

Foeniculum vulgare Mill.: Chemical composition, *in vitro* anti-diabetic and anti-inflammatory assessment

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Fennel is a culinary spice of the Apiaceae family and is traditionally used as medicine for a wide range of ailments accompanied by digestive, respiratory, gastrointestinal and hormonal imbalance systems. Fennel has an anti-inflammation property to relieve normal cough/cold and abdominal pain. This research was assessed to analyse the chemical composition and biological activities in hexane and methanol extract of fennel seeds collected from the Terai region in the Uttarakhand state. Chemical composition was examined in polar (methanol) and non-polar (hexane) extracts of fennel seeds using GC-MS technique. An *in vitro* anti-diabetic and anti-inflammatory assay was observed in fennel extract. A statistical approach was done by cluster analysis in fennel seeds. D-arabitol (29.51%), Octadecadienoic acid (25.80%), Methyl pyrrolidine (23.41%), Guanosine (15.73%), Xanthine riboside (16.10%) were major components identified in methanol extract of fennel. Octadecenoic acid, palmitic acid, oleamide, trimesic acid, and linoleic acid were observed to major in the range of 79.43, 12.61, 32.76, 17.66, and 35.18 in the hexane extract of fennel, respectively. The highest α -amylase and α -glucosidase inhibition potential were observed in the hexane extract of FNL-125 ($IC_{50}=227.77\pm 0.64$ $\mu\text{g/mL}$) and FNL-120 ($IC_{50}=230.02\pm 0.62$ $\mu\text{g/mL}$), respectively. Hexane extract of FNL-125 ($IB_{50}=676.44\pm 0.96$ $\mu\text{g/mL}$) exhibits superior anti-inflammatory activity. These fennel seeds have effective biological activities in hexane extract, suggesting their use for drug purposes. A statistical approach was undertaken with cluster analysis in fennel seeds that could be used to develop a new variety of fennel that possesses prominent chemical constituents with effective pharmacological activities and to be used in pharma industries.

Keywords: Anti-diabetic, Anti-inflammatory, Apiaceae, Fennel, *Foeniculum vulgare* Mill., GC-MS

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Introduction

Due to numerous advances in medicine and nutrition, the plant plays a vital role in human health and has been studied since ancient times. Awareness of the benefits of plants in food as rich additives leads researchers to discover how these ingredients influence human health^{1,2}. Medicinal plants are used to prepare various drugs individually or in combination or as the key source of raw material for other medicines³. Some spices are rich in phenol content and exhibit effective antioxidant and anti-diabetic activity. Alkaloids, phenols, amino acids and fatty acids are natural phytochemicals biologically estimated for their medicinal activity⁴. The anti-diabetic activity was determined by per cent inhibition of the transport of glucose across yeast cells⁵. Inflammation is a natural

defensive response for inactivating or killing the invading species, eliminating the irritants and setting the stage for tissue repair. Natural products have made a significant contribution to the development of modern medicine. The rich resources of the plant kingdom can be a new source of new compounds with essential anti-inflammatory activities⁶. Bioactive components isolated from spices can potentially control the growth of food-borne bacteria⁷. The medicinal value of a plant is due to the presence of certain special substances such as alkaloids, resins, glycosides, oils, gums, and tannins. The active concepts usually remain concentrated in the root cells of the plants⁸. *Foeniculum vulgare* Mill. commonly known as fennel, is a small, erect, aroma herb of the Apiaceae genus. Fennel fruits are one of the leading plant-based natural spice⁹. Researchers have recorded a variety of chemical components and various therapeutic impacts of this fennel herb¹⁰. *F. vulgare* herbs have numerous culinary and traditional medicinal benefits. Capillaceum and Piperitum are two important

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subspecies of this plant, which are the key constituents of essential oil, fatty acids, phenolic compounds, etc¹¹. The fennel plant was endemic to Mediterranean and Southern European regions but is now widely spread in the world's temperate and tropical regions and is therefore widely grown. It is also a very economical and common medicinal herb in China¹². Fennel seed is a potent source of natural antioxidant¹³. Fennel has become widespread in many regions of the world and is used as a medicinal and culinary herb¹⁴. Higher phytochemicals were found in methanol extract of fennel seeds¹⁵. Fennel seed contains about 2-4% essential oil¹⁶. Essential oil composition varies based on the environment, season and taxonomy of plant material¹⁷. Phenylpropanoids are the major component of fennel, which includes α - phellandrene, fenchone, and α - pinene¹⁸⁻²⁰. Fennel seed tends to be used for antimicrobial, anti-inflammatory and antispasmodic activity²¹. The antispasmodic potential of fennel is used to heal respiratory disorders and pediatric colic^{22,23}. Fennel seed contains a rich amount of flavonoids and glycosides used for drug purpose²⁴. In the current study, we examined the chemical composition and pharmacological assessment of hexane and methanol extract of fennel seeds collected from the Terai region in Uttarakhand, India. The research results can help provide information about these fennel varieties and their future research applications.

Materials and Methods

Collection and extraction of plant material

Fennel seeds were collected from the Vegetable Research Centre (VRC) GBPUA&T Pantnagar in May 2019 for experimental analysis. These varieties were developed in the Pantnagar Terai region, which lies at a Latitude of 29° N, Longitude of 79.380° E and an Altitude of 243.84 MSL viz. FNL -119, FNL-120, FNL-123, FNL-125, FNL-126 and FNL-PM. Fennel seeds were shade-dried in the laboratory and ground to fine powder using a mixture grinder. A 10 g powder sample of fennel was taken and mixed in the ratio 1:5 with 50 mL of hexane and methanol solvent, respectively and left for 72 h with occasional stirring. The mixture was then centrifuged at 4000 rpm for 10 min. The supernatant was carefully collected and concentrated using a vacuum rotatory evaporator at 40°C and stored in a deep freezer for further analysis.

Chemical composition in fennel seeds

Phytochemical components present in hexane and methanol extract of fennel were determined by

GC-MS instrumentation at Advanced Instrumental Research Facility, Jawaharlal Nehru University (JNU) New Delhi. GC-MS analysis was performed using gas chromatograph HP 6890 with a mass selective detector MS 5973 fitted with HP-5 MS fused silica column. The carrier gas was He, with a column flow rate of 1.21 mL/min and a linear velocity of 40.1 cm/sec. A 1 μ L sample was injected with a split ratio of 50.0. Column oven and injection temperatures were maintained at 60.0 and 260.0°C, respectively. The pressure of 76.7 kPa was maintained, the total flow rate was 64.7 mL/min, and the purge flow rate was 3.0 mL/min. The oven temperature was programmed at 60°C to 210°C for 3 min with final isothermal status of 10 min. The chemical constituents of hexane and methanol extract were recognised by comparing their mass spectra with those in NIST-MS, FFNSC Wiley Library. The resolution and peak purity were evaluated for each analyte peak from the nearest eluting peak Adams, 2007²⁵.

Anti-diabetic activity

The present study evaluated the potential inhibitory evidence for fennel extracts on α -amylase and α -glucosidase enzymes as per Kidane *et al.*²⁶.

α -amylase enzyme inhibitory activity

Firstly, the extract was placed in the test tube with five concentrations (200, 400, 600, 800, 1000 μ g/mL) and 0.02 M sodium phosphate buffer solution containing the α -amylase enzyme solution was added. This reaction mixture was pre-incubated at 25°C for 10 min. A starch solution of 1% was added in 0.02 M sodium phosphate buffer and incubated for 10 min at 25°C. For the termination of the reaction, 2 mL of DNS reagent (40 mM) was added, followed by incubation for 5 min in a water bath and then cooled at room temperature. At last, the reaction mixture was diluted with distilled water. Absorbance was recorded at 540 nm against the reagent blank. Acarbose and voglibose were used as standard. α -amylase inhibitory activity was calculated in terms of % inhibition = $[(A-B)/A] \times 100$ where, A= Absorbance of control B=Absorbance of sample. IC₅₀ values were calculated for all the extracts of *F. vulgare* by plotting a straight line between % inhibition and concentration.

α -glucosidase enzyme inhibitory activity

This procedure was initiated by incubating maltose substrate with tris buffer (pH 8.0) and five extract concentrations (200-1000 μ g/mL) at 35°C. To this, added α -glucosidase enzyme into the above reaction

mixture to initiate the reaction. DNS colour reagent was added at the termination of the reaction. Absorbance was noted at 540 nm. α -glucosidase inhibitory activity was calculated in terms of % inhibition = $[(A-B)/A] \times 100$ where, A= Absorbance of control B= Absorbance of sample. IC₅₀ values were calculated by plotting a straight line between % inhibition and concentration.

Anti-inflammatory activity

Anti-inflammatory activity in fennel seeds was performed as per Banerjee *et al.*²⁷. Egg albumin of 0.2 and 2.8 mL of phosphate buffer (pH 6.4) are added to the 2 mL sample extract of five different concentrations (200, 400, 600, 800, 1000 μ g/mL). The above reaction mixture was incubated for 15 min in BOD at 37°C. After incubation, the mixture was heated for 5 min at 70°C and kept aside for cooling. Take the absorbance at 660 nm against the reagent blank. Diclofenac was used as a standard for the standard curve. Anti-inflammatory activity or protein denaturation was calculated by % inhibition = $[(A-B)/A] \times 100$, where A= Absorbance of control B=

Absorbance of sample. IB₅₀ values were calculated by plotting a straight line between % inhibition and concentration.

Statistical analysis

All the experiments were conducted in triplicates, and results were analysed as Mean \pm SD (standard deviation). Cluster analysis was observed using SPSS-20 software applying Ward's method with Squared Euclidean distance.

Results and discussion

Chemical composition in fennel seeds extract

GC-MS technique analysed the comparative evaluation of various phytoconstituents present in hexane and methanol extract of fennel seeds. The phytoconstituents in fennel seeds were recognised by comparing their mass spectra with those in NIST-MS, Wiley Library. Chemical compositions in hexane and methanol extract of fennel seeds are presented in Tables 1 and 2, respectively.

GC-MS identified 106 constituents in the hexane extract and 125 components in the methanol extract of

Table 1 — % Composition in hexane extract of fennel seeds

S. No.	Compound name	K.I.	% Composition in hexane extract of fennel seeds					
			FNL-119	FNL-120	FNL-123	FNL-125	FNL-126	FNL-PM
1	Tetradecane	1399	1.88	-	4.32	5.36	-	-
2	Heptylhexanoate	1459	2.76	-	-	-	-	-
3	Heptadecane	1700	3.58	3.83	-	-	0.63	-
4	β -Caryophyllene	1418	0.68	-	-	0.32	-	-
5	Dodecane	1199	2.90	-	-	-	-	-
6	Butan-2-one	-	1.89	-	-	-	-	-
7	Lauryl chloride	1446	0.65	-	-	-	-	-
8	Nonadecane	1900	0.91	-	-	-	-	-
9	Hept-2-ene	729	2.30	-	-	-	-	-
10	Pentadecanoic acid	1820	4.54	6.77	2.70	-	-	-
11	Stearic acid	2124	2.29	29.10	-	-	-	-
12	Phthalic acid	1917	0.62	-	-	0.28	-	-
13	13-Hexyloxacyclotridec-10-en-2-one	-	1.97	-	-	-	-	-
14	Linoleic acid	2152	4.67	3.65	4.45	2.30	3.84	-
15	Palmitoleic acid	1953	32.76	-	-	-	-	-
16	Methyl stearate	2088	1.63	1.37	1.87	-	2.57	-
17	Hexadecadienoic acid	-	1.07	-	5.04	-	3.84	-
18	Palmitic acid	2046	4.28	3.78	5.04	9.97	13.46	4.27
19	Methyl eicosenate	2339	0.82	-	-	-	-	-
20	Cyclohexane	677	0.50	-	-	-	-	-
21	Oleamide	2375	7.92	1.54	-	12.61	-	-
22	4-acetamido-1-hexanol	-	1.69	-	-	-	-	-
23	1,2-dideuterioethane	-	0.24	-	-	-	-	-
24	<i>cis</i> -9-hexadecenal	1759	1.02	-	-	-	-	-
25	Ethyl oleate	2179	0.22	-	-	-	-	-

(Contd.)

Table 1 — % Composition in hexane extract of fennel seeds

S. No.	Compound name	K.I.	% Composition in hexane extract of fennel seeds					
			FNL-120	FNL-123	FNL-125	FNL-126	FNL-PM	FNL-119
26	Diisooctyl phthalate	2519	0.60	-	-	-	-	-
27	Tetradecenyl acetate	1789	2.33	-	-	-	-	-
28	Oleic acid	2144	2.12	-	35.18	-	-	-
29	Stigmasterol	3213	2.97	1.63	-	-	-	0.64
30	β -sitosterol	3202	1.88	-	1.96	-	-	-
31	Tetradecyl cyanide	-	2.73	-	-	-	-	-
32	Hydroxy cineole	1716	-	1.03	-	-	-	-
33	2,6-Nonadienoic acid	-	-	2.64	-	-	-	-
34	Limonene-1,2-diol	1321	-	1.56	-	-	-	-
35	2,6-dimethyl-4-heptanone	962	-	1.27	-	-	-	-
36	Eicosapentaenoic acid	2339	-	2.25	-	-	-	-
37	3',5'-Dimethoxyacetophenone	-	-	3.17	-	-	-	-
38	4-(<i>p</i> -Methoxyphenyl)-1-butanol	917	-	2.16	-	-	-	-
39	Limonene dioxide	1294	-	2.66	-	-	-	-
40	benzyl alcohol	1113	-	1.43	-	-	-	-
41	Hexadecanol	1879	-	1.91	3.82	-	-	-
42	Benzenepropanoic acid	1324	-	2.07	-	-	0.71	-
43	Isopropyl palmitate	2011	-	2.67	6.06	-	-	-
44	13-Hexyloxacyclotridec-10-en-2-one	-	-	1.54	-	-	-	-
45	Hexadecanal	1794	-	2.34	-	-	-	-
46	Pentadecanoic acid	1866	-	2.05	-	-	-	-
47	Squalene	2663	-	0.60	1.06	-	-	-
48	6-octadecenoic acid	2188	-	0.67	2.63	-	7.46	-
49	Nonacosane	475	-	1.42	-	-	-	-
50	Methyl petroselinate	2104	-	1.77	-	-	-	-
51	Nonacosane-10-one	3089	-	1.76	1.12	-	-	1.36
52	3-decyn-2-ol	991	-	-	1.20	-	-	-
53	Vinyl cyclohexane	813	-	-	0.48	-	-	-
54	<i>E</i> -8-Hydroxylinalool	1338	-	-	1.14	-	-	-
55	Pinanol	1103	-	-	0.64	-	-	-
56	α - <i>trans</i> Bergamotene	1434	-	-	0.43	-	-	-
57	Diacetylacetone	1554	-	-	0.94	-	-	-
58	Eicosapentaenoic acid	2339	-	-	2.58	-	-	-
59	Hexadecane	1600	-	-	1.88	0.50	1.52	-
60	Phenyl acetic acid	1236	-	-	0.98	-	-	-
61	Heptenyl acetate	1427	-	-	1.79	-	-	-
62	Benzoic acid	1313	-	-	2.11	-	-	-
63	Methyl palmitate	1926	-	-	2.06	-	-	-
64	Isopropyl palmitate	2012	-	-	6.06	-	-	-
65	Undecylenoyl chloride	-	-	-	2.01	-	-	-
66	Cyclopentadecanone	1676	-	-	2.34	-	-	-
67	Hexadecanolide	1842	-	-	2.20	-	-	-
68	Cithrol A	-	-	-	1.17	-	-	-
69	Calarene epoxide	1592	-	-	1.88	-	-	-
70	Dodecane	1200	-	-	-	1.45	0.73	-
71	Decanol	1271	-	-	-	3.41	3.41	-
72	Lactic acid	2159	-	-	-	1.24	-	-
73	Dodecenyne	-	-	-	-	1.54	-	-

(Contd.)

Table 1 — % Composition in hexane extract of fennel seeds

S. No.	Compound name	K.I.	% Composition in hexane extract of fennel seeds					
			FNL-120	FNL-123	FNL-125	FNL-126	FNL-PM	FNL-119
74	Methoxyacetic acid	-	-	-	-	1.18	-	-
75	Resorcinol monoacetate	1382	-	-	-	0.99	-	-
76	Epoxy cyclopentanol	-	-	-	-	3.07	-	-
77	Hydroxyl benzoic acid	1558	-	-	-	1.37	-	-
78	<i>L</i> -Proline	3240	-	-	-	2.82	-	-
79	13-Hexyloxacyclotridec-10-en-2-one	2071	-	-	-	2.00	-	-
80	Octadecenoic acid	2188	-	-	-	4.44	22.83	79.43
81	Hexadecanamide	2150	-	-	-	8.03	-	-
82	Tridecanone	1494	-	-	-	0.92	-	-
83	Hydroxypentadecanoic acid	-	-	-	-	4.59	-	-
84	Oleoyl chloride	2241	-	-	-	0.65	-	-
85	Octadecenamide	2375	-	-	-	6.75	6.20	-
86	Pentatriacontane	3500	-	-	-	1.40	-	-
88	Dihydroxypropylelaidate	-	-	-	-	0.32	-	-
89	Octanoic acid	1191	-	-	-	0.33	-	-
90	Trimesic acid	1892	-	-	-	17.66	12.76	-
91	2-decene	1003	-	-	-	-	1.01	-
92	Oxacycloheptadec-8-en-2-one	1925	-	-	-	-	2.24	-
93	Myristic amide	1921	-	-	-	-	4.84	-
94	9-Octadecenoic acid	2085	-	-	-	-	3.45	-
95	9,12-Octadecadienoic acid	2092	-	-	-	-	3.83	6.06
96	Glycidylolate	-	-	-	-	-	0.72	-
97	Trimellitic acid	-	-	-	-	-	6.23	-
98	Oxalic acid	748	-	-	-	-	2.82	-
99	Tetradecenal	1619	-	-	-	-	-	0.42
100	Ethyl Oleate	2179	-	-	-	-	-	1.02
101	9-Octadecenamide	2228	-	-	-	-	-	0.27
102	Palmitaldehyde	1795	-	-	-	-	-	0.66
103	Cyclohexanoneoxime	1074	-	-	-	-	-	1.47
104	9-Octadecenal	2137	-	-	-	-	-	0.65
105	6-Ethyl-3-decanol	1312	-	-	-	-	-	0.50
106	Butylphosphonic acid	-	-	-	-	-	-	1.89

Table 2 — % Composition in methanol extract of fennel seeds

S. No.	Compound name	K.I.	% Composition in methanol extract of fennel seeds					
			FNL-119	FNL-120	FNL-123	FNL-125	FNL-126	FNL-PM
1	<i>d</i> -arabitol	1766	29.51	26.15	10.59	9.54	15.97	-
2	Dodecane	1268	0.87	-	-	-	1.77	-
3	Hexadecanoic acid	2000	4.44	2.54	2.08	2.45	2.96	4.20
4	Guanosine	3067	15.73	-	-	-	6.27	8.09
5	3,4-Dimethoxyacetophenone	1515	1.82	4.80	5.11	3.48	-	1.99
6	Octadecane	1800	0.69	-	-	-	1.30	1.05
7	Megastigmatrienone	1696	0.25	0.32	-	-	-	-
8	(<i>E</i>)-4-Propenylsyringol	1652	2.15	-	-	-	-	-
9	2-Cyclohexenol	1244	0.37	0.55	-	-	-	-
10	<i>trans</i> -sinapyl alcohol	2034	0.96	1.33	1.50	-	-	-
11	Oxacyclododecenone	-	0.64	-	-	-	-	-

(Contd.)

Table 2 — % Composition in methanol extract of fennel seeds

S. No.	Compound name	K.I.	% Composition in methanol extract of fennel seeds					
			FNL-119	FNL-120	FNL-123	FNL-125	FNL-126	FNL-PM
12	9,12-Octadecadienoic acid	2134	25.80	3.20	2.97	-	-	-
13	Methyl stearate	2128	0.89	0.68	0.60	0.76	-	1.15
14	(Z)-9-Octadecenamide	2375	1.43	0.41	0.28	1.52	-	-
15	Floctafenine	3132	0.39	-	-	-	-	-
16	Undecenal	1308	3.15	-	2.34	-	-	-
17	Glycidylpalmitate	-	0.84	-	-	-	-	-
18	Eicosanoic acid	2339	0.32	-	-	-	-	-
19	Oleoyl chloride	2241	1.48	-	-	-	-	-
20	Caprylic acid	1179	4.19	3.79	-	5.58	5.55	1.58
21	Octadecenal	2137	0.92	-	-	-	-	-
22	Tetradecenyl acetate	1789	0.94	-	-	-	-	-
23	Nonacosanone	454	0.76	-	1.54	-	-	-
24	Stigmasterol	3170	0.44	1.62	1.32	3.91	4.31	-
25	Decane	1015	-	1.26	1.83	-	-	-
26	1,3-Propanediol	1785	-	23.93	-	-	-	-
27	Undecane	183	-	1.30	-	-	-	-
28	Tetramethylchromanolglucoside	-	-	0.29	-	-	-	-
29	2,6-Dimethoxyphenol	2269	-	0.98	-	-	-	-
30	Cyclopentafuranone	1060	-	1.00	-	-	-	-
31	2-Cyclohexenol	890	-	0.55	-	-	-	-
32	Phenylpropanoic acid	1324	-	0.30	-	0.52	0.49	0.70
33	13-Hexyloxacyclotridec-10-en-2-one	2071	-	0.29	-	-	-	-
34	Octadecenoic acid	2188	-	15.47	2.01	-	-	-
35	9,12-Octadecadien-1-ol	2052	-	0.31	2.01	-	-	-
36	Ethyl Oleate	2445	-	0.33	-	0.71	0.50	1.10
37	Graveolone	-	-	0.69	1.27	-	-	-
38	Hexadecanal	1811	-	2.35	-	-	-	-
39	Hydroxypentadecanoic acid	2111	-	0.60	-	-	-	-
40	Undecandioic acid	-	-	0.32	-	-	-	-
41	Triclosan	2114	-	0.21	-	-	-	-
42	Octadecenol	2060	-	0.82	-	-	-	-
43	Nonacosan-10-one	3089	-	0.44	-	6.98	9.10	6.82
44	Oxiranedodecanoic acid	-	-	0.70	-	-	-	-
45	<i>p</i> -vinyl guaiaicol	1284	-	-	1.14	-	-	-
46	Hexadecane	1612	-	-	0.41	0.62	-	-
47	Epoxy-linalooloxide	1468	-	-	1.49	-	-	-
48	Methyl pyrrolidine	1646	-	-	23.41	-	-	-
49	Eicosatrienoic acid	2324	-	-	2.72	-	-	-
50	Cyclohexanone	1282	-	-	6.91	-	-	-
51	<i>o</i> -Methylphenylbutanol	-	-	-	3.27	-	-	-
52	Allylsyringol	2543	-	-	3.04	-	1.10	-
53	6-Octadecenoic acid	-	-	-	15.68	20.74	22.55	46.78
54	9,12-Octadecadienoate	2092	-	-	0.30	-	-	-
55	Ethyl octadecenoate	2193	-	-	0.89	-	-	-
56	2-Undecenal	1364	-	-	2.34	2.76	-	-
57	Eicosanedioic acid	2543	-	-	0.51	-	-	-
58	Oleic acid	2144	-	-	1.16	-	-	0.40
59	1,5-Bis (methylamino)-3-oxapentane	-	-	-	4.37	-	-	-

(Contd.)

Table 2 — % Composition in methanol extract of fennel seeds

S. No.	Compound name	K.I.	% Composition in methanol extract of fennel seeds					
			FNL-119	FNL-120	FNL-123	FNL-125	FNL-126	FNL-PM
60	Butylphosphonic acid	-	-	-	4.21	-	-	2.44
61	17-Pentatriacontene	-	-	-	0.95	-	-	-
62	Thunbergol	2073	-	-	0.71	1.76	1.93	-
63	Dodecane	202	-	-	-	0.50	-	-
64	Tetradecane	236	-	-	-	1.09	-	1.28
65	Xanthine riboside	2713	-	-	-	16.10	-	-
66	6,9,12-Octadecatrienol	2077	-	-	-	1.09	-	-
67	α -Santalol	1671	-	-	-	1.12	-	-
68	(<i>E</i>)-Isoferulic acid	1767	-	-	-	1.83	-	-
69	<i>Cis</i> - β -santalol	1713	-	-	-	0.44	-	-
70	4-Piperidinol	1053	-	-	-	0.72	-	-
71	1,2-benzenedicarboxylic acid	1868	-	-	-	0.60	-	-
72	9,12-Octadecadienoic acid	2092	-	-	-	3.84	-	7.17
73	Tetrahydro-6-tridecyl-	2196	-	-	-	0.64	-	-
75	15-Hydroxypentadecanoic acid	2111	-	-	-	0.79	-	-
76	Palmitoyl chloride	1924	-	-	-	0.79	-	-
77	4,8,12,16-Tetramethylheptadecan-4-olide	2364	-	-	-	0.38	-	-
78	9-Octadecenamide	2397	-	-	-	0.47	-	0.78
79	9-Octadecenol	2358	-	-	-	1.63	1.95	-
80	<i>bis</i> -(2-ethylhexyl) phthalate	2519	-	-	-	0.43	-	-
81	Tetracosahexane	-	-	-	-	0.64	-	-
82	Behenic alcohol	2456	-	-	-	0.55	-	-
83	Benzopyrene	-	-	-	-	1.24	-	-
84	β -pregnane	-	-	-	-	1.72	-	-
85	γ -sitosterol	3790	-	-	-	1.85	-	-
86	Tridecane	1299	-	-	-	-	0.38	-
87	3- <i>tert</i> -Butyl-4-hydroxyanisole	1513	-	-	-	-	2.28	-
88	2-phenylacetic acid	1236	-	-	-	-	0.15	-
89	2-Pentadecanone	1698	-	-	-	-	0.49	-
90	Palmitic acid	2000	-	-	-	-	2.96	-
91	Octacyclododecenone	-	-	-	-	-	0.58	-
92	Phosphonic acid	1142	-	-	-	-	0.13	-
93	Tridecanedial	1690	-	-	-	-	0.61	-
94	<i>trans</i> -anethole	1190	-	-	-	-	0.33	-
95	Cyclopentadecanone	1676	-	-	-	-	3.39	-
96	9-Octadecenoic acid	2103	-	-	-	-	1.02	-
97	2-Nonadecanone	2101	-	-	-	-	0.19	-
98	Cyclooctanone	929	-	-	-	-	0.97	-
99	Octadecanoic acid	2161	-	-	-	-	0.53	-
100	Methyl undecenolide	-	-	-	-	-	0.28	-
101	Cyclohexane	665	-	-	-	-	0.57	-
102	Di- <i>n</i> -octyl phthalate	2741	-	-	-	-	0.47	-
103	<i>trans</i> -9-Octadecenoic acid	2109	-	-	-	-	0.31	-
104	Squalene	2663	-	-	-	-	0.48	-
105	1-eicosanol	2282	-	-	-	-	0.62	-
106	Succinic acid	1132	-	-	-	-	1.04	-
107	Benzopyranol	-	-	-	-	-	1.35	-

(Contd.)

Table 2 — % Composition in methanol extract of fennel seeds

S. No.	Compound name	K.I.	% Composition in methanol extract of fennel seeds					
			FNL-119	FNL-120	FNL-123	FNL-125	FNL-126	FNL-PM
108	10-Nonadecanone	2082	-	-	-	-	1.42	-
109	Fucosterol	3315	-	-	-	-	1.46	-
110	Nonadecatriene	1934	-	-	-	-	-	0.63
111	2-methoxyphenol	1086	-	-	-	-	-	1.27
112	cyclooct-2-en	904	-	-	-	-	-	0.35
113	7-Hexadecenoic acid	1976	-	-	-	-	-	0.42
114	Hexadecanoic acid	2923	-	-	-	-	-	4.20
115	Methyl undecanal	1644	-	-	-	-	-	1.89
116	Methyl-12-oxo-9-dodecenoate	1800	-	-	-	-	-	0.69
117	Oleic Acid	2144	-	-	-	-	-	-
118	cyclopentadecanone	1851	-	-	-	-	-	0.93
119	Oxacyclohexadecan-2-one	2242	-	-	-	-	-	0.81
120	Hexadecenoic acid	2223	-	-	-	-	-	0.73
121	9-octadecenamamide	2375	-	-	-	-	-	0.78
122	Heptadecene	1700	-	-	-	-	-	0.59
123	Stigmasta-5,22-dien-3-ol	3138	-	-	-	-	-	3.10
124	10-Nonadecanone	2082	-	-	-	-	-	1.24
125	β -sitosterol	3407	-	-	-	-	-	1.20

fennel seeds. The major component in the hexane extract of FNL -119 was palmitic acid (32.76%), and in the methanol extract was Octadecadienoic acid (25.80%). Octadecenoic acid (79.43%) was noted as major in the hexane extract, and 6-Octadecenoic acid (46.78%) was observed higher in the methanol extract of FNL-PM. The common component found in the hexane extract of fennel seeds was Palmitic acid. The common components identified in the methanol extract were d-arabitol, caprylic acid, stigmaterol, and hexadecanoic acid, which have pharmaceutical applications. d-arabitol have anticarcinogenic agent. The volatile class was identified in fennel seeds, including aldehydes, esters, ketones and alcohols. Oleic acid and oleamide are the common fatty acids observed in the hexane extract of fennel seeds. Trans-anethole and phenylpropanoic acid are the phenylpropanoid class identified in the fennel seeds' methanol extract. Trans-sinapyl alcohol, being cinnamyl alcohol, is noted in methanol extract. Squalene is a polyunsaturated hydrocarbon observed in methanol extract. Graveolone belongs to the class of pyranocoumarins and is found in methanol extract of fennel seeds. Floctafenine is an NSAID drug found in fennel seeds. Previous results demonstrated that in the methanol extract of *F. vulgare*, the main compounds identified were *trans*-anethole (31.49%), 2-pentanone (25.01%), fenchone (11.68%) and benzaldehyde-4-methoxy (8.01%)²⁸. It was observed in previous results fennel seed extract has

p-allylanisole (71.40%), *d*-limonene (12.35%) and α -terpinene (5.17%)²⁹. *trans*-anethole, fenchone, limonene, fenchyl acetate, α -pinene and *trans* β -ocimene were major constituents in fennel volatile oils³⁰. A simple comparison of characterised composition in the present study revealed that fennel seeds have different qualitative and quantitative values. Hence, these fennel seeds are different genotypes, which may be due to genetic variation or climatic conditions.

Anti-diabetic activity

α -amylase and α -glucosidase enzyme inhibition activity in hexane and methanol extract is shown in Table 3.

α -amylase and α -glucosidase enzyme inhibition activity

In the hexane extract, higher α -amylase and α -glucosidase inhibition activity was observed in FNL-125 (IC_{50} =227.77 \pm 0.64 μ g/mL) and FNL-120 (IC_{50} = 230.02 \pm 0.62 μ g/mL), respectively. In methanol extract, higher α -amylase inhibition potential was observed in FNL-119 (IC_{50} = 642.19 \pm 1.14 μ g/mL) and α -glucosidase inhibition activity in FNL-123 (IC_{50} = 305.35 \pm 1.75 μ g/mL). α -amylase and α -glucosidase inhibition potential exhibited higher in the hexane extract followed by the methanol extract. In the previous study, inhibition levels of α -amylase and α -glucosidase in methanol extract were observed up to 8 h with 82.43 and 82.26% data value³¹. Previous results demonstrated that *n*-

Table 3 — α -amylase and α -glucosidase IC₅₀ value in hexane and methanol extract of fennel seeds

S. No.	Fennel	Extract	α -amylase IC ₅₀ value (μ g/mL)	α -glucosidase IC ₅₀ value (μ g/mL)
			Mean \pm SD	Mean \pm SD
1	FNL -119	Hexane	234.61 \pm 0.68 ^{bc}	284.83 \pm 0.51 ^d
		Methanol	642.19 \pm 1.14 ^c	324.53 \pm 0.65 ^d
2	FNL -120	Hexane	250.75 \pm 1.52 ^c	230.02 \pm 0.62 ^a
		Methanol	696.72 \pm 1.15 ^d	323.91 \pm 1.09 ^d
3	FNL -123	Hexane	229.02 \pm 0.89 ^a	265.45 \pm 0.50 ^c
		Methanol	737.69 \pm 1.62 ^f	305.35 \pm 1.79 ^c
4	FNL -125	Hexane	227.77 \pm 0.64 ^a	262.07 \pm 1.60 ^c
		Methanol	730.55 \pm 0.66 ^c	325.18 \pm 3.20 ^d
5	FNL -126	Hexane	239.0 \pm 0.89 ^{cd}	344.45 \pm 1.74 ^f
		Methanol	785.09 \pm 1.22 ^g	350.85 \pm 1.07 ^f
6	FNL -PM	Hexane	240.58 \pm 1.73 ^d	319.70 \pm 1.11 ^e
		Methanol	807.93 \pm 1.48 ^h	337.26 \pm 1.73 ^e
7		Acarbose*	231.56 \pm 2.04 ^a	234.52 \pm 3.14 ^a
8		Voglibose*	241.84 \pm 2.74 ^b	244.89 \pm 4.97 ^b

*= standard. Values are mean of three replicates \pm SD. Within a column, mean value followed by same alphabetic letter are significantly not different according to Tukey's test ($P < 0.005$).

Table 4 — IB₅₀ value in hexane and methanol extract of fennel seeds

S. No.	Fennel	Extract	IB ₅₀ value (μ g/mL)
			Mean \pm SD
1	FNL-119	Hexane	912.78 \pm 1.77 ^f
		Methanol	943.98 \pm 3.02 ^d
2	FNL-120	Hexane	677.95 \pm 1.56 ^b
		Methanol	704.77 \pm 1.20 ^b
3	FNL-123	Hexane	815.51 \pm 1.79 ^e
		Methanol	997.53 \pm 2.31 ^f
4	FNL-125	Hexane	676.44 \pm 0.96 ^b
		Methanol	1072.92 \pm 2.47 ^g
5	FNL-126	Hexane	760.41 \pm 3.36 ^c
		Methanol	966.77 \pm 1.66 ^e
6	FNL-PM	Hexane	791.22 \pm 1.10 ^d
		Methanol	796.46 \pm 2.75 ^c
7		Diclofenac*	431.45 \pm 4.18 ^a

*= standard. Values are mean of three replicates \pm SD. Within a column, mean value followed by same alphabetic letter are significantly not different according to Tukey's test ($P < 0.005$).

butanol, ethyl acetate, and benzene extract of *F. vulgare* seeds exhibit higher α -glucosidase inhibitory effect than the α -amylase inhibitory effect compared to the standard acarbose values³². In the previous study, the fennel leaf extract exhibits α -glucosidase inhibition was IC₅₀= 203.80 \pm 1.3 μ g/mL³³. This research shows that the hexane extract of FNL-125 and FNL-120 exhibited potent anti-diabetic activity compared to the standard acarbose and voglibose values.

Anti-inflammatory activity

IB₅₀ value for protein denaturation in hexane and methanol extract of fennel seeds is shown in Table 4. The IB₅₀ value depicted the anti-inflammatory potential assessed by its ability to inhibit denaturation.

In the hexane extract of fennel seeds, maximum anti-inflammatory activity was found in FNL-125 (IB₅₀= 676.44 \pm 0.96 μ g/mL) and minimum activity was observed in FNL-119 (IB₅₀= 912.78 \pm 1.77 μ g/mL). In the methanol extract, maximum anti-inflammatory activity was found in FNL-120 (IB₅₀= 704.77 \pm 1.20 μ g/mL), and minimum activity was observed in FNL-125 (IB₅₀= 1072.92 \pm 2.47 μ g/mL). It has been reported that oral administration (200 mg/kg) of *F. vulgare* fruit methanolic extract exhibited inhibitory properties against acute and subacute inflammatory diseases and type IV allergic reactions, revealing a central analgesic effect. The malondialdehyde MDA (as an indicator of lipid peroxidation) level considerably decreased in the methanol extract of *F. vulgare*

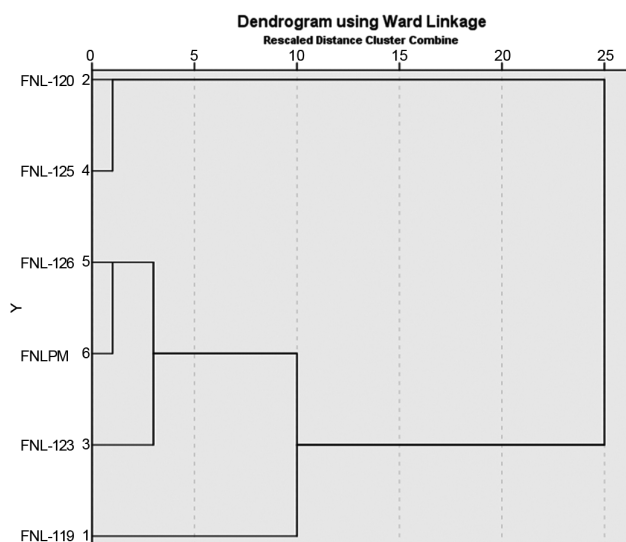


Fig. 1 — Cluster analysis in hexane extract of fennel seeds.

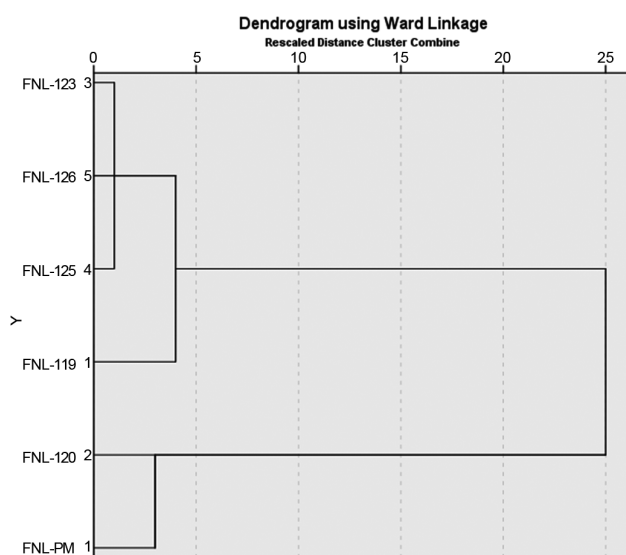


Fig. 2 — Cluster analysis in methanol extract of fennel seeds.

relative to the control group ($P < 0.05$)³⁴. In the previous study, the anti-inflammatory potential of *F. vulgare* extracts is higher for 5-lipoxygenase inhibitions³⁵. The present study reveals that anti-inflammatory activity was comparatively higher in hexane extract, followed by methanol extract.

Cluster analysis

Cluster analysis was done based on GC-MS composition, anti-diabetic activity and anti-inflammatory activity in hexane and methanol extract of fennel to demonstrate the similarities in fennel seeds grouped into three clusters, presented in their respective dendrograms in Fig. 1 and 2 as follows.

Clustering in hexane extract of fennel seeds

Group 1: FNL-119

Group 2: FNL-120, FNL-125

Group 3: FNL-123, FNL-126, FNL-PM

Clustering in methanol extract of fennel seeds

Group 1: FNL-119

Group 2: FNL-120, FNL-PM

Group 3: FNL-123, FNL-125, FNL-126

On cluster analysis, three clusters are obtained in hexane and methanol extract of fennel. This statistical approach depicts a new variety of fennel that could be developed with eminent pharma activities.

Conclusion

In this research, the chemical composition of hexane and methanol extract of fennel was characterised using GC-MS. Diterpene like thunbergol, Sesquiterpene like α -santalol, cannabinoid like β -Caryophyllene, pheromone like guaicol, lignin like sinapyl alcohol, essential oil component like α -bergamotene, protein like *L*-proline, sterol like stigmasterol are bioactive compounds observed in the fennel seeds extract. Fumaric acid, linoleic acid, pinacol, etc., are food additive agents that enhance food flavour. Our study reveals that fennel has excellent anti-diabetic and anti-inflammatory properties. Cluster analysis was done to obtain the cluster, which showed the importance of these fennel seeds. Further investigation is required to isolate the active constituents in these fennel varieties that can be used in the pharma industries for medication.

Conflict of interest

The authors declare no conflicts of interest.

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