8:2) which enables the distribution of different component(s) in the fraction extracts. The distribution was not that visible to the naked eye, but with the help of iodine crystals and 10 % Sulphuric acid, there was visualization.

Fractions 4-14 gave a coloured concentrate which was later washed with methanol to give a white powder. It was observed that fractions from 30-60 showed similar

TLC profiles and fractions from 62-90–also showed similar TLC profiles. They were individually pooled to obtain various single fractions and the labeled E (62-80) was used for further purification on a smaller column. They were all concentrated at room temperature for analysis.

Purification

A sub column with diameter of 3 cm and a length of 50 cm was washed with already prepared Hexane and Ethyl acetate solvent system in a ratio of 8:2 by volume to facilitate compact packing. A slurry of the silica gel was prepared with the solvent mixture. The system was pre-conditioned for 5 minutes. About 2.60 g of pooled labelled E was mixed with silica gel and allowed to dry, but it did not dry because the sample was oily. The pooled fraction was loaded on top of the silica gel and the eluents were collected with a 50 cm^3 container. The TLC profile of each fraction was carried out with n-hexane-ethyl acetate solvent mixture (9:1 respectively) because it was observed that purely n-hexane couldn't move the spot while ratio of (8:2) n-hexane-ethyl acetate mixture was too polar for the compounds. Fraction 2 gave a single spot on the TLC plate, fraction 3 gave three spots on the TLC plate while fraction 4 to 19 showed two spots that were not clearly separated and were also more polar than the initial fractions. Fraction 20 to 24 showed a single spot too but couldn't be resolved by the 9:1 Hexane to Ethyl acetate solvent system. An 8:2 solvent system was used.

Fraction 2 was concentrated and it gave colourless oily liquid.

Table 12. Profile for the first column purification.

Fraction Numbers	Pooled labelled	Colour
1-13	A	Light brown
15-28	B	Colourless
30-45	C	Light yellow
47-60	D	Yellow
62-80	E	Light green
81-90	F	Light green

Table 13. TLC values for pooled E fraction.

Fractions	Rf	No of spot
1	-	0
2	0.76	1
3	0.76, 0.69, 0.65	3
4-19	0.65, 0.38	2
20-24	0.38	1

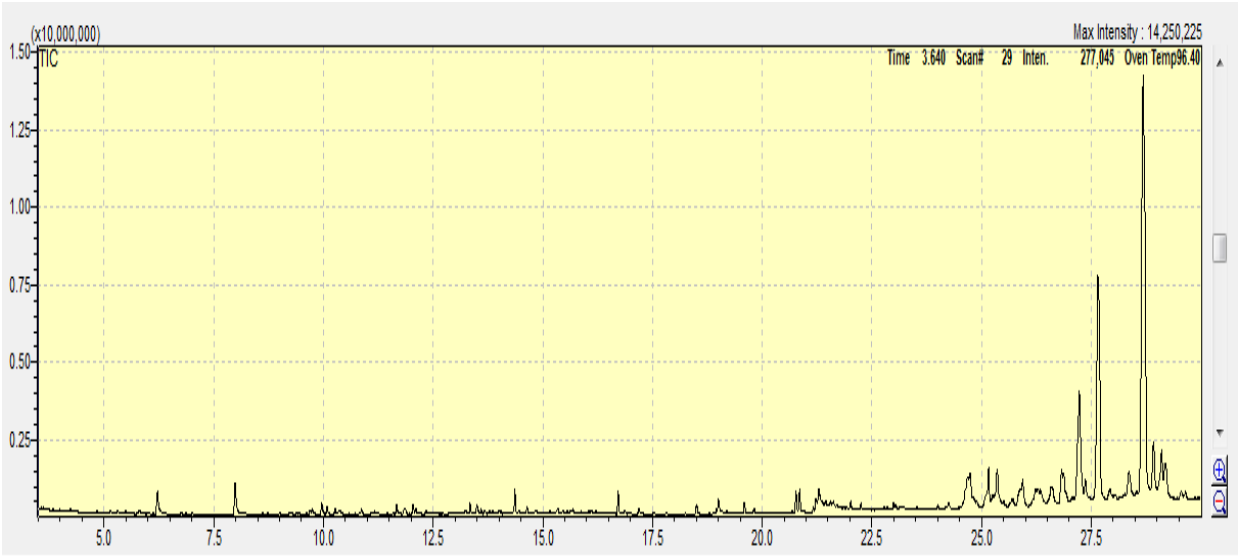
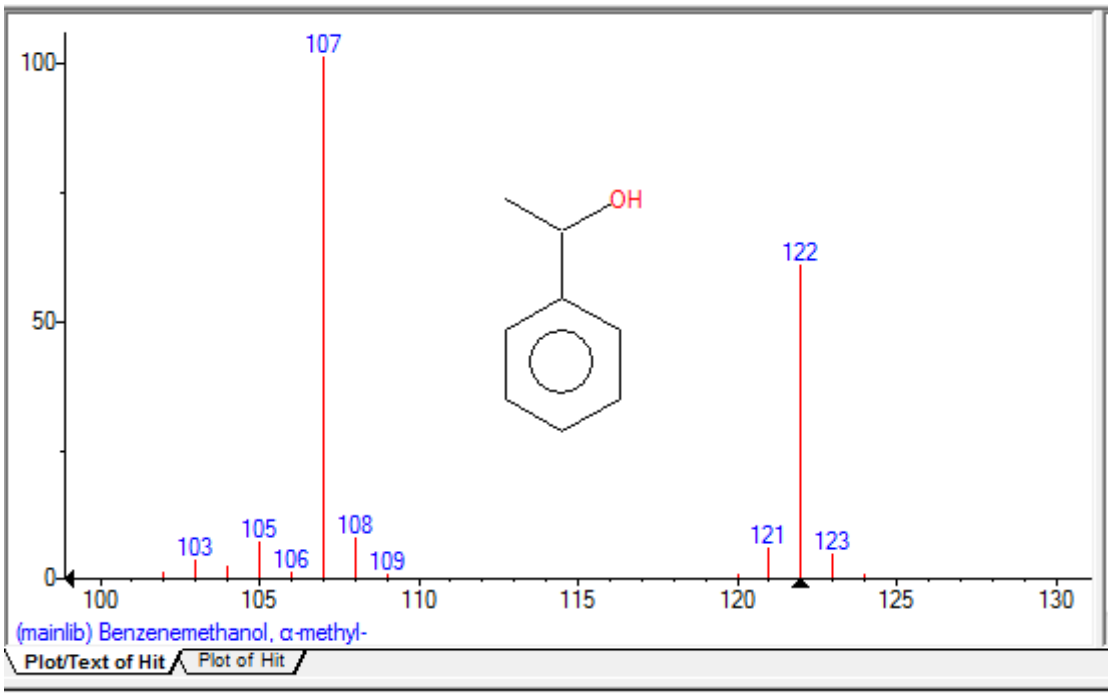
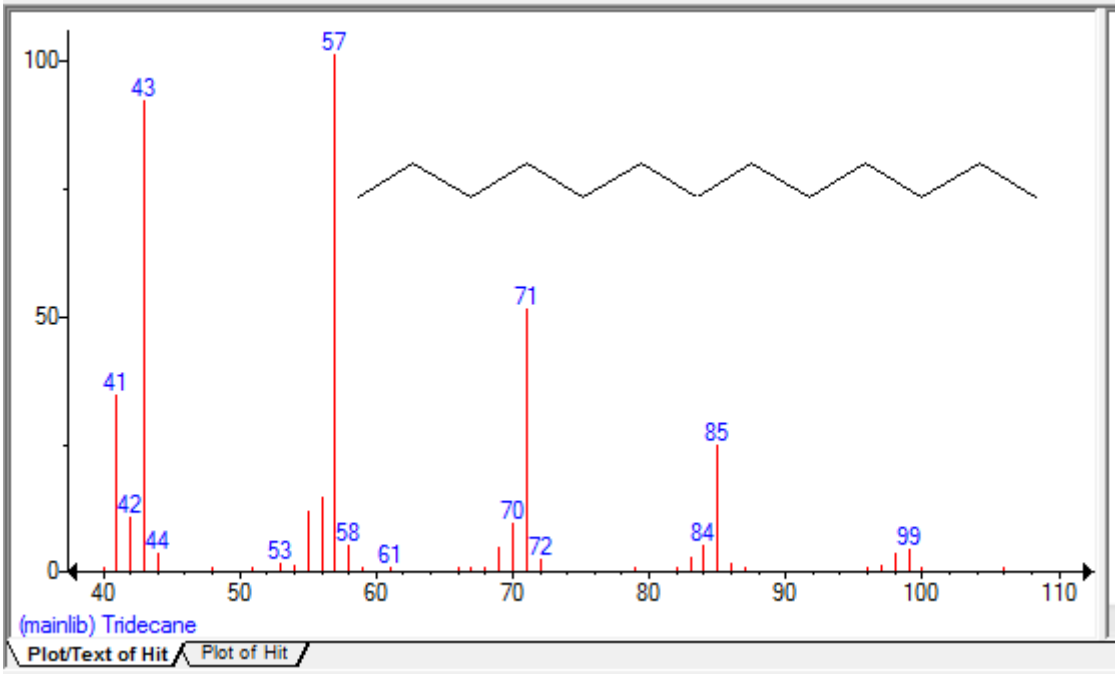


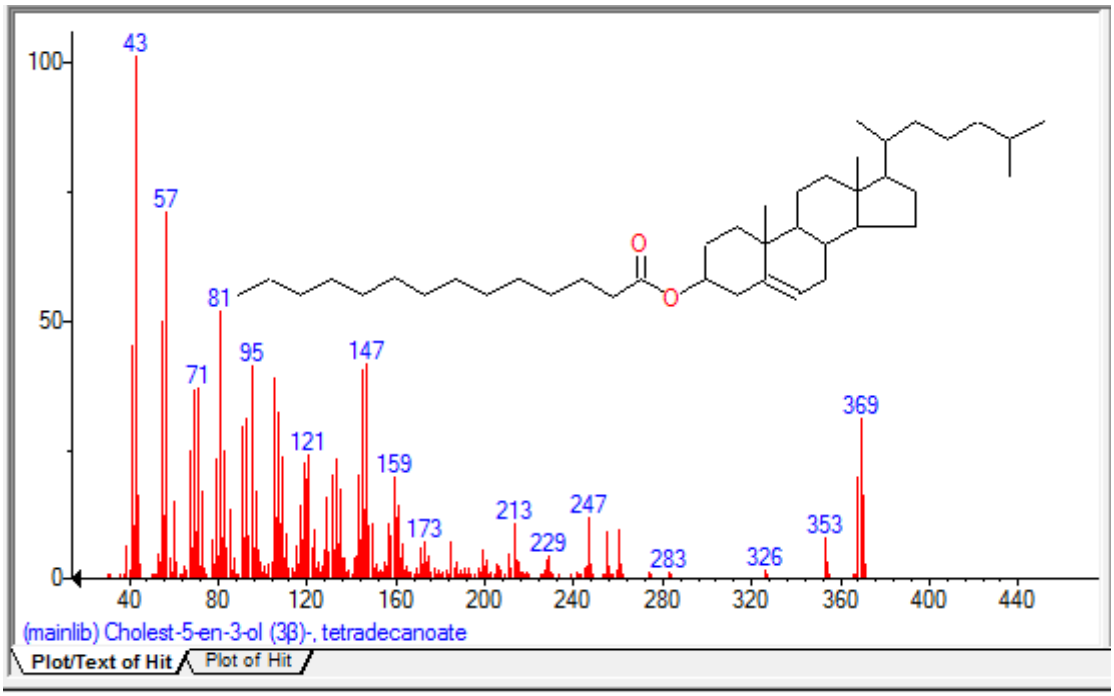
Figure 1. GCMS of Sample in ethylacetate solvent



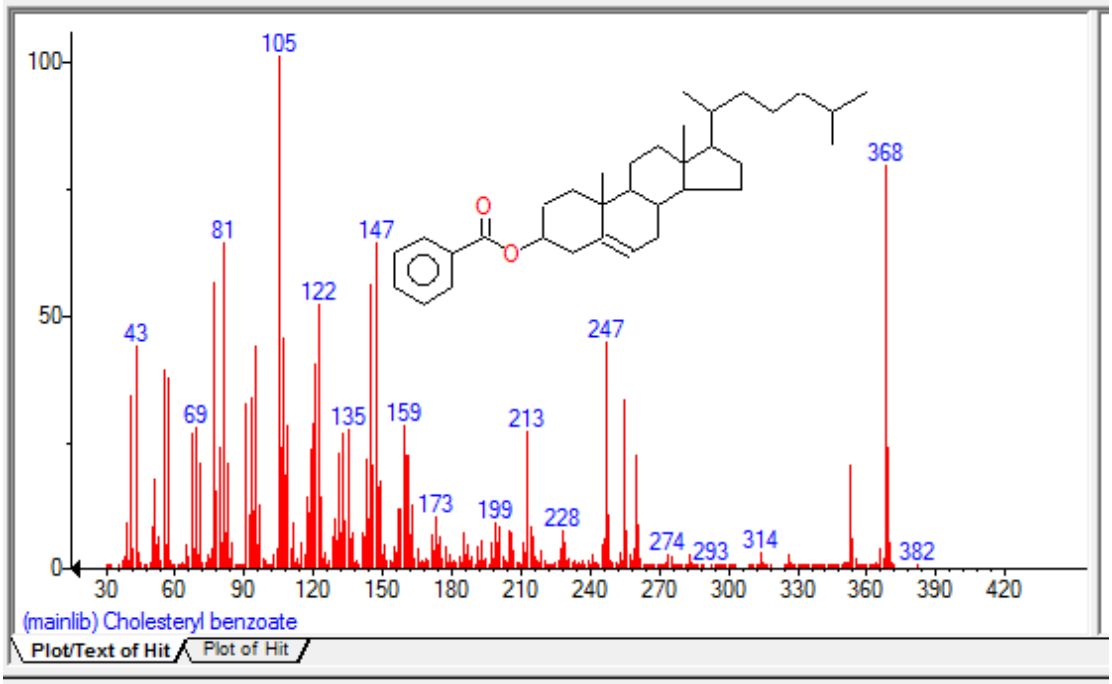
Appendix A: Library match of sample 2 in ethyl acetate.



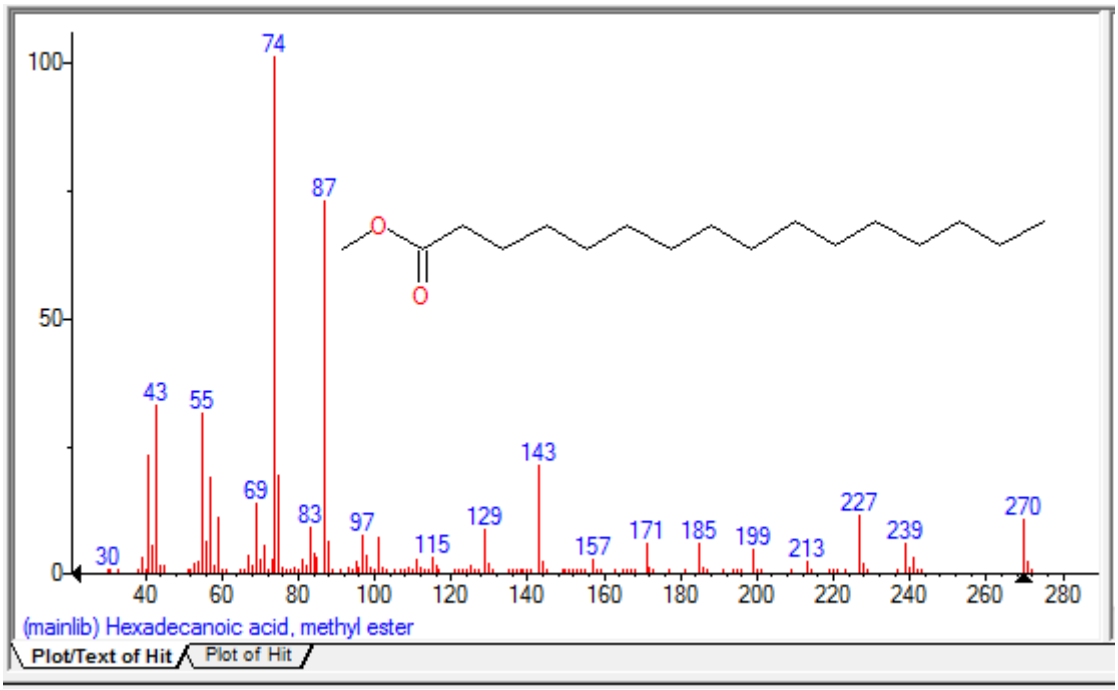
Appendix B: Library match of sample 2 in ethyl acetate



Appendix C: Library match of sample 2 in ethyl acetate.



Appendix D: Library match of sample 2 in ethyl acetate.



Appendix E: Library match of sample 2 in ethyl acetate.

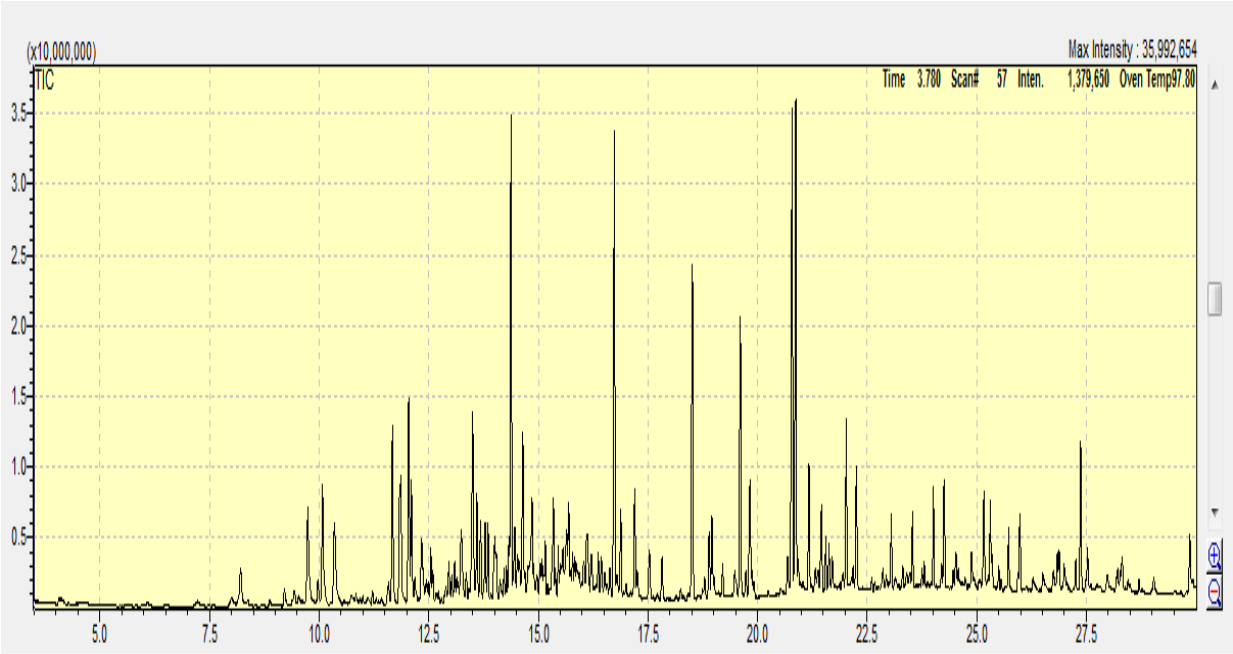
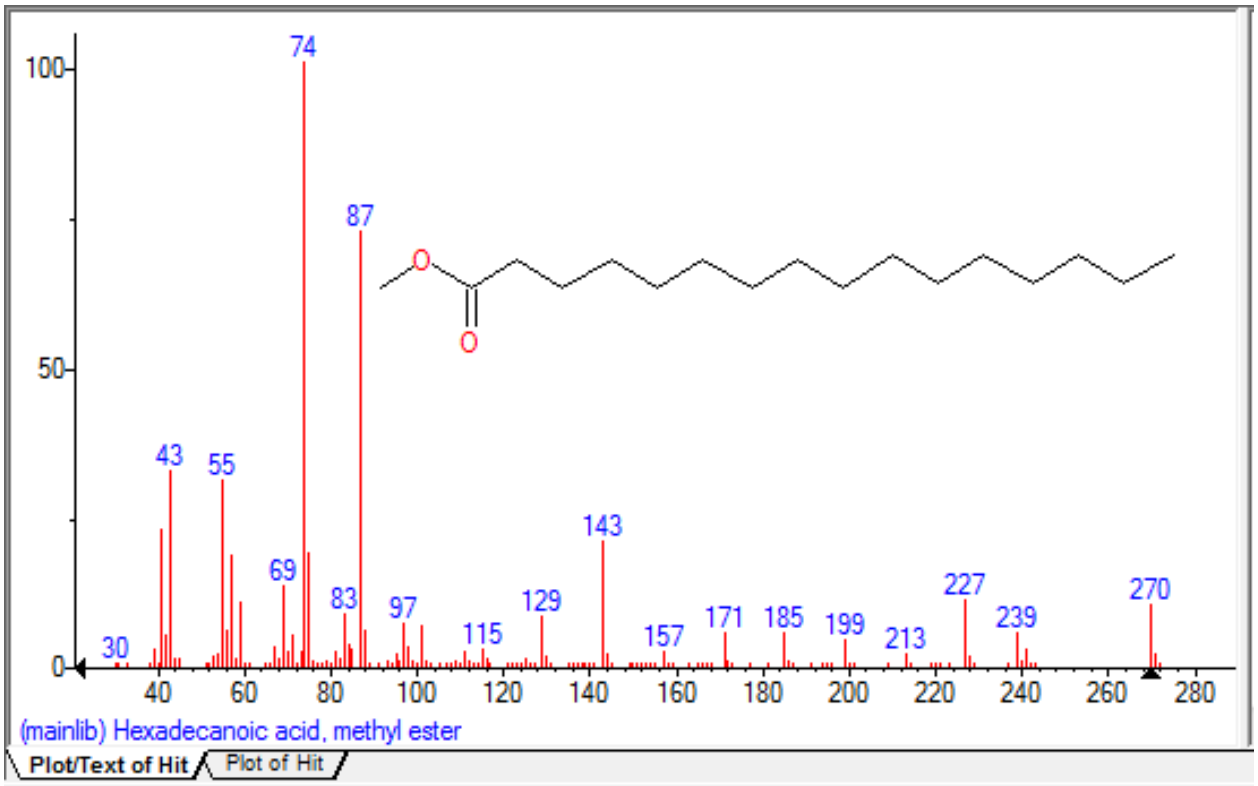
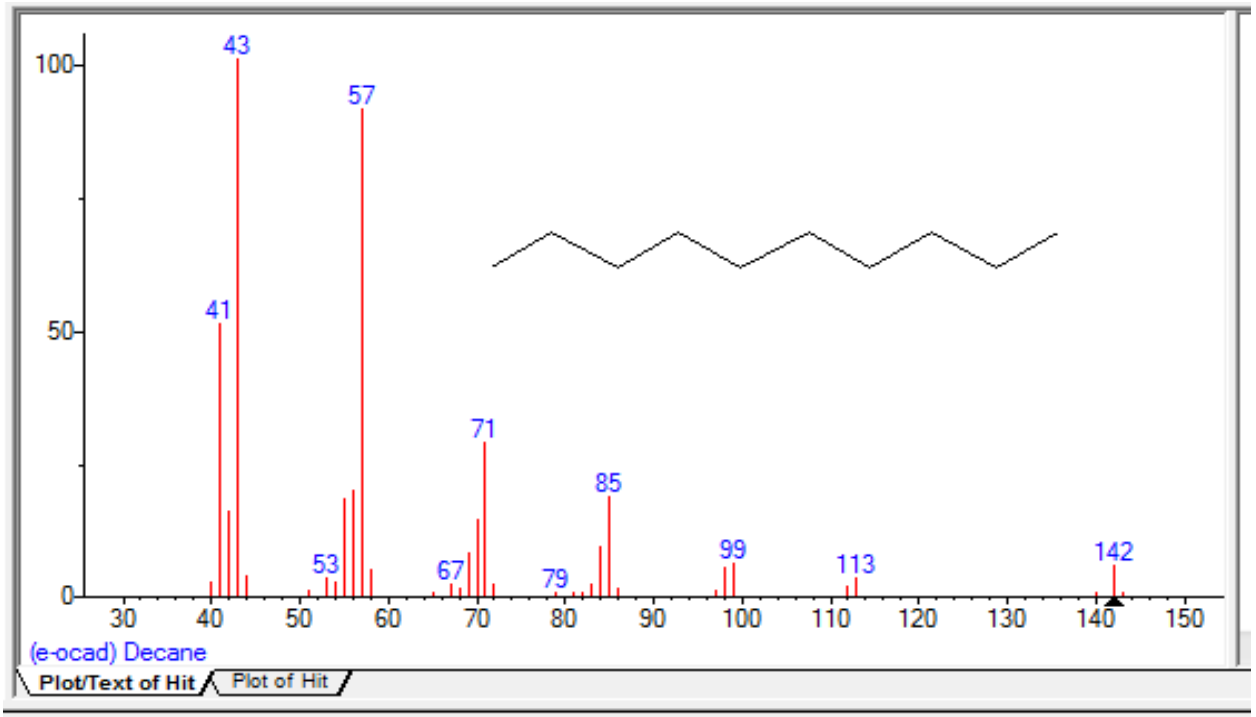


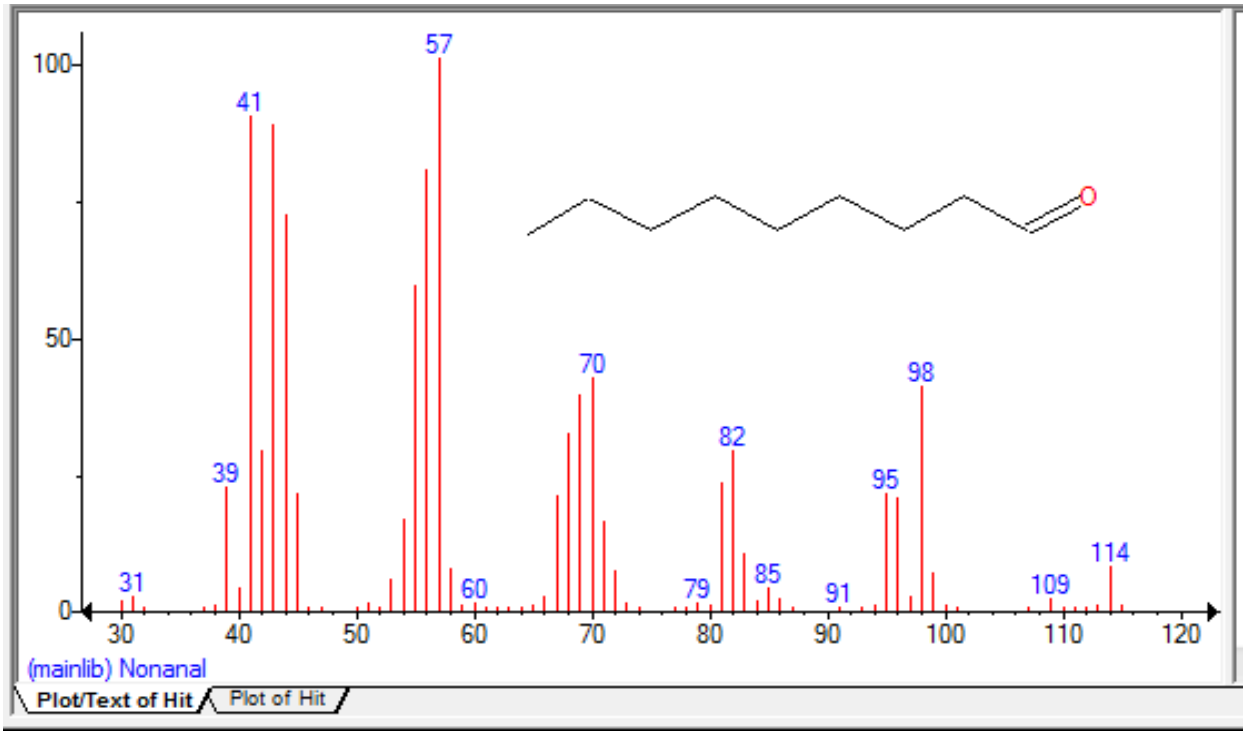
Figure 2. GCMS of sample in Hexane/Acetone 4:1 solvent system.



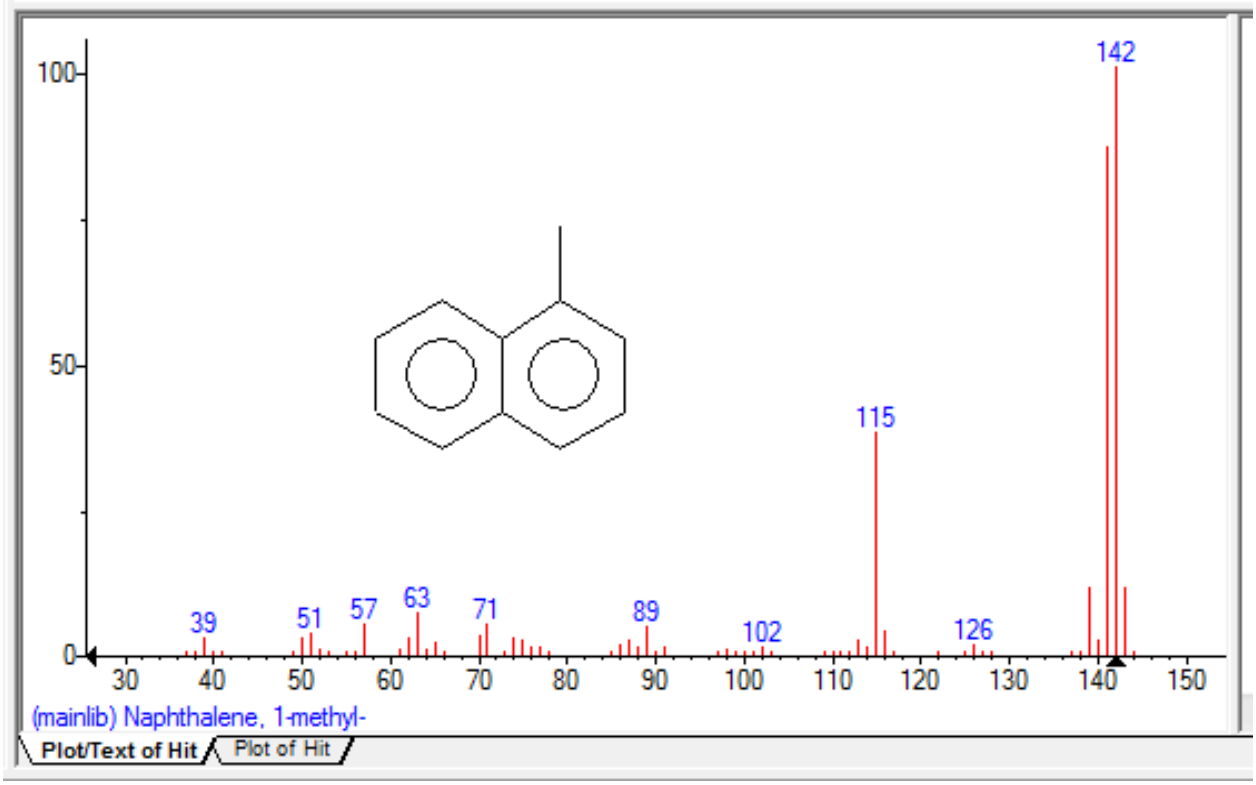
Appendix F: Library match of sample 2 in Hexane: Acetone (4:1).



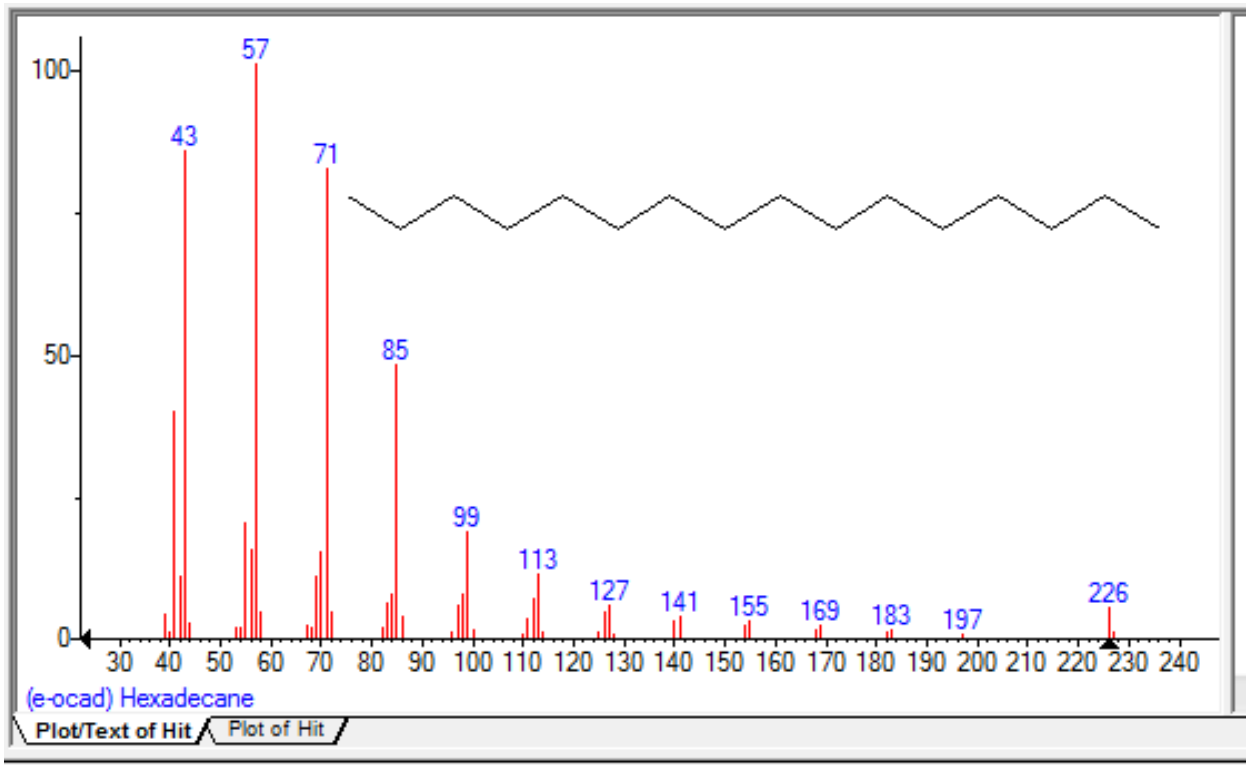
Appendix G: Library match of sample 2 in Hexane: Acetone (4:1)



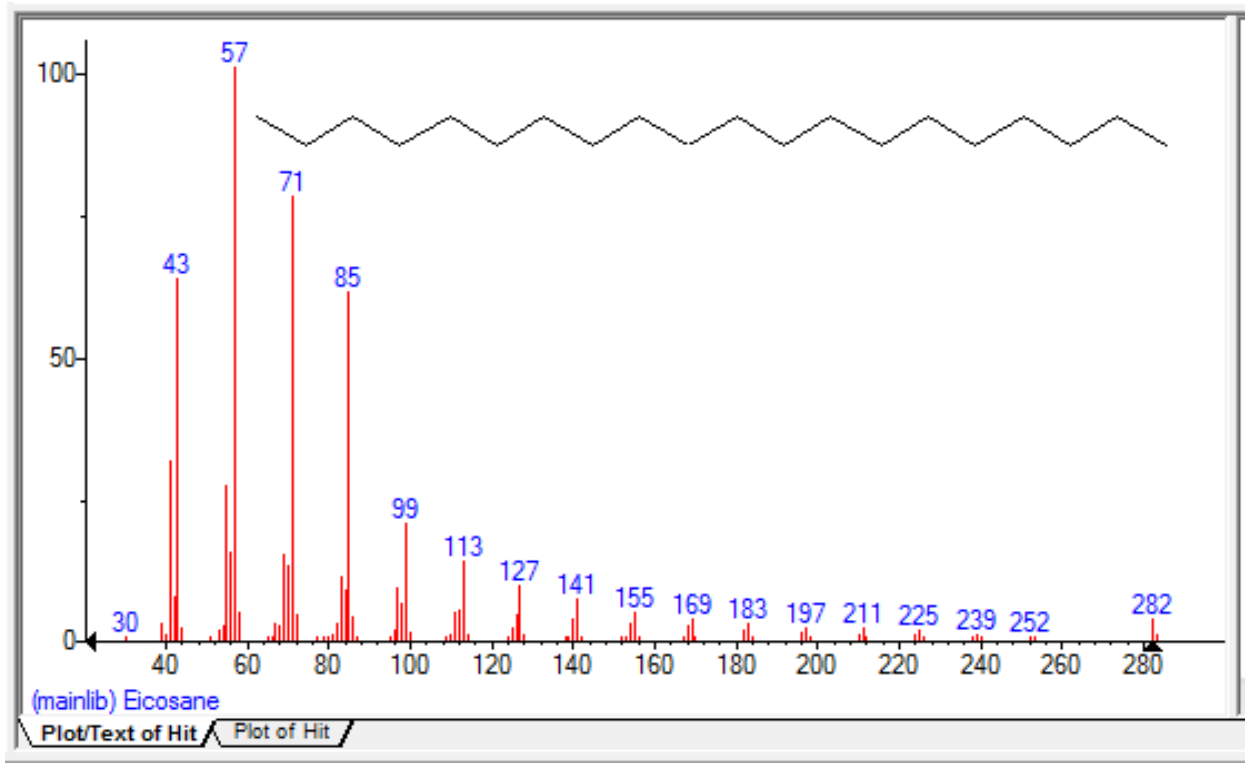
Appendix H: Library match of sample 2 in Hexane: Acetone (4:1).



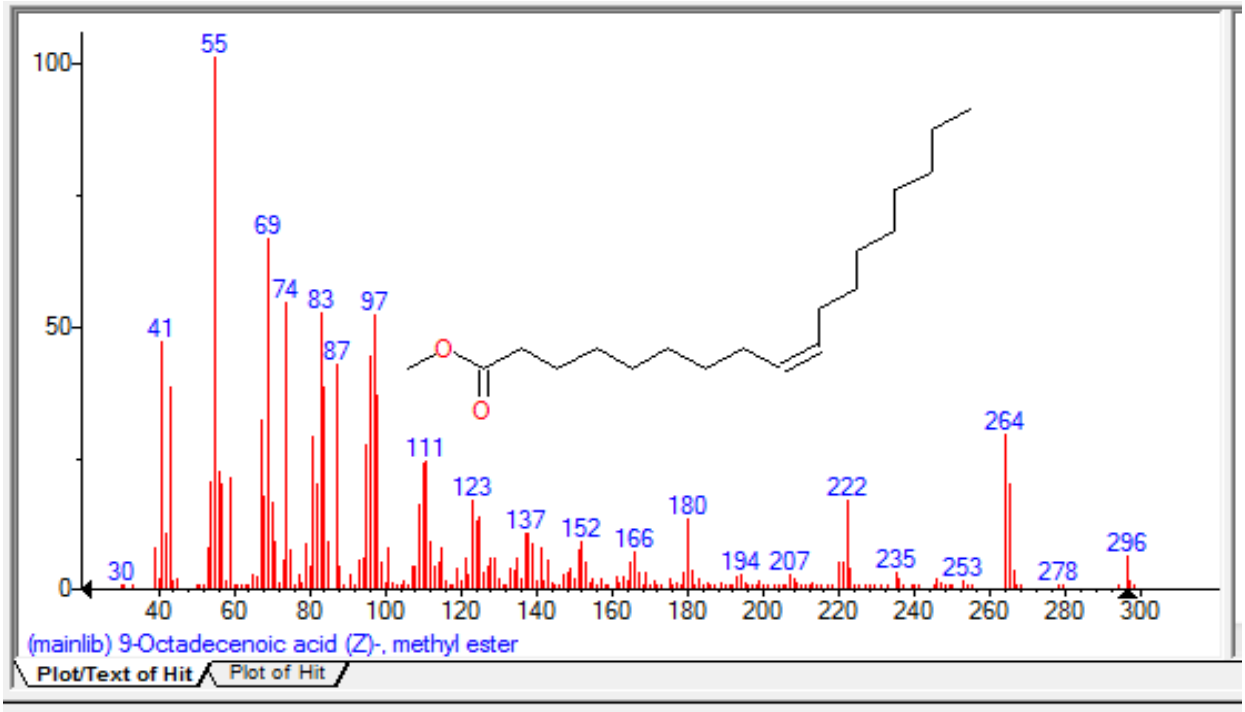
Appendix I: Library match of sample 2 in Hexane: Acetone (4:1)



Appendix J: Library match of sample 2 in Hexane: Acetone (4:1).

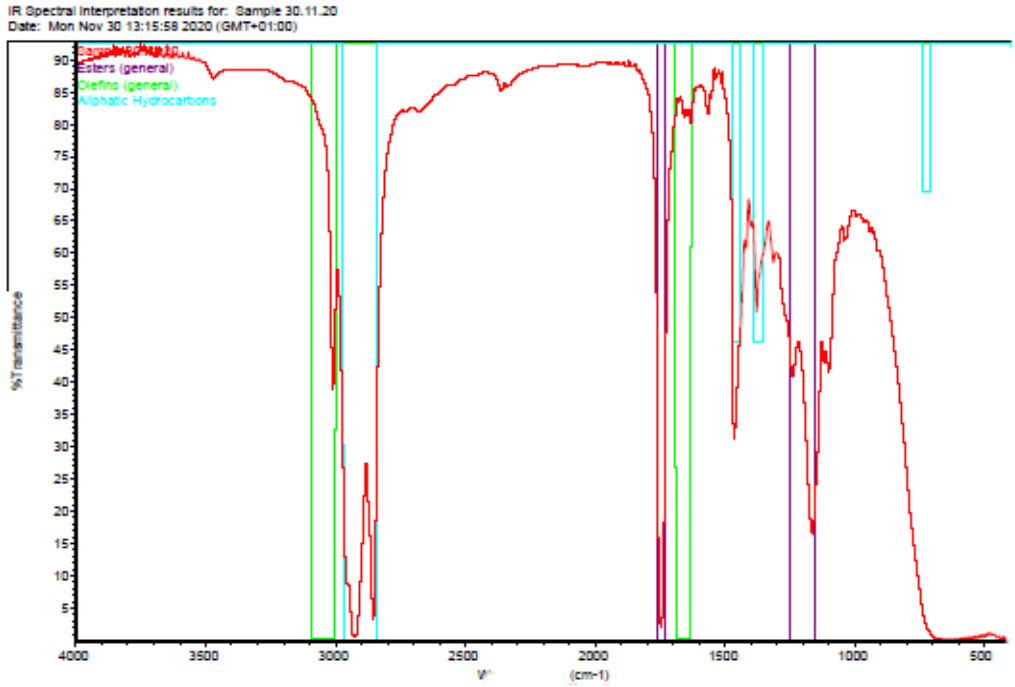


Appendix K: Library match of sample 2 in Hexane: Acetone (4:1)

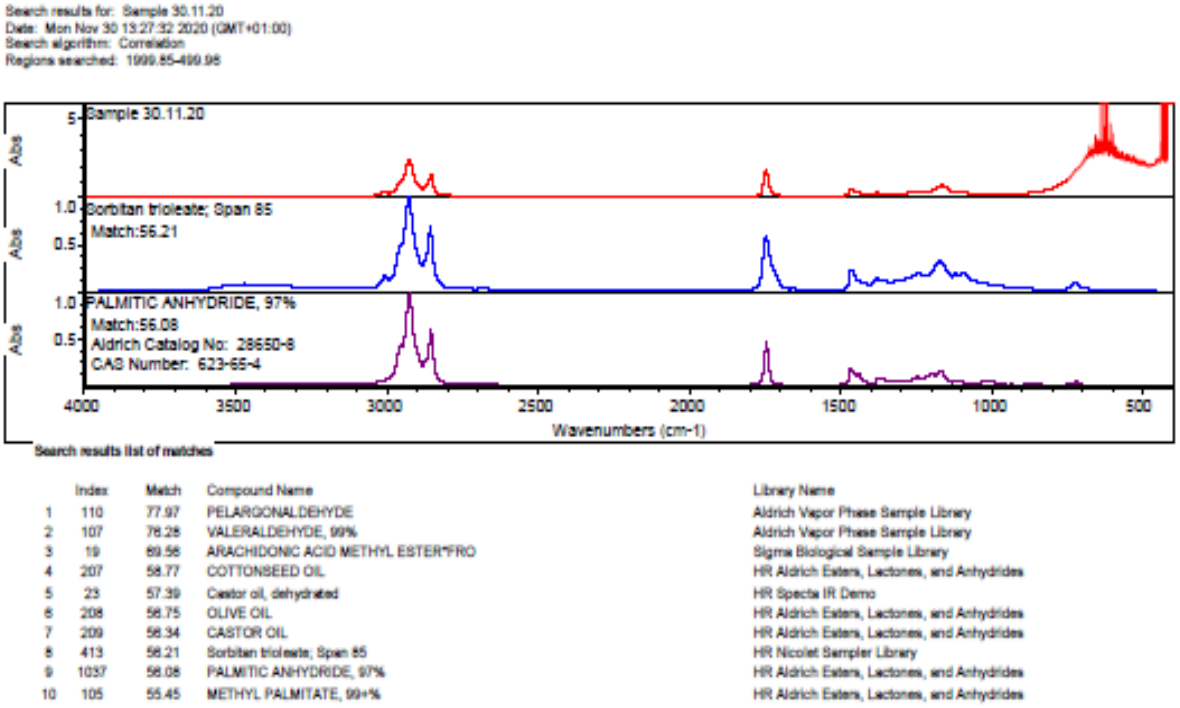


Appendix L: Library match of sample 2 in Hexane: Acetone (4:1).

Trigonella foenum-graecum S. was extracted by maceration and then subjected to screening. Crude concentrate showed phyto-compounds such as fats and oil, flavonoid, tannins, carbohydrate, saponins, and glycoside. A pure compound was gotten after purification which was labeled sample 2 which was a colourless oil with a characteristic taste.



Appendix M: FTIR spectral of Sample 2



Appendix N: FTIR library of sample 2

Table 14. Sample 2 FTIR Result.

Frequency(cm^{-1})	Assignment
2935	C-H stretching
2860	C-H stretching
1375	C-H Bending
1820	C=O Stretching
1055	C-O Stretching

Table 15. GCMS of Sample 2 in Ethylacetate.

Compound	R.T(secs)	Lib. Match (%)	Molecular mass	Assignment
Benzenemethanol	7.60	87.90	123	OH,C-C,C=C,C-O
Tridecane	8.21	81.30	99	C-C
MethylHexadecanoic acid	18.51	92.80	270	C-C,C=O,C-O,
Cholest-5-en-3-ol(Tetradecanoate)	27.23	73.70	369	C-C,C=C,C=O,C-O
Cholesteryl benzoate	27.65	70.70	382	C-C,C=C,C=O,C-O

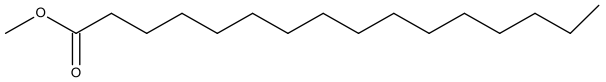
Table 16. GCMS of Sample 2 in Hexane/Acetone 4:1.

Compound	R.T(seconds)	Lib. Match (%)	Molecular mass	Assignment
Decane	4.13	90.10	142	C-C
Nonanal	6.08	93.30	114	C-C,C=O,C-O
Naphthalene	10.08	93.70	142	C-C,C=C
Hexadecane	14.38	91.50	226	C-C
MethylHexadecanoic acid	18.51	94.60	270	C-C,C=O,C-O
Eicosane	19.61	93.90	282	C-C
9-Octadecenoic acid	20.87	93.60	296	C-C,C=C,C=O,C-O,

Compound 2 showed an absorption bands at 2935 and 2860 cm^{-1} . Frequencies include 1820 cm^{-1} as a result of C=O and 1375 cm^{-1} for C-H. The frequency at 1055 cm^{-1} is due to C-O . These assignments are in good agreements with reported values result with previous literature for molecular structure of Methylhexadecanoic acid, a methyl hexadecanoate with CAS Registry number 112-39-0 and SDBS number 7831.

From the above findings, an ester of Hexadecanoic acid was isolated from n-hexane fraction of the seed of *Trigonella foenum-graecum*. Although the activity of the

isolated compound was not determined due to insufficient quantity.



MethylHexadecanoic acid

CONCLUSION

The phytochemical investigation showed the presence of vital natural products. The fractions of the plant were evaluated for their biological activities and it was found that the n-hexane fraction possesses spectrum of antibacterial activity against certain bacterias. A known pharmacologically active saturated fatty acid, (MethylHexadecanoic acid) was isolated and characterized from the n-hexane fraction of the seed using FT-IR and GCMS.

RECOMMENDATION

This research was limited to the phytochemical studies and antibacterial screening of the fractions of Fenugreek. During the extraction process, it will be advised for the researcher to use large quantity of the seed of T. foenum-graecum in order to have large quantity of individual fractionated extract for glass column chromatography. However, work needs to be carried out on its fractionation using a more polar solvent like butanol and also the isolation of the ethyl acetate or even the butanol fraction. It will also be recommended to use aqueous Ethanol for the extraction since it content large amount of phenolics and Tannins.

Competing Interests: The authors declare that there are no competing interests.

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