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Preparation and GC-MS analysis of sweet mustard oil

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Abstract

The inclusion of mustard oil in the Indian diet is due to its numerous health benefits, but its nutritional value is limited by the high levels of erucic acid. To address this issue and impart a sweet flavor, coriander seeds were added to create Sweet Mustard Oil. The objective of this study was to produce Sweet Mustard Oil and identify its bioactive chemical constituents using GC-MS analysis through Trace 1300 series gas chromatography (Thermo scientific). The experimental conditions of the GC-MS system involved a run time of 48.36 minutes, sample injection volume of 1 ml, a maximum temperature of 350 °C, and mass spectral scan range of 40 to 500 atomic mass units. Data evaluation of GC-MS chromatogram was performed using GC/MSD Chem Station software. The separated compounds were named and their molecular weight and structure were determined by comparing the mass spectra with data from National Institute of Standards and Technology (NIST) libraries. The GC-MS analysis of Sweet Mustard Oil showed the presence of 50 different compounds, with the major compounds being Benzoic acid, 4-ethoxy, ethyl ester (24.88%), Alpha phenyl alpha tropylacetaldehyde tosylhydrazone (8.82%), ζ Sitosterol (6.74%), Glycerol 1palmitate (4.70%), and 9,12 Octadecadienoic acid (Z,Z), 2-hydroxy1(hydroxymethyl) ethyl ester (13.24%). Other compounds identified included various alkanes, alcohols, esters, and acids. This information provides insight into the composition of Sweet Mustard Oil and its potential applications in various industries.

Keywords: Sweet mustard oil, GC-MS analysis, polyphenols, antioxidants

1. Introduction

The Indian subcontinent is a major producer of oilseeds, with Indian mustard (*Brassica juncea* L.) being a prominent crop belonging to the Brassicaceae family. In recent times, there has been a growing demand for vegetable oils that are rich in mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA) due to consumers' desire for a balanced and healthy diet ^[1].

Mustard oil is a vital component in the Indian diet due to its ability to increase the good HDL cholesterol ratio and provide omega-3 and omega-6 fatty acids that lower cancer risk ^[3]. The significance of Mustard oil stems from its antibacterial, antifungal, and anti-carcinogenic properties, high oleic acid content (which increases shelf life), natural antioxidants, tocopherol, phytosterols, vitamin K, and polyphenols ^[4]. Compared to other vegetable oils, mustard oil boasts superior nutritional attributes such as low saturated fatty acid content (6.6 g/100 g) and considerable amounts of linoleic (18:2 n-6 LA, 19.7 g/100 g) and α -linolenic (18:3 n-3 ALA, 9.6 g/100 g) acids, which are two essential fatty acids. Consequently, it is an excellent source of n-3 polyunsaturated fatty acids (PUFA) for individuals who do not consume non-vegetarian foods, especially of marine origin. However, the presence of high amounts of erucic acid in mustard oil limits its nutritional advantages ^[5]. Therefore, it is imperative to reduce the level of erucic acid in mustard oil.

Coriandrum sativum L. (Umbelliferae) is a spice native to Europe and Asia and widely known as coriander. Its dried fruits are called coriander seeds, which are commonly used in food, perfumes, and cosmetics to impart flavor ^[6-14]. Coriander has several reported biological activities, including anticancer, neuroprotective, anxiolytic, hypnotic, anticonvulsant, analgesic, anti-inflammatory, and antidiabetic effects ^[15-22]. Additionally, it has been found to exhibit antioxidant activity both *in vitro* and *in vivo*, which may be related to its chemical composition, including high levels of total phenolic compounds and flavonoids that can mitigate oxidative damage ^[23-30]. Furthermore, coriander has demonstrated anxiolytic activity in the CNS ^[31-34], which may contribute to its reported health benefits.

The therapeutic properties and integration of coriander into daily life make it a highly valuable functional food. To reduce the erucic acid level in mustard oil and add a sweet flavour, coriander seeds were incorporated, resulting in the creation of Sweet Mustard Oil. To identify significant compounds in Sweet Mustard Oil, a GC-MS analysis was conducted, which is a breakthrough in compound analysis and structure elucidation due to its high sensitivity in detecting compounds as low as 1 mg^[35]. This analysis was carried out as a part of the present study.

2. Materials and Methods

2.1 Collection and preparation of Sweet mustard oil

To prepare Sweet Mustard Oil, we purchased Mustard oil and dry seeds of *Coriandrum sativum* from a local market in Benad, Jaipur, Rajasthan, India. We added 25 g of coriander seeds to 250 ml of mustard oil in a glass bottle and kept it under direct sunlight for 10 weeks. Afterwards, we filtered the oil with a sieve to separate it from the seeds and left it in sunlight for 2 days. This prepared oil was named as Sweet Mustard Oil and used for GC-MS analysis.

2.2 GC-MS Analysis

The analysis was conducted using a Trace 1300 series gas chromatography (Thermo scientific) with a sample injection volume of 1 ml. The GC-MS run time for the sample was 48.36 minutes, with a maximum temperature of 350 °C. The temperature was ramped four times to provide a range of temperatures to the compounds. Initially, the temperature was set at 50 °C for 2 minutes and then programmed to rise to 150 °C at a rate of 5 °C/min, held at this temperature for 2 minutes. Then, the temperature was raised at the rate of 8 °C/minute until it reached 200 °C, held for 2 minutes, followed by a rise to 240 °C at a rate of 8 °C/min, held at this temperature for 2 minutes. Finally, the temperature was programmed to 280 °C at a rate of 10 °C/min and held at this temperature for 8 minutes. The sample was injected in splitless mode, and the mass spectral scan range was set between 40 and 500 amu (atomic mass unit) with mass spectra recorded in the electron ionization (EI) mode. Data evaluation of GC-MS chromatogram was performed using GC/MSD Chem Station software, and the separated compounds' name, molecular weight, and structure were ascertained by comparing the mass spectra with data from National Institute of Standards and Technology (NIST) libraries.

3. Results

The results pertaining to GC-MS analysis led to the identification of a number of compounds from the GC fractions of Sweet mustard oil. These compounds were identified through mass spectrometry attached with GC. The results of the GC-MS was interpreted by using a database of the National Institute of Standards and Technology (NIST)

library having more than 2,00,000 patterns. The GC-MS spectrum of the unknown constituent was then compared with the known components stored in NIST-14 library. The results of present study are tabulated in Table 1.

GC-MS analysis of Sweet Mustard oil revealed the presence of 50 different compounds namely 2,4 Dimethyl-1-1-heptene (1.17%), Stearic acid, 3-(octadecyloxy) propyl ester (0.38%), Decane (0.80%), Nonane, 2, 6-dimethyl (0.56%), Nonane, 2, 6-dimethyl (0.72%), 2-Undecanethiol, 2methyl (0.73%), Cyclooctane, 1,4-dimethyl,trans (1.01%), Dodecane (1.61%), Anethole (0.48%), 2-Isopropyl-5-methyl-1-heptanol (1.55%), Dichloroacetic acid, tridecyl ester (1.37%), 1- Docosene (1.07%), Tetradecane (1.21%), Hexadecane, 2,6,11,15 tetra methyl (0.40%), 1-Iodo-2-methyl undecane (0.50%), Pentadecane (0.99%), Phenol, 2,6-bis (1,1-dimethylethyl) (2.32%), Benzoic acid, 4-ethoxy, ethyl ester (24.88%), 1 Hexadecanol,3,7,11,15 tetramethyl (0.41%), 1Hexadecanol, 2 methyl (0.55%), Acetic acid, 3,7,11,15tetramethylhexadecyl ester (0.57%), 1Dodecanol, 2hexyl (0.46%), Nonadecane (1.47%), Heptadecane (1.67%), Benzene, 1,1'(2butene1,4diyl) bis (0.42%), Heptacosane (0.38%) Trichloroacetic acid, hexadecyl ester (0.73%), Tert-Hexadecanethiol (0.51%) Octadecane (0.96%) Z,Z,Z 4,6,9 Nonadecatriene (0.72%), Tetradecane, 2,6,10 trimethyl (1.16%) Phthalic acid, isobutyl octadecyl ester (0.63%), Octacosane (0.47%), cis Vaccenicacid (0.77%), Glycerol 1palmitate (4.70%), Eicosanoic acid (0.46%), nPropyl9,12octadecadienoate (0.52%), Alpha phenyl alpha troylacetaldhyde tosylhydrazone (8.82%), Butyl 9,12 octadecadienoate (18.23%), 9,12 Octadecadienoicacid (Z,Z), 2-hydroxy1(hydroxymethyl) ethyl ester (13.24%), 6,9,12,15 Docosatetraenoicacid, methyl ester (3.45%), 1Heptatriacotanol (2.45%), Hexadecanoic acid, 1(hydroxymethyl)1,2ethanediy ester (3.94%), Phthalic acid, di (2propylpentyl) ester (1.46%), Stigmasterol (2.64%), ζ Sitosterol (6.74%), Squalene (0.83%), d-Mannitol, 1decylsulfonyl (0.53%), Linoleic acid ethyl ester (1.22%), ζ Tocopherol (1.59%).

The GC-MS spectrum confirmed that the presence of 50 major components with the retention time 5.46, 8.86, 9.87, 10.08, 10.20, 12.16, 12.29, 15.87, 18.30, 18.71, 18.94, 19.17, 21.41, 23.12, 23.41, 24.26, 24.36, 24.88, 24.99, 25.22, 25.49, 25.75, 26.92, 28.99, 29.30, 29.77, 30.00, 30.13, 30.76, 32.53, 32.70, 33.70, 34.56, 36.63, 39.03, 39.23, 40.96, 41.25, 41.61, 41.68, 41.76, 41.84, 42.00, 42.36, 43.05, 45.11, 45.70, 46.75, 47.64, 49.64 respectively (Figure 1). The name, molecular weight, molecular formula and structure of the component of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The identified constituents from sweet mustard oil possess the therapeutic properties (Table 2).

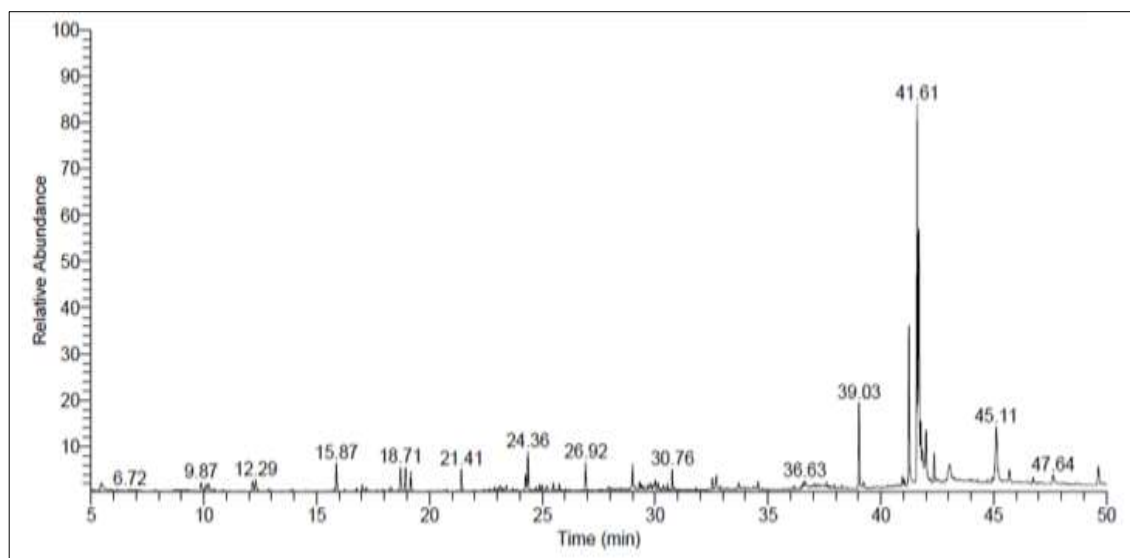


Fig 1: GC-MS chromatogram of constituent's sweet mustard oil

Table 1: Components identified in the sweet mustatrd oil.

S. No.	Retention Time	Area	Area%	Compound Name
1.	5.46	136406108.25	1.17	2,4 Dimethy-l-Iheptene
2.	8.86	44942568.16	0.38	Stearic acid, 3 (octadecyloxy) propyl ester
3.	9.87	93821033.88	0.80	Decane
4.	10.08	65390108.98	0.56	Nonane, 2, 6-dimethyl
5.	10.20	84148833.94	0.72	Heptane, 5-ethyl 2-methyl
6.	12.16	85231614.95	0.73	2-Undecanethiol, 2methyl
7.	12.29	117961894.15	1.01	Cyclooctane, 1,4-dimethyl,trans
8.	15.87	188885701.65	1.61	Dodecane
9.	18.30	55683267.77	0.48	Anethole
10.	18.71	181896295.88	1.55	2-Isopropyl-5-methyl-1-heptanol
11.	18.94	1160216910.95	1.37	Dichloroacetic acid, tridecyl ester
12.	19.17	1160216910.95	1.07	1 Docosene
13.	21.41	124728770.46	1.21	Tetradecane
14.	23.12	142080790.47	0.40	Hexadecane, 2,6,11,15 tetra methyl
15.	23.41	46902867.29	0.50	1Iodo 2 methyl undecane
16.	24.26	58960733.19	0.99	Pentadecane
17.	24.36	115335202.25	2.32	Phenol, 2,6-bis (1,1-dimethylethyl)
18.	24.88	271476607.32	24.88	Benzoic acid, 4-ethoxy, ethyl ester
19.	24.99	50297569.22	0.41	1 Hexadecanol,3,7,11,15 tetramethyl
20.	25.22	48279074.03	0.55	1Hexadecanol, 2 methyl
21.	25.49	63852364.77	0.57	Acetic acid, 3,7,11,15tetramethylhexadecyl ester
22.	25.75	67091332.99	0.46	1Dodecanol, 2hexyl
23.	26.92	53709648.03	1.47	Nonadecane
24.	28.99	171493420.61	1.67	Heptadecane
25.	29.30	195848480.01	0.42	Benzene, 1,1'(2butene1,4diyl) bis
26.	29.77	49648992.27	0.38	Heptacosane
27.	30.00	44104103.05	0.73	Trichloroacetic acid, hexadecyl ester
28.	30.13	85482325.85	0.51	Tert-Hexadecanethiol
29.	30.76	60232137.93	0.96	Octadecane
30.	32.53	112758037.77	0.72	Z,Z,Z 4,6,9 Nonadecatriene
31.	32.70	83830990.97	1.16	Tetradecane, 2,6,10 trimethyl
32.	33.70	135929144.37	0.63	Phthalic acid, isobutyl octadecyl ester
33.	34.56	73511073.28	0.47	Octacosane
34.	36.63	54509815.07	0.77	cis Vaccenicacid
35.	39.03	89667893.25	4.70	Glycerol 1palmitate
36.	39.23	550308037.53	0.46	Eicosanoic acid
37.	40.96	54290853.68	0.52	nPropyl9,12octadecadienoate
38.	41.25	60775716.80	8.82	Alpha phenyl alpha tropylacetaldehyde tosylhydrazone
39.	41.61	1032049141.09	18.23	Butyl 9,12 octadecadienoate
40.	41.68	2133194716.27	13.24	9,12 Octadecadienoic acid (Z,Z), 2 hydroxy 1 (hydroxymethyl) ethyl ester
41.	41.76	1549814966.96	3.45	6,9,12,15Docosatetraenoicacid, methyl ester
42.	41.84	403679881.98	2.45	1Heptatriacotanol
43.	42.00	287134700.85	3.94	Hexadecanoic acid, 1 (hydroxymethyl)1,2ethanediyl ester
44.	42.36	461558894.32	1.46	Phthalic acid, di(2propylpentyl) ester

45.	43.05	170620972.61	2.64	Stigmasterol
46.	45.11	308493899.35	6.74	çSosterol
47.	45.70	788228865.43	0.83	Squalene
48.	46.75	97329563.21	0.53	dMannitol, 1decylsulfonyl
49.	47.64	61842532.52	1.22	Linoleic acid ethyl ester
50.	49.64	185685261.09	1.59	çTocopherol

Table 2: Therapeutic potential of the components identified in the sweet mustard oil.

Phytoconstituents identified in Sweet Mustard Oil	Properties
Anethole	Flavouring substance, measuring 13 times sweeter than sugar. Used in seasoning and confectionery applications, oral hygiene products, and in small quantities in natural berry flavors.
Dichloroacetic acid, tridecyl ester	inhibit the activity of enzyme pyruvate 1dehydrogenase kinase
Hexadecane, 2,6,11,15 tetra methyl (Croctetane)	Biomarker and often associated with anaerobic methane oxidation.
Phenol, 2,6 bis (1,1dimethylethyl)	It prevents gumming in aviation fuels. Work as UV stabilizer and antioxidants for hydrocarbon-based products ranging from petrochemicals to plastics.
Octadecane	A bacterial metabolite and a plant metabolite.
Tetradecane, 2,6,10 trimethyl	Sesqui-terpenoids
Octacosane	Antimicrobial, antioxidant, and antiinflammatory
cisVaccenic acid	Omega-7 fatty acid. lowered total cholesterol, lowered LDL cholesterol and lower triglyceride levels. Alkaline phosphatase inhibited 25% by vaccenic acid in osteoblasts.
Eicosanoic acid	anti-inflammatory
Alpha phenyl alpha tropylacetaldehyde tosylhydrazone	Alpha-lipoic acid or ALA antioxidant
6,9,12,15Docosatetraenoic acid, methyl ester	Anticholesterol compound
1Heptatriacotanol	anti-hypercholesterolemic effects
Phthalic acid, di(2propylpentyl)ester	Antimicrobial
Stigmasterol	Having potential to reduce the risk of cardiovascular diseases.
çSosterol	Lowering cholesterol levels and improving symptoms of an enlarged prostate (benign prostatic hyperplasia or BPH). strong oxygen scavenging abilities and anti-tumor activities
Squalene	strong oxygen scavenging abilities and anti-tumor activities
dMannitol, 1decylsulfonyl	Sugar alcohol elevates blood plasma osmolality resulting in enhanced flow of water from tissues, including the brain and cerebrospinal fluid, into interstitial fluid and plasma. As a result, cerebral edema, elevated intracranial pressure, and cerebrospinal fluid volume and pressure may be reduced.
Linoleic acid ethyl ester	Essential fatty acid anti-inflammatory properties.
çTocopherol	naturally-occurring fat-soluble vitamin E antioxidant and cytoprotective activities prevents protein oxidation and inhibits lipid peroxidation, thereby maintaining cell membrane integrity and protecting the cell against damage.

4. Discussion

The incorporation of coriander seeds into mustard oil to create Sweet Mustard Oil has opened up a new avenue for producing healthy vegetable oils with added flavor. The combination of the two plants has resulted in a product with several beneficial compounds, including plant sterols, essential fatty acids, and antioxidants that can mitigate oxidative damage. Moreover, the addition of coriander seeds has reduced the level of erucic acid in mustard oil, which has been a limiting factor in its nutritional value.

The GC-MS analysis conducted on sweet mustard oil revealed a complex mixture of 50 different compounds, including alkanes, alkenes, alcohols, esters, phenols, and terpenoids. Among the identified compounds, benzoic acid, 4-ethoxy, ethyl ester was found to be the most abundant, accounting for 24.88% of the total compounds. This compound has been reported to possess antifungal and antibacterial properties [36]. Another interesting compound found in the sweet mustard oil was alpha-phenyl alpha-tropylacetaldehyde tosylhydrazone, which is a derivative of tropine and has various pharmacological activities, including anticholinergic and analgesic effects [37, 38]. Additionally, the presence of çSosterol, a plant sterol, was

detected, which has been reported to have anti-inflammatory, anti-cancer, and cholesterol-lowering effects [39, 40].

Furthermore, several compounds identified in the sweet mustard oil, such as linoleic acid ethyl ester, 6,9,12,15 docosatetraenoic acid methyl ester, and stigmasterol, have previously been reported to have beneficial health effects [41-43]. Butyl 9,12-octadecadienoate was also found to be a major compound in the analyzed sweet mustard oil, with antifungal activity against various fungi, including *Aspergillus niger* and *Candida albicans* [44]. Phenol, 2,6-bis(1,1-dimethylethyl), which was present in the sweet mustard oil at 2.32% of the total compounds, has been reported to have antioxidant activity and may protect against oxidative stress-induced damage [45]. The presence of various fatty acids, including stearic acid, eicosanoic acid, and linoleic acid ethyl ester, may also contribute to the nutritional value of sweet mustard oil, with reported health benefits including anti-inflammatory and cholesterol-lowering effects [46].

5. Conclusion

In the present study, the GC-MS analysis of Sweet Mustard Oil revealed the presence of fifty compounds. The oil constituents identified has potent anticancer and antioxidant activities, which can be used to reduce the damages caused by free radicals in the body and therefore contribute to the prevention of diseases related to the oxidative stress and also for the management of diabetes, inflammation and microbial infections. Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principle components in the oil. The identification of constituents would be helpful for further detailed studies.

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7. Conflicts of interest

We certify that there is no conflict of interest with any commercial or financial organization that could be construed as a conflict of interest.

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