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Mass Spectral Analysis of Solvent Extracts of Fenugreek Seeds

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Fenu greek seeds were extracted using the solvents in the order of n-hexane, chloroform and methanol based on their polarity. Residues from each solvent extract was collected separately and purified by column chromatograpy after running TLC. They were subjected to gas–chromatography (EI) mass as well as FAB–mass sepectroscopy and analysed.

Key Words: Mass spectra, TLC, Gas chromatography, Extracts, Fenugreek seeds.

INTRODUCTION

Fenugreek (*Trigonella Foenum-gracuem*) is well known for its medicinal values. It belongs to the order Leguminales and family Leguminacea. The seeds are medicinally used as carminative and tonic[1]. It found useful in dropsy, chronic cough, in external and internal swelling and in hair decay[1]. Autidiabetic[2] activity and hypoglycemic effect of fenugreek seeds had been established in clinical and animal models[2–4]. Diosgenin[5] and sapogenins[6] in Trigonella were detected and extracted. Yamogenin glycosides[1] and 4-hydroxy isoleucine[8], isolated from fenugreek seeds were characterised by a combination of chemical, IR, PMR spectroscopic and X-ray crystallographic techniques. Chemical composition of fenugreek seeds and leaves were discussed[9]. Metabolic changes in structural carbohydrates and minerals at three developmental stages of fenugreek leaves were reported. Various biological usefulness of fenugreek seeds were discussed[10]. Though there were several extraction reports on fenugreek seeds, a complete extraction followed by mass spectral (GC-EI and FAB) analysis has not yet reported. Herein we report GC-(EI) and FAB mass spectral analysis of residues from different solvent extracts of fenugreek seeds.

RESULTS AND DISCUSSION

Fenugreek seeds were successively extracted using the solvents in the order of n-hexane, chloroform and methanol based on the polarity. Semi solid brown coloured residues from each solvent extract was collected separately. All the residues were purified by column chromatography after running TLC. Benzene, benzene-pet ether (1 : 1) and methanol were the solvents used for column chromatography. The residues thus obtained were subjected to GC (EI) as well as FAB mass spectral analysis.

Analsis of n-hexane extract

The residue from n-hexane extract were column chromatographed by using benzene-pet ether (1 : 1) (I), benzene (II) and methanol (III). The mass from each portion was collected separately.

Analysis of n-hexane extract (I): FAB mass spectral pattern of n-hexane extract using benzene-pet ether (1:1) as eluent showed several fragmented peaks with appreciable intensity (Table-2).

Analysis of n-hexane extract (II): GC (EI) mass spectrum for n-hexane extract using benzene as an eluent showed five peaks with five different retention time. (Table-1) FAB mass spectrum showed several fragmented peaks with appreciable intensity (Table-2). The spectrum clearly revealed a huge molecular mass with m/z 1888.4. It's EI spectra showed the similar fragmentation pattern in the lower molecular mass level and the pattern resembles with that of compounds 2,6,10-trimethyl dodecane, 9-n-octylheptadecane, 7-n-hexyleicosane, 24-s-stigmast-5-en-3-β-ol and 6-formyl-3-methyl-2-oxo-4-hexanoic acid.

Analysis of n-hexane extract (III): When methanol was used as an eluent GC (EI) mass spectrum of n-hexane extract revealed the presence of four peaks with four different retention time. (Table-1). FAB mass spectrum showed several fragmented peaks with appreciable intensities. (Table-2) The spectrum clearly revealed a huge molecular mass with m/z 1942.1 . It's EI spectra shows similar fragmentation in the lower molecular mass level and the pattern resembles with that of compounds 4-octadecanoic acid, 9,12-octadecadienoic acid, 1-octadecanoic acid and 9,12,15-octadecatrienoic acid methylester. So the EI spectral fragments with low m/z may be possible fragments from the huge molecular mass compound.

Analysis of chloroform extract

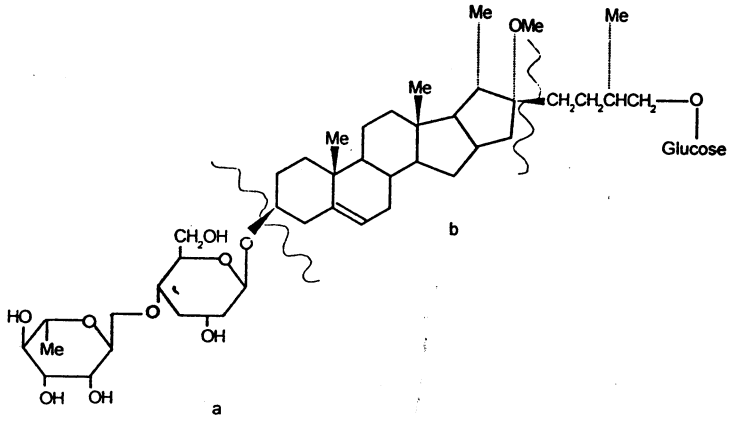
On elution of chloroform extract using benzene and benzene—pet ether (1 : 1), no residues were obtained. But residue was obtained upon elution with methanol. It's GC (EI) mass spectrum revealed the presence of four peaks with four different retention times. (Table-1) FAB mass spectrum resembled with that of benzene-pet ether (1 : 1) eluted n-hexane extract (Table-2). Also the EI spectra showed similar fragmentation pattern in the lower molecular level and the pattern resembles with that of compounds pentadecanol, 7-N-hexyleicosane, tetracosanol and 9,12,15-octatrienoic acid 2,3-dihydroxy propylester.

Analysis of methanol extract

As in the case of chloroform extract, here also no residues were obtained upon elution with benzene and benzene-pet ether (1 : 1). But residue was obtained when eluted with methanol. The FAB mass spectrum clearly revealed similar fragmentation with that got for n-hexane extract eluted with methanol. (Table 2) Also the EI spectra of methanol extract showed the similar fragmentation pattern in the lower molecular mass level and the pattern resembled with that of octadecanoic acid. (Table-1).

In all the cases, none of EI-mass spectrum gave molecular ion peak (M+) but

FAB mass spectrum gave M+. But EI spectra was helpful for finding low m/z fragmentation pattern; In all, the mass spectral pattern obtained for all, gave us the closest resemblence with the pattern of a yamogenin glycoside (Structure-1). More over from the EI mass spectra of n-hexane extract, it seems a possible fragmentation at points a and b in structure I with exceptionally low m/z region and resembles with that of EI mass spectra of hexane extract with GC retention time of 3.00 sec. Hence all the mass spectra obtained attributes to the structure-1 which has not been supplemented with mass spectral studies earlier.



Structure 1

EXPERIMENTAL

All the solvents used were of spectro grade. Silica gel of 60–120 mesh and 100–200 mesh were used for column-chromatography and TLC respectively. Mass spectra were recorded in a V.G. analytical 7070E instrument equiped with VG11-250 data acquistion system. Based on the polarity of the solvents, extraction was done in the order of n-hexane, chloroform and methanol.

n-Hexane extraction:

For 20 g of powdered fenugreek seeds, 400 mL of n-hexane was taken. First 50 mL of n-hexane was poured into the Round bottomed flask. Then 20 g of powdered sample was packed air tightly in the soxhlet apparatus. Through the top of the apparatus first 150 mL of n-hexane was added slowly until all the material became wet. Care was taken that the level of solvent in the capillary tube should be equal to the stuffed powder. Then the round bottomed flask fitted with soxhlet apparatus was heated using heating mantle keeping the temperature between 50–55°C for the first 15 min. The temperature was reduced to 30°C, after the solvent in the round bottomed flask gets bubbled. This was refluxed for three days. Each day a subsequent 50 mL of n-hexane was added and refluxed. Similar procedure was adopted for chloroform and methanol extraction also.

TABLE-1
GC (EI) MASS SPECTRAL DATA OF EXTRACTS

No. of peaks	Retention Time (sec)	m/z (intensity)	Probable Compound
		n-Hexane extract II using benzene as an eluent	
5.	0.300	57(100) 43 (88), 71 (56), 85 (32), 97(24), 124(8), 140(4), 164(1), 181(1),195(1).	2,6,10-Trimethyl dodecane
	0.833	57(100), 43(76) 71(68), 96(56), 110(36), 124(20),139(16), 153(12), 169(4), 183(4), 223(4), 210(4), 323(4), 337(2)	9-N-octylhepta, decane and 7-N-Hexyleicosane
	1.183	57(100), 43(68), 71(68), 85(44), 110(24), 124(20), 139(16), 153(12), 169(4), 183(4), 223(4), 210(4), 323(4), 337(2),	7-N-Hexyleicosane
	2.275	43(100), 54(92), 69(56), 95(32), 134(16), 121(12), 147(8), 161(4), 175(4), 262(4), 396(2)	24-s-stigmast-5-en-3-β-ol
	5.408	43(100), 53(80), 69(56), 83(40), 97(32), 111(16), 135(12), 159(4)	6-formyl-3-methyl-2-oxo-4-hexenoic acid.
		n-Hexane extract III using methanol as an eluent	
4.	0.482–0.749	43(100), 57(72), 69(48), 95(32), 109(20), 123(8),	9-octa decanoic acid
	0.982–1.221	54(100), 67(64), 81(48), 95(60), 109(36), 122(12), 136(8), 149(4), 280(4)	9,12-octa decadienoic acid
	2.017	43(100), 57(60), 71 (32), 94(32), 121 (24), 139(8), 158(4), 262(4),	1-octadecanoic acid
	4.073–5.674	81(100), 67(92), 95(68), 108(44), 43(40), 121(24), 135(12), 149(8), 262(4),	9,12,50-octadecatrienoic acid methyl ester
		Chloroform extract using methanol as an eluent	
4.	0.083	57(100), 43(92), 82(56), 96(44), 110(20), 124(12), 135(12), 149(10), 166(4)	Pentadecanol
	1.158	57(100), 43(84), 71 (64), 96(44), 110(28), 149(24), 124(14), 155(4), 181(4), 195(5),279(4)	7-N-Hexyleicosane
	2.058	43(100), 55(76), 69(48), 83(48), 97(28), 111(16), 125(4), 139(4), 336(4)	teteracosanol
	4.566–5.388	67(100), 83(84), 108(52), 43(28), 121(20), 149(8)	9,12,15 octadeca trienoic acid, 2,3-dihydroxy propyl ester.
		Methanol extract using methanol as an eluent	
	1.181–1.809	43(100), 55(80), 69(44), 97(32), 83(28), 111(8), 129(8), 149(4), 171 (4), 183(4), 262(4), 313(4).	1-octadecanoic acid

TABLE-2
FAB-MASS SPECTRAL DATA OF EXTRACTS

Extract	Eluent	m/z (intensity)
1. n-hexane	benzene-petether 1 : 1	106.5(95.8), 124.3(73.0), 151.9(54.4), 184.5(52.6), 257.3(52.2) 279.3(70.7), 313.6(47.8), 339.4 (42.9), 355.6(64.8), 467.6(34.5), 493.5(19.8), 507.3(21.2), 577.5(47.7), 601.4(26.1), 715.8(13.8), 762.0(11.3), 804.9(12.2), 855.8(10.0), 887.2(11.2), 967.7(10.7),
2. n-hexane	benzene	107.10(100.0), 204.70(55.3), 244.10(36.8), 315.40(25.9), 397.40(51.5), 454.40(14.6), 507.10(10.8), 576.50(10.5), 640.50(11.3), 723.50(9.90), 787.10(8.60), 867.50(6.90), 982.40(7.00), 1045.2(7.20), 1147.7(10.5), 1556.3(5.90), 1728 (6.10), 1888.4(4.5),
3. n-hexane	methanol	101.2(98.0), 202.8(54.9), 307.3(100), 391.1(33.9), 438.4(10.9), 577.4(9.00), 639.6(6.70), 798.3(7.80), 826.5(5.60), 947.7(6.40),1070.4(6.60), 1155.1 (5.70), 1208.5(5.80), 1270.8(5.30), 1329(5.90),1414.5 (6.10), 1727(5.90), 1896.4 (5.40), 1942.1 (5.00),
4. chloroform	methanol	170.1(100), 196.7(15.8), 263.3(22.6), 337.2(24.5), 431.3(9.70), 522.2(8.90), 597.5(18.4), 647.4(13.2), 705.8(18.1), 786.9(8.40), 832.1 (5.70)
5. methanol	methanol	123.9(66.3), 167.3(50.1), 206.1(100), 263.2(50.1), 313.4(27.0),337.2(65.2), 391.1(24.3), 431.3(20.4), 475.2(17.1), 515.1(15.8), 537.5(12.5), 575.6(36.4), 634.6(16.1), 669.2(12.1), 695.5(11.7), 749.9(12.6), 790.8(12.4), 842.3(14.9), 895.5(13.8), 914.0(12.5), 969.4(14.5), 1023.4(10.3), 1079.4(8.70), 1081.4(5.20), 1149.2(11.0), 1179.3(10.8), 1214.3(8.60), 1348.4(8.60), 1384.4(11.4), 1422.4(8.80), 1483.8(6.90).

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