



PHYTOCHEMICAL ANALYSIS OF TRIGONELLA FOENUM- GRAECUM L. AND MUSA PARADISIACA L .BY HR-LCMS ANALYSIS

Dr. Abubakar S Bawazir¹, Sumaiya Z Khan^{2*}

¹Department of pharmacognosy, Y. B. Chavan College of pharmacy, Aurangabad.

^{2*}Department of pharmacognosy, Y. B. Chavan College of pharmacy, Aurangabad.
Email: ksumaiya40@gmail.com

***Correspondence author:** Sumaiya Z Khan

*Department of pharmacognosy, Y. B. Chavan College of pharmacy, Aurangabad.
Email: ksumaiya40@gmail.com

ABSTRACT:

With the widespread use of medicinal plant products in the biotechnological and pharmaceutical industries, phytochemical analysis of medicinal plants has grown in importance and challenging. The present study was aimed at bioactive constituent analysis from selected medicinal plants such as *Trigonella foenum-graecum L.* (TF), *Musa paradisiaca L.*(MP), by HR-LCMS (High Resolution-Liquid Chromatography Mass Spectrometry) analysis. TF and MP are highly special plants with a wide range of therapeutic applications. We examined the primary chemical components found in hydro-ethanol extract of seeds of TF and ethanol extract of fruits & peels of raw MP through HR-LCMS techniques. The compounds of TF seeds extract includes Hellicoside, Kaempferol 3-rhamnoside 7-xyloside, Eupachloroxin, Phrymarolin, Cynaropi crin ,Cetirizine, Gestodene , Ibogaine. The compounds of raw MP fruits & peels extract includes Cyclothialidine , Pimentol, Amaroswerin, Ketotifen, Foeniculoside III, Camellianin A, Herbacetin 3,8-diglucoside, Cerivastatin ,Quercetin 3-glucoside 7- Xyloside, Imiquimod. Total twenty three compounds of TF seeds extract and twenty four compounds of raw MP fruits & peels extract were identified based on their retention time and measured accurate mass. Because of the presence of bioactive compounds in extracts of TF seeds and raw MP fruits & peels , they can be further studied for their biological activity.

Keywords: HR-LCMS, *Trigonella foenum-graecum L.*, *Musa paradisiaca L.*, Bioactive compounds.

INTRODUCTION

Global human health is maintained by the use of bioactive compounds extracted from medicinal plants, as is generally accepted. Homeopathy, Unani, and Ayurveda (3700 B.C.) are among ancient medical systems that reference the use of medicinal plant products to treat a variety of human ailments.[1,2] *Trigonella foenum graecum L.*, or fenugreek, has its origins in central Asia and has been traded since 2000 BCE for medicinal purposes.[3]

It features trifoliolate leaves, noduled roots, many branches, white buds, and conspicuous yellow seeds.[4] .Under typical environmental conditions, the plant produces multiple single and double-long pods that are 10–15 cm long and contain 17–20 seeds. [5] There is evidence that each part of this plant has a variety of therapeutic uses. The mucilage of fenugreek seeds has anti-inflammatory

properties.[6] Fenugreek seed powder is having hyperlipidemic activity.[7] It also contains Diosgenin and Galactomannan which is responsible for anti-diabetic action. [8]

Grown in tropical and subtropical regions, *Musa paradisiaca L.* is commonly used for its nutritional properties. Phytochemical screening revealed the presence of alkaloids, tannins, steroids, saponin, carbohydrates and flavonoids in *Musa paradisiaca L.* peels.[9]

In conventional medicine, *Musa paradisiaca L.* fruit, peels, and leaves have been used.[10]

Banana peels contain bioactive chemicals that have been found to have antibacterial, anti-hypertensive, anti-inflammatory, and anti-diabetic activities. These substances include glycosides, flavonoids, anthocyanins, tannins, alkaloids, phlobatannins, and terpenoids.[11]

MATERIAL AND METHOD

Two plants *Trigonella foenum graecum L.* (TF) seeds and raw fruits with peel of *Musa paradisiaca L.* (MP) were selected for the study which have been reported for its anti-diabetic activity. They were procured from a farm in aurangabad. Both were subjected to extraction by soxhlet extraction method by using hydro-ethanolic solvent for TF seeds and ethanolic solvent for raw fruits with peel of MP.

The resultant dried extract were soluble in water hence subjected to HR-LCMS analysis.

High Resolution-Liquid Chromatography Mass Spectroscopy for bioactive compound Identification:

The resulting extract's phytochemical composition from TF seeds and raw fruits with peel of MP was analyzed.

The sample's HR-LCMS analysis was done at the Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Pawai in Mumbai.

Table 1: HR-LCMS analysis parameters

Parameters	Conditions
Samples	Extract of TF seeds and extract of MP raw fruits with peels
Ion source	Dual AJS ESI
Detector	MS Q-TOF
Model	G6550A
Mass resolution	0.01%
Scanning rate	Minimum range :120 (m/z) and maximum range : 1200 (m/z)
Gas chromatography temperature and speed	250°C and 13 ml/min.
Sampler model	G4226A HIP
Ejection rate	100 µl/min
Auxiliary speed	100 µl/min
Solvent	Acetonitrile: water

RESULT AND DISCUSSIONS:

Trigonella foenum-graecum (TF) seeds ethanolic extract was subjected for HR LC-MS analysis. The biological active compounds present in seed extract shown in table no 2 and mass spectra in figure no.1. Total twenty three peaks were observed in HR LC-MS analysis of *Trigonella foenum-graecum* seed. NIST Library Analysis & Structures of Compounds are presented in figures 2 to 24.

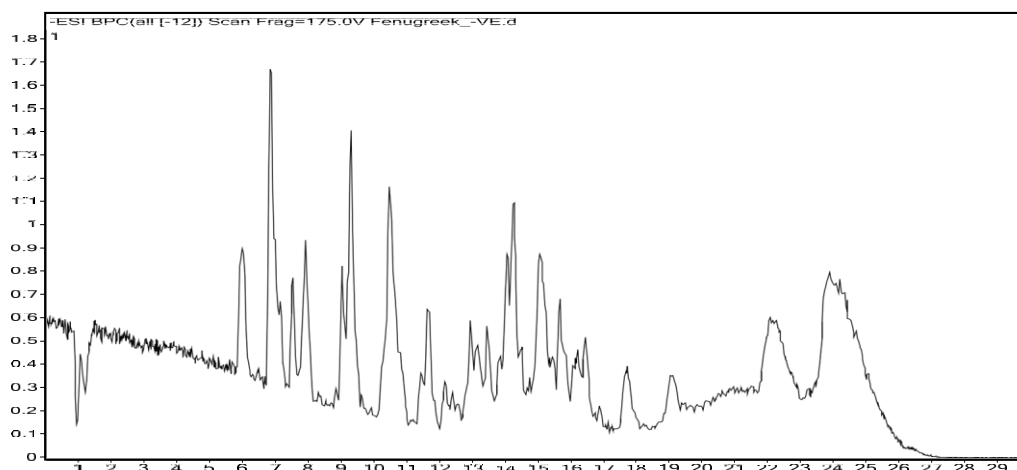


Figure 1: HR-LCMS chromatogram of ethanolic extract of TF seeds.

Table 2: HR-LCMS analysis of ethanolic extract of TF seeds

Sr. no.	Name of the Compound	Retention Time (RT)	Molecular Weight	Molecular Formula	DB Difference (ppm)
1	Hellicoside	1.141	656.194	C ₂₉ H ₃₆ O ₁₇	2.5
2	Kaempferol 3-rhamnoside 7-xyloside	5.824	564.151	C ₂₆ H ₂₈ O ₁₄	-5.03
3	Eupachloroxin	6.058	428.125	C ₂₀ H ₂₅ Cl O ₈	-2.56
4	Phymarolin I	6.517	488.136	C ₂₄ H ₂₄ O ₁₁	-7.93
5	Cynaropicrin	6.934	346.142	C ₁₉ H ₂₂ O ₆	-1.65
6	Eupatundin	7.01	376.153	C ₂₀ H ₂₄ O ₇	-1.37
7	Calaxin	7.529	344.128	C ₁₉ H ₂₀ O ₆	-5.06
8	Cetirizine	7.915	388.139	C ₂₁ H ₂₅ Cl N ₂ O ₃	42.21
9	N-Feruloylglycyl-L-phenylalanine	7.93	398.147	C ₂₁ H ₂₂ N ₂ O ₆	1.9
10	3Hydroxymugineic acid	8.077	336.118	C ₁₂ H ₂₀ N ₂ O ₉	-4.45
11	Elephantin	9.009	374.139	C ₂₀ H ₂₂ O ₇	-5.19
12	Chlormadinone acetate	9.513	404.176	C ₂₃ H ₂₉ Cl O ₄	-0.76
13	13-Hydroxy-5'-O-methylmelledonal	10.561	462.19	C ₂₄ H ₃₀ O ₉	-2.37
14	9-chloro-10-hydroxy-hexadecanoic acid	10.774	306.1959	C ₁₆ H ₃₁ Cl O ₃	0.81
15	Etoposide glucuronide	12.185	764.224	C ₃₅ H ₄₀ O ₁₉	-9.31
16	,4"-bis(N-feruloyl)serotonin	12.916	702.259	C ₄₀ H ₃₈ N ₄ O ₈	14.55
17	Gestodene	13.076	310.195	C ₂₁ H ₂₆ O ₂	-5.46
18	Ibogaine	13.344	310.21	C ₂₀ H ₂₆ N ₂ O	-18.7
19	Irbesartan	15.67	428.237	C ₂₅ H ₂₈ N ₆ O	-9.83
20	Astemizole	15.89	458.249	C ₂₈ H ₃₁ F N ₄ O	-0.74
21	Salannin	18.862	596.298	C ₃₄ H ₄₄ O ₉	1.46
22	3,7-Dihydroxy-25-methoxycucurbita-5,23-dien-19-al	23.507	486.372	C ₃₁ H ₅₀ O ₄	-1.89
23	3,6-Epoxy-5,5',6,6'-tetrahydro-b,b'-carotene-3',5,5',6'-tetrol	24.568	618.427	C ₄₀ H ₅₈ O ₅	1.69

NIST Library Analysis & Structures of Compounds

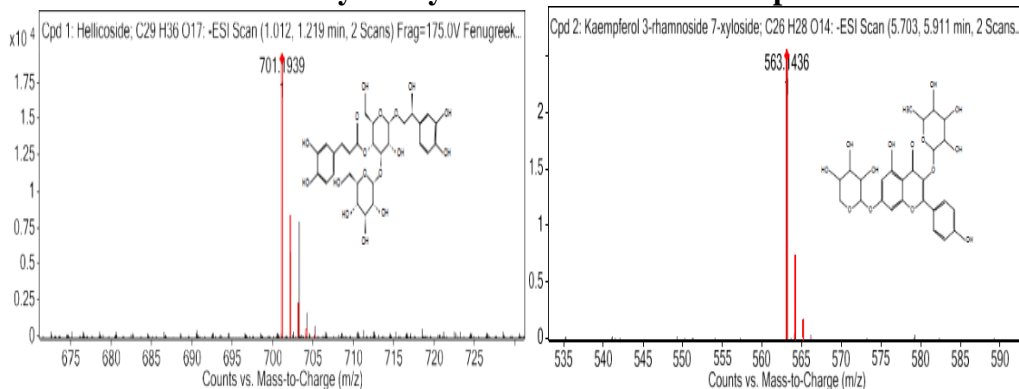


Figure 2: Hellicoside

Figure 3: Kaempferol 3-rhamnoside 7-xyloside.

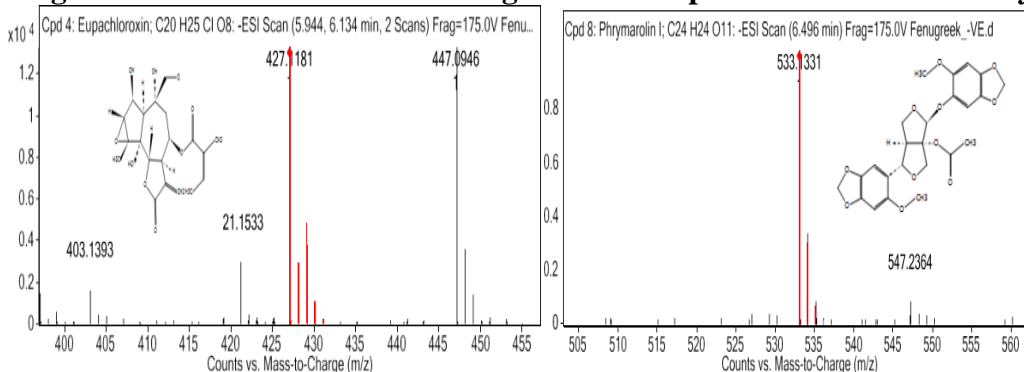


Figure 4: Eupachloroxin

Figure 5: Phymarolin I

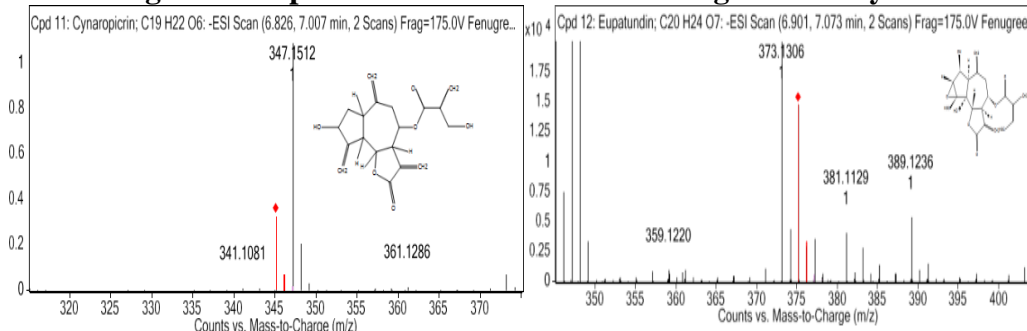


Figure 6: Cynaropicrin

Figure 7: Eupatundin

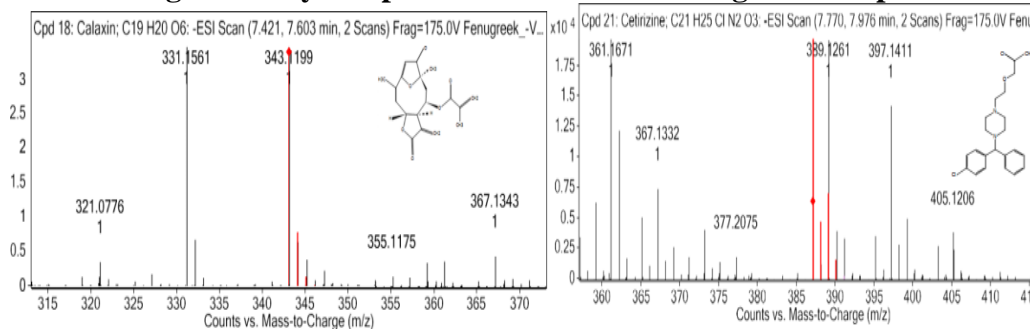


Figure 8 :Calaxin

Figure 9: Cetirizine

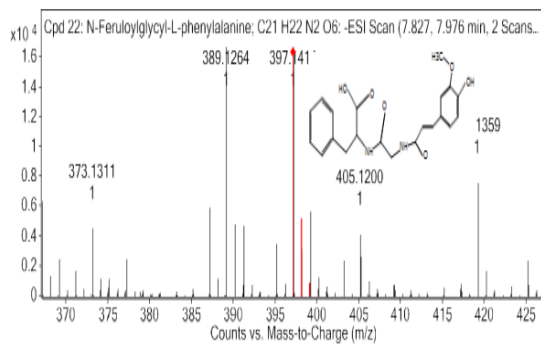


Figure 10 :N-Feruloylglycyl-L-phenylalanine

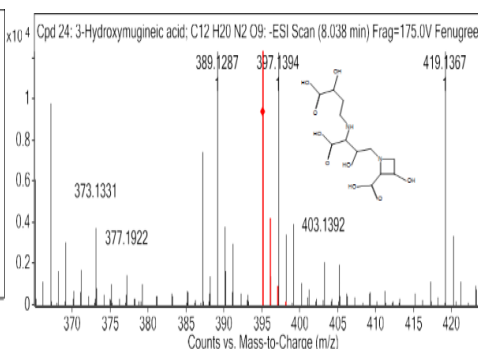


Figure 11: Hydroxymugineic acid.

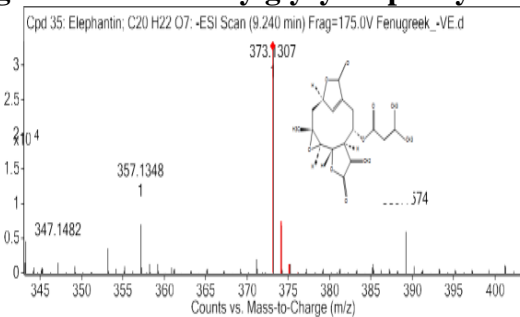


Figure 12: Elephantin

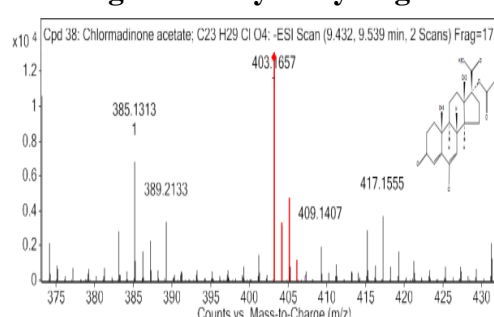


Figure 13: Chlormadinone acetate

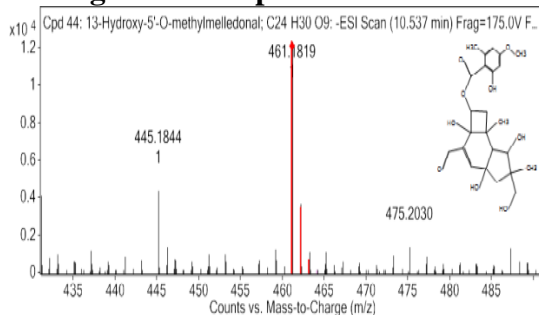


Figure 14: 13-Hydroxy-5'-O-methylmelledonal

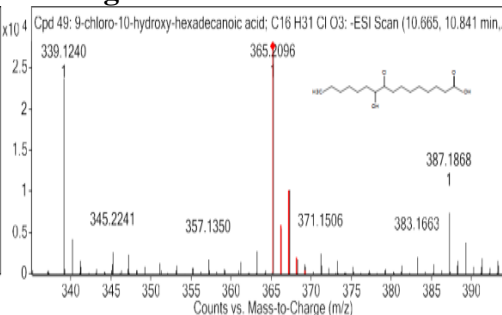


Figure 15: 9-chloro-10-hydroxy-hexadecanoic acid.

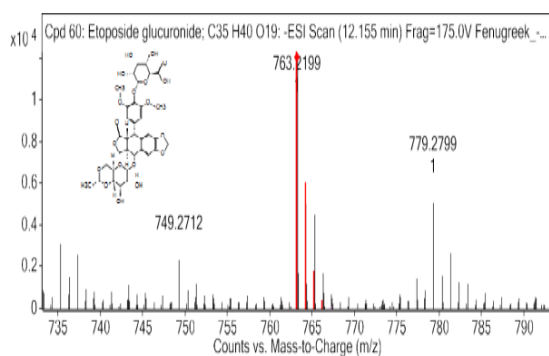


Figure 16 :Etoposide glucuronide

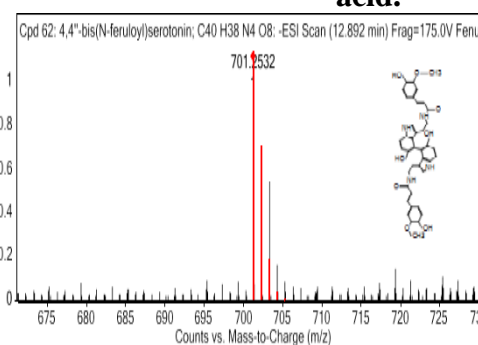


Figure 17 :4,4''-bis(N-feruloyl) serotonin

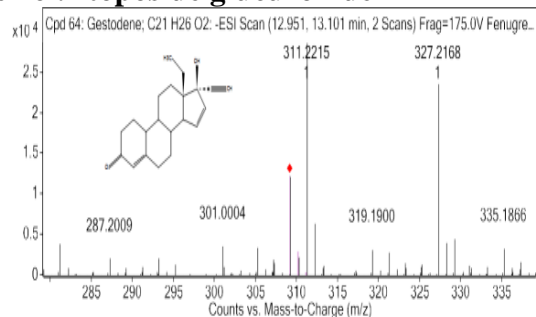


Figure 18: Gestodene

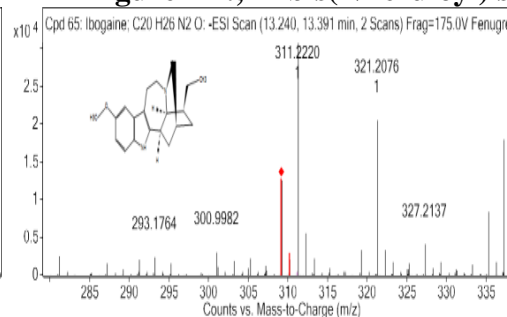


Figure 19 :Ibogaine

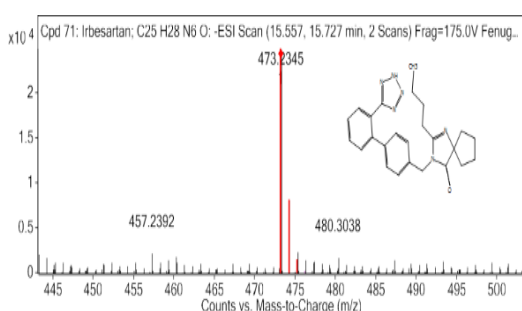


Figure 20 :Irbesartan

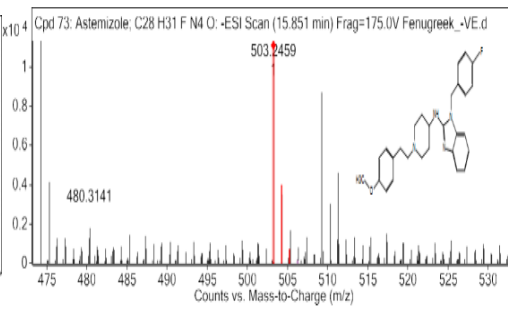


Figure 21 :Astemizole

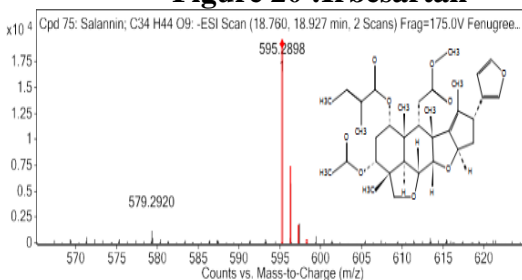


Figure 22 :Salannin

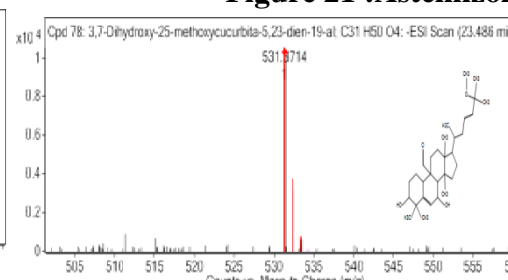


Figure 23 : 3,7-Dihydroxy-25-methoxycucurbita-5,23-dien-19-al

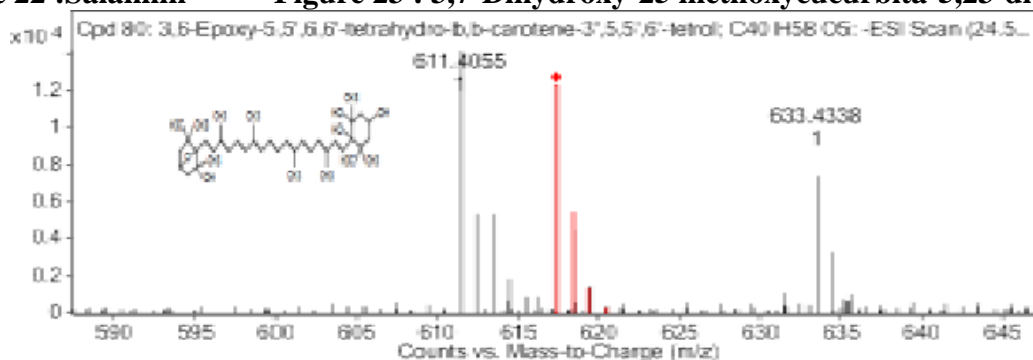


Figure 24:3,6-Epoxy-5,5',6,6'-tetrahydro-b,b-carotene-3',5,5',6'-tetrol

HR-LCMS analysis of raw *Musa paradisiaca* fruits with peel extract:

The raw *Musa paradisiaca* (MP) fruits with peel extract was subjected for HR LC-MS analysis. The biological active compounds present in raw MP fruits with peel extract is shown in table 3 and mass spectra in figure 25 . Total twenty four peaks were observed in HR LC-MS analysis of raw MP raw fruits with peel extract. NIST Library analysis & structures of compounds are shown in figures 26 to 53.

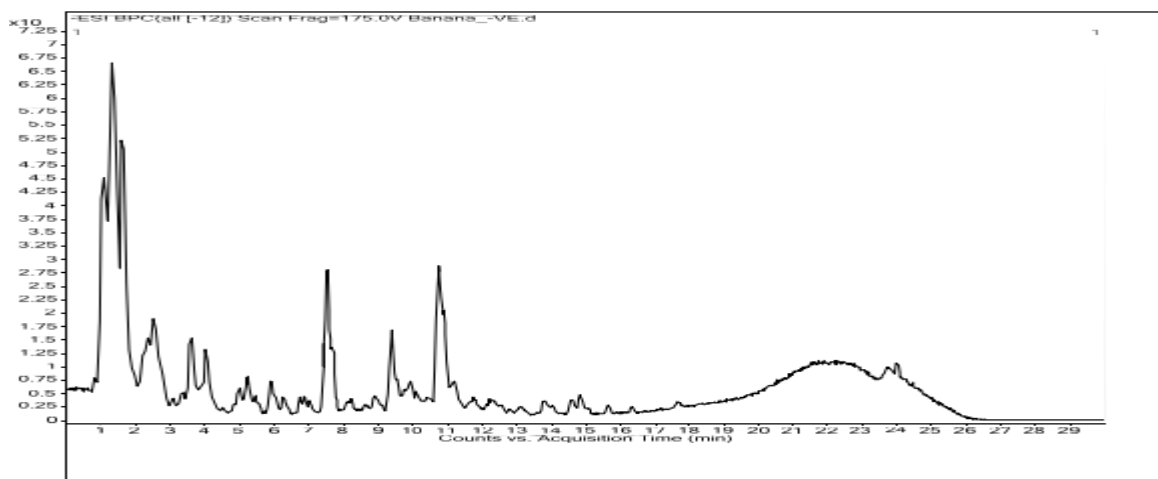


Figure 25 :HR-LCMS chromatogram of raw MP fruits with peel extract

Table 3 : HR-LCMS analysis of raw MP fruits with peel extract.

Sr. No	Name	Mass	RT	Formula	DB Diff (ppm)
1	Cyclothialidine	641.1925	0.932		
2	Pimentol	494.1434	0.993	C23 H26 O12	-2.06
3	Amylopectin	828.2794	1.012	C30 H52 O26	-5.72
4	Debromohymenialdine	245.0913	1.312	C11 H11 N5 O2	0.02
5	(R)-Rutaretin 1'-(6"-sinapoylglucoside)	630.2011	1.601	C31 H34 O14	-9.96
6	Kaempferol 3-(2"-rhamnosyl-6"-acetylgalactoside) 7- rhamnoside	782.229	1.808	C35 H42 O20	0.02
7	Amaroswerin	602.1644	2.487	C29 H30 O14	-1.43
8	Ketotifen	309.1185	2.448	C19 H19 N O S	0.85
9	Foeniculoside III	1004.2977	2.952	C54 H52 O19	12.52
10	Metolachlor	283.1375	2.978	C15 H22 Cl N O2	-12.66
11	Camellianin A	620.1716	3.371	C29 H32 O15	4.01
12	Bakankoside	357.1414	3.829	C16 H23 N O8	2.65
13	Azelastine	381.1609	4.283	C22 H24 Cl N3 O	-0.21
14	Herbacetin 3,8-diglucoside	626.1496	6.127	C27 H30 O17	-2.13
15	Cerivastatin	459.2445	6.332	C26 H34 F N O5	-5.26
16	Quercetin 3-glucoside 7-xyloside	596.1384	6.339	C26 H28 O16	-1.16
17	Dinorcapsaicin	277.1649	6.598	C16 H23 N O3	10.4
18	7alpha-1(10->19)-Abeo-7-acetoxybacun-9(11)-ene	496.2144	7.099	C28 H32 O8	-9.43
19	Imiquimod	240.13	9.336	C14 H16 N4	6.04
20	Trimethobenzamide	388.1886	10.769	C21 H28 N2 O5	29.03
21	9-chloro-10-hydroxy hexadecanoic acid	306.194	11.671	C16 H31 Cl O3	7.17
22	PE(18:3(9Z,12Z,15Z)/18:1(11 Z))	739.5186	21.492	C41 H74 N O8 P	-4.64
23	Unknown bound 35		1.987		
24	Unknown Compound 76		21.139		

NIST Library Analysis & Structures of Compounds

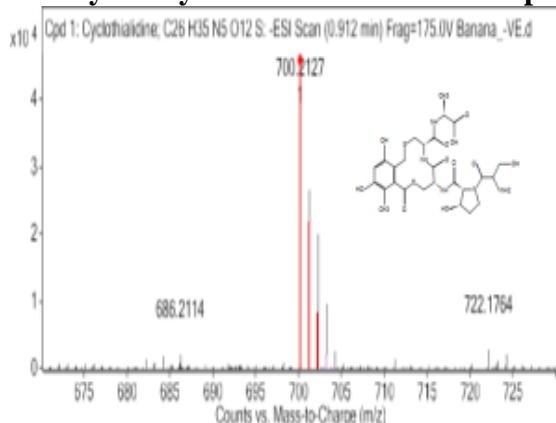


Figure 26 :Cyclothialidine

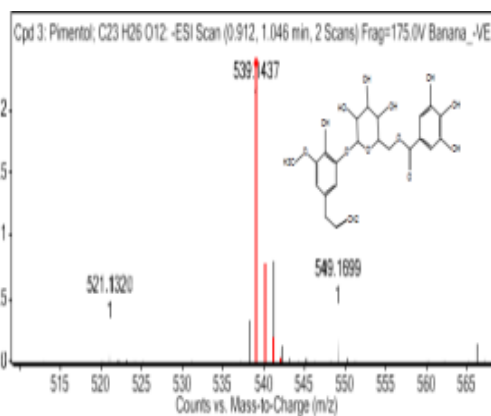


Figure 27 :Pimentol

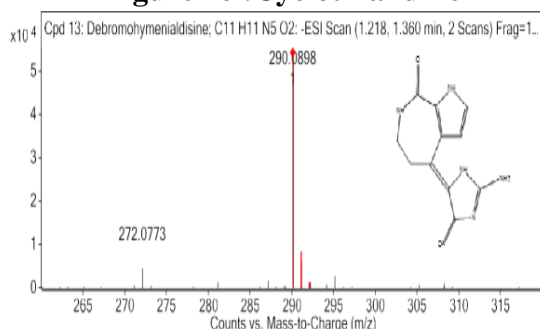


Figure 28: Amylopectin

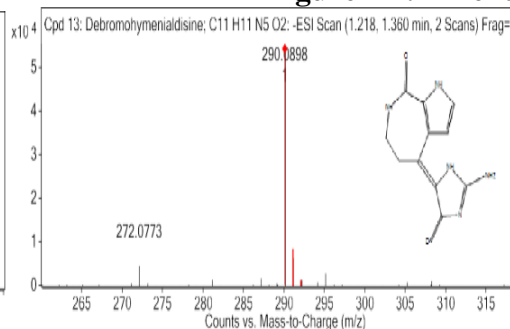


Figure 29:Debromohymenialdisine

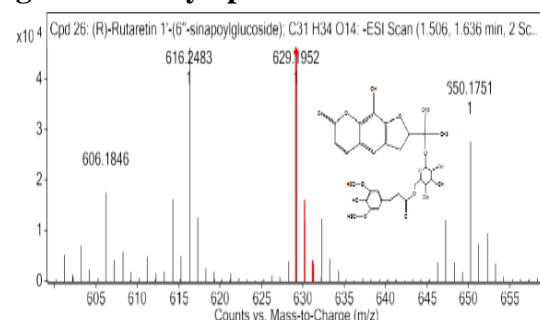


Figure 30: (R)-Rutaretin 1'-(6''-Sinapoylglucoside)

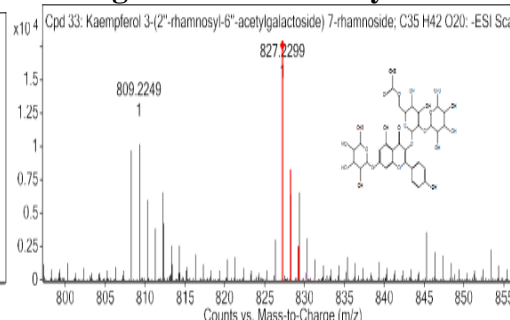


Figure 31: Kaempferol 3-(2''- rhamnosyl-6''-acetylgalactoside) 7-rhamnoside

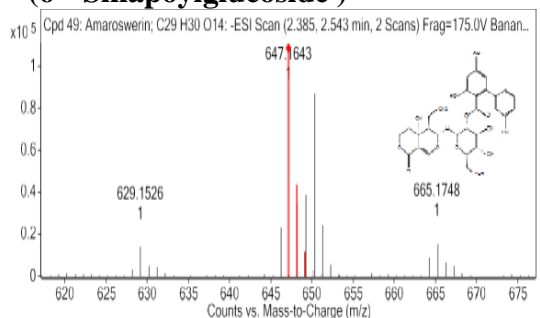


Figure 34:Amaroswerin

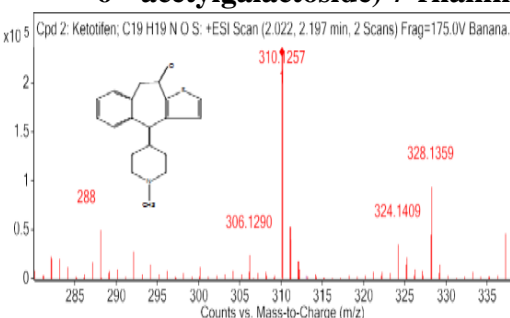


Figure 35: Ketotifen

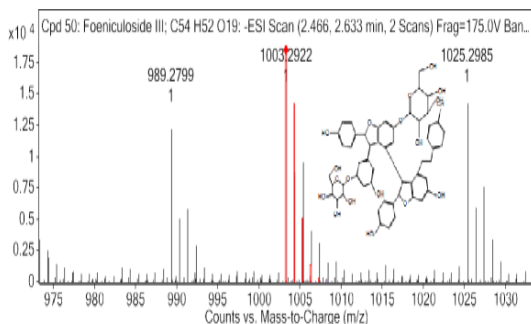


Figure 36 :Foeniculoside III

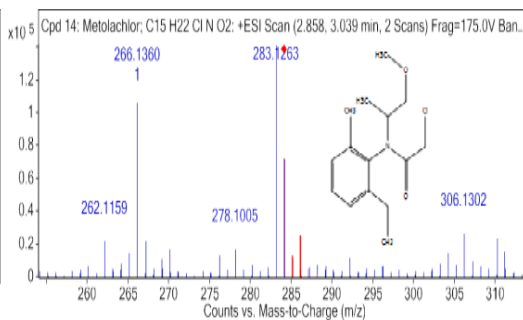


Figure 37 :Metolachlor

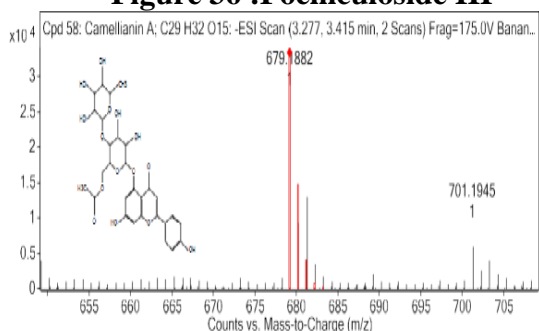


Figure 38 :Camellianin A

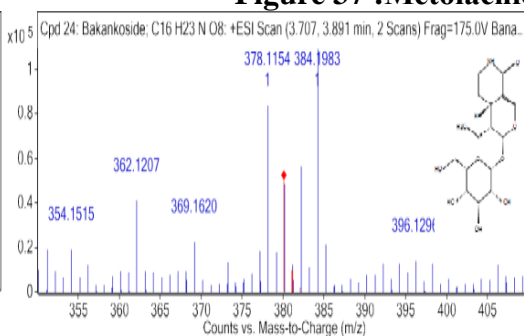


Figure 39: Bakankoside

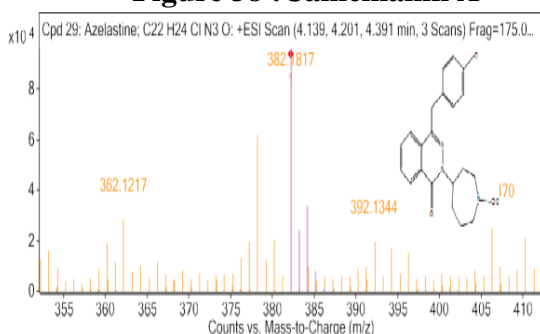


Figure 40:Azelastine

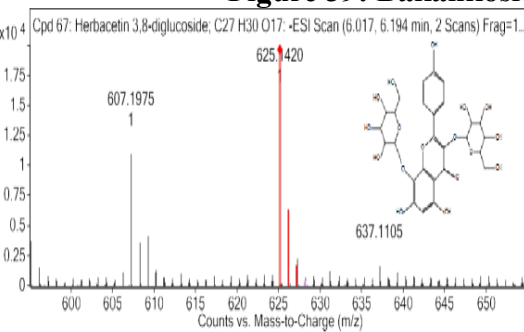


Figure 41 :Herbacetin 3,8-diglucoside.

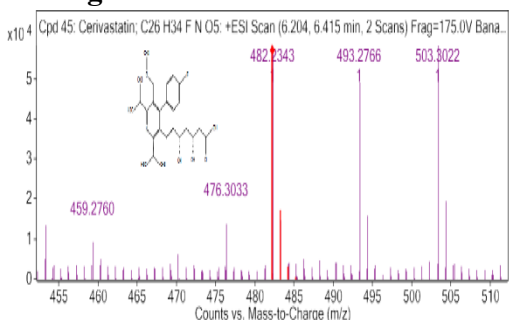


Figure 42: Cerivastatin

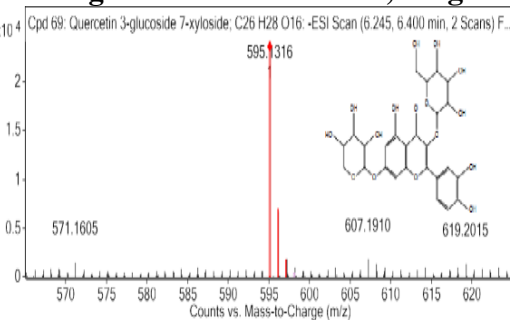


Figure 43: Quercetin 3-glucoside 7- Xyloside

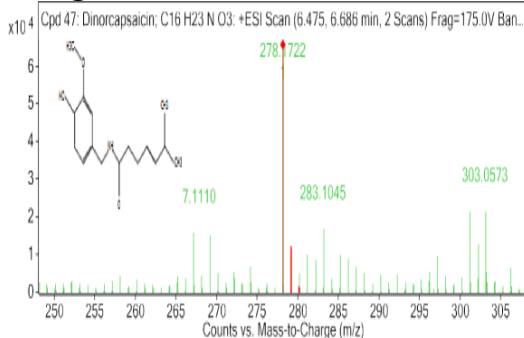


Figure 46: Dinorcapsaicin

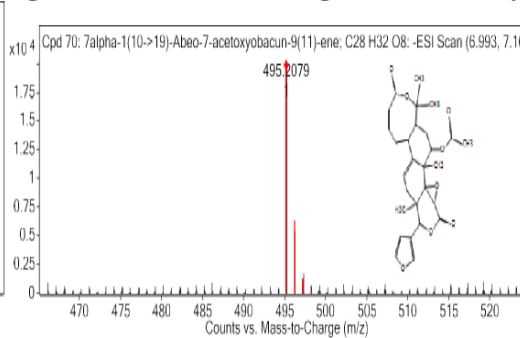


Figure 47: 7alpha-1(10->19)-Abeo-7-acetoxybacun-9(11)-ene

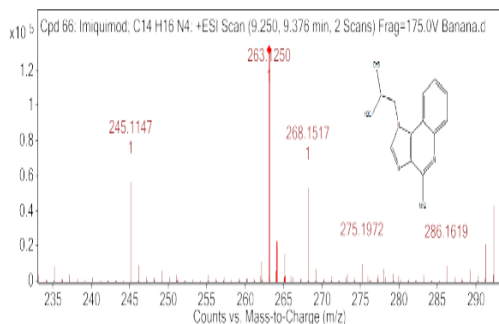


Figure 48: Imiquimod

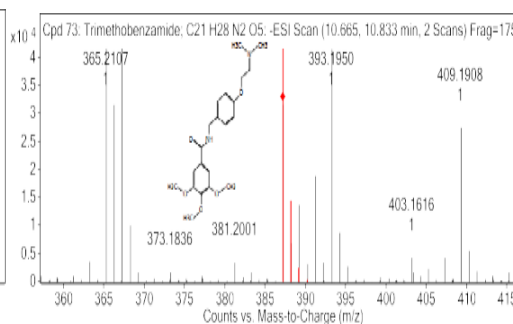


Figure 49: Trimethobenzamide

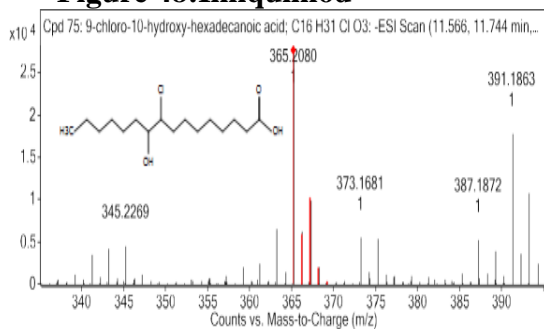


Figure 50: 9-chloro-10-hydroxy

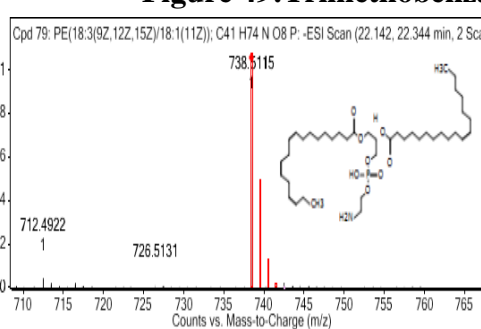


Figure 51: PE(18:3(9Z,12Z,15Z)/18:1(11Z))

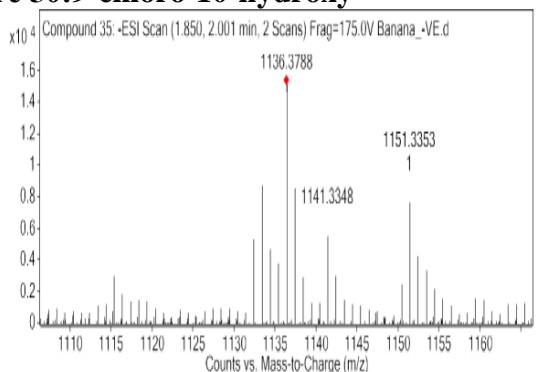


Figure 52: Unknown Compound 35

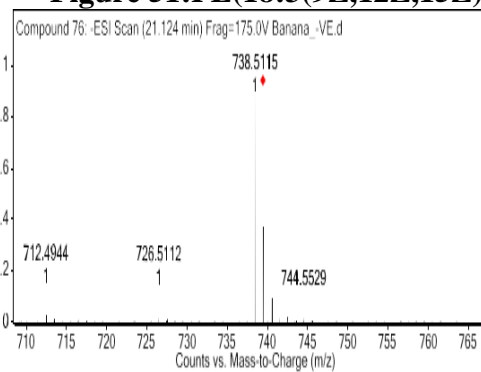


Figure 53: Unknown Compound 76

CONCLUSION:

Hydro-ethanolic extract of *Trigonella foenum-graecum L* seeds and ethanolic extract of raw *Musa paradisiaca L* fruits with peel confirms the presence of 23 and 24 bioactive compounds respectively by HR-LCMS analysis which may be responsible for various biological activity and can be a topic of research for treatment of various diseases including diabetes mellitus, hypercholesterolemic, cancer, hypertension, piles.

REFRECES:

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