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ORIGINAL ARTICLE

Chemical composition of fennel seed extract and determination of fenchone in commercial formulations by GC–MS method

Prawez Alam[1] · Maged S. Abdel-Kader[1,2] · Mohammed H. Alqarni[1] · Hala H. Zaatout[2] · Syed Rizwan Ahamad[3] · Faiyaz Shakeel[4]

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Abstract In the present study, various phytoconstituents of methanolic extract of *Foeniculum vulgare* were identified using gas-chromatography mass spectrometry (GC–MS) method. GC–MS method was also applied for the analysis of biomarker fenchone in extract and eight different commercial formulations. The mass of prepared extract and formulations A–D and H (commercial herbal mixtures and commercial extract) used for the analysis of fenchone was 10 g. However, the mass of formulations E–G (soft gelatin capsules) was 100 mg. Fifty seven different phytoconstituents were identified in the methanolic extract of *F. vulgare* using GC–MS technique. The main compounds identified were trans-anethole (31.49%), 2-pentanone (25.01%), fenchone (11.68%) and benzaldehyde-4-methoxy (8.01%). Several other compounds were also identified in higher amounts and some compounds were identified in trace amounts. Many compounds have been reported for the first time in the methanolic extract of *F. vulgare*. The amount of fenchone was found to be maximum in plant extract (9.789 mg/g) in comparison with other commercial formulations by the proposed GC–MS technique. In three different commercial formulations (F, G and H), the amount of fenchone was obtained as more than 1.0 mg/g. However, in five different commercial formulations (A, B, C, D and E), the amount of fenchone was recorded as less than 0.1 mg/g. This method could be utilized for the analysis of fenchone contents in the commercial formulations containing fenchone as an active ingredient. The results obtained in this work could be useful in standardization of commercial formulations containing fenchone.

Keywords Fenchone · *Foeniculum vulgare* · Gas-chromatography mass-spectrometer · Phytoconstituents · Standardization

Introduction

Foeniculum vulgare Mill. [chemical structure: supplementary Fig. 1 (Figure S1)] is a biennial or perennial medicinal crop/herb which belongs to the family Apiaceae (Bahmani et al. 2015). Essential oils are the main constituents of *F. vulgare* seed which are being used as flavoring agents in various food, cosmetic and pharmaceutical products (Piccaglia and Marotti 2001; Shahat et al. 2011; Bahmani et al. 2015). Various therapeutic activities such as hepatoprotective (Ozbek et al. 2003), antispasmodic (Reynolds 1982), diuretic (Shahat et al. 2011), anti-inflammatory, analgesic, antioxidant (Choi and Hwang 2004; Badgujar et al. 2014; Kontogiorgis et al. 2016; Majdoub et al. 2017), antibacterial (Elagayyar et al. 2001; Diao et al. 2014; Shahat et al. 2011), antimicrobial (Roby et al. 2013), antifungal (Singh et al. 2006), anti-diabetic (Saleem et al.

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✉ Faiyaz Shakeel
faiyazs@fastmail.fm

[1] Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O. Box 173, Al-Kharj 11942, Saudi Arabia

[2] Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Alexandria 21215, Egypt

[3] Central Laboratory, Department of Pharmaceutical Chemistry, Research Center, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

[4] Kayyali Chair for Pharmaceutical Industries, Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

2017), anti-neurological (Cioanca et al. 2016) and anti-cancer activity (Anand et al. 2008) have been reported in essential oils of *F. vulgare*.

Several studies have been carried out to determine the different phytoconstituents of *F. vulgare* in literature (Diaz-Maroto et al. 2006; Ozcan et al. 2006; Mojab et al. 2007; Cosge et al. 2009; Renjie et al. 2010; Anubhuti et al. 2011; Shahat et al. 2011; Hammouda et al. 2013; Rodriguez-Solana et al. 2014; Acimovic et al. 2015; Bahmani et al. 2015; Shojaiefar et al. 2015; Upadhyay 2015; Abdel Karm et al. 2017; Shahmokhtar and Armand 2017; Ahmad et al. 2018). The main components which have been reported in literature are phenylpropanoid derivatives and monoterpenoids in essential oils of *F. vulgare* (Diaz-Maroto et al. 2006; Ozcan et al. 2006; Renjie et al. 2010; Shahat et al. 2011). In literature, various compounds such as trans-anithole, fenchone, α-pinene, β-pinene and camphene have been reported as the biomarkers of the essential oils of *F. vulgare* (Renjie et al. 2010; Shahat et al. 2011; Bahmani et al. 2015; Upadhyay 2015; Abdel Karm et al. 2017; Ahmad et al. 2018).

Gas-chromatography mass-spectrometry (GC–MS) technique has been reported as one of the most important analytical techniques for the identification of phytoconstituents of plant materials (Renjie et al. 2010; Shahat et al. 2011; Acimovic et al. 2015; Bahmani et al. 2015; Upadhyay 2015; Ahmad et al. 2018). It offers several advantages over other analytical techniques for profiling of chemical compositions of plant materials (Renjie et al. 2010; Shahat et al. 2011; Bahmani et al. 2015). Fenchone (Figure S2) is one of the biomarkers of essential oils of *F. vulgare* which is present in various commercially available formulations. Chemical composition of essential oils of *F. vulgare* using GC–MS technique had been reported extensively in literature (Cosge et al. 2009; Renjie et al. 2010; Anubhuti et al. 2011; Shahat et al. 2011; Hammouda et al. 2013; Acimovic et al. 2015; Bahmani et al. 2015; Upadhyay 2015; Abdel Karm et al. 2017; Shahmokhtar and Armand 2017). Chiral GC method has been reported for the analysis of fenchone in its essential oils (Ravid et al. 1992). Nevertheless, the analysis of its biomarker fenchone in various commercial formulations has not been reported in literature. Therefore, in this work, different phytoconstituents of methanolic extract of *F. vulgare* were determined using GC–MS technique. The proposed GC–MS technique was applied further for the analysis of fenchone contents in extract and eight different commercial formulations.

Materials and methods

Materials

Standard fenchone, ethanol, ethyl acetate (EA) and hexane were obtained from "Sigma-Aldrich (St. Louis, MO, USA)". Herbal mixtures and various commercial formulations were purchased from local market in Riyadh, Saudi Arabia and Alexandria, Egypt. HNO_3, perchloric acid and hydrogen peroxide were obtained from "E-Merck (Hamburg, Germany)". Water was collected from "Milli-Q Water Purification Unit" in the Laboratory. All the solvents were of chromatography grade and other chemicals used were of analytical reagent (AR) grade.

Plant material

The seeds of *F. vulgare* Mill. were purchased from the local market of "Al-Kharj, Saudi Arabia". The seeds were identified by comparison with voucher specimen at the "Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia".

Preparation of standard solutions and calibration

Accurately weighed 10 mg of standard fenchone (purity 99%) was dissolved in methanol in a 10 ml volumetric flask. About 1.0 ml of accurately measured standard was transferred to another 10 ml volumetric flask and completed the volume with methanol in order to obtain standard concentration of 100 µg/ml. Serial dilutions were made from standard solution in order to obtain the concentrations of fenchone in the range of 1–100 µg/ml. The GC–MS response of each concentration was recorded and calibration curve was plotted between the concentration of fenchone and GC–MS response. The experiments were performed in triplicates.

Sample preparation for the analysis of fenchone in the methanolic extract of fennel seeds and herbal mixtures

For seed extract, 10 g of fennel seeds were extracted to exhaustion with methanol at room temperature. After filtration, the combined methanol extract was evaporated under reduced pressure using rotary vacuum evaporator. The resulted extracts were separately transferred to 50 ml volumetric flasks and completed the volume to 50 ml.

For marketed herbal mixtures (A–D), 10 g of each mixture were separately extracted to exhaustion with methanol at room temperature. After filtration, the

combined methanol extracts were evaporated under reduced pressure using rotary vacuum evaporator. The resulted extracts were separately transferred to 10 ml volumetric flasks and completed the volume to 10 ml. All the sample matrices were investigated in triplicates.

Sample preparation for the analysis of fenchone in soft gelatin capsules

HPLC syringe was used to obtain the oily contents of the capsules. From each formulation (E–G), 100 mg samples were taken and dissolved in 10 ml of methanol in volumetric flasks. The volume was completed to 10 ml. These experiments were performed in triplicates.

Sample preparation for the analysis of fenchone in baby instant drink

From the instant baby drink granules (H), 10 g of samples were taken and dissolved in 50 ml of methanol, filtered and completed the volume to 50 ml in volumetric flask. Experiments were performed in triplicates.

GC–MS analysis of methanolic extract of *F. vulgare*

Around 2 µl of sample extract was injected into the system with the split mode (split ratio 1:20). To perform the analysis, "Perkin Elmer GC–MS coupled with Clarus 600 T mass Spectrometer (USA)" was used. The system was composed of an auto-sampler unit, auto-injector unit and a gas chromatograph Clarus 600 coupled with a single quadrupole mass spectrometer. For the analysis of the samples, "TurboMass Solution Software Version 5.4" was used in GC–MS analysis. The samples were separated on Elite 5 MS (30 m × 0.25 mm i.d., 0.25 µm film thickness) capillary CG column (Perkin Elmer, USA). Analyses were performed using helium as a carrier gas at a constant pressure mode (65.2 kPa). The separation was carried out in a gradient temperature program. The oven temperature was maintained at 40 °C for 2 min, ramped to 100 °C at 5 °C/min for 2 min and again increased at a rate of 5 °C/min to 300 °C, ramped with the grade 5 °C/min and held for 5 min. The total run time was 61 min. The injector, ion source and interface temperature were set at 280 °C, 240 °C and 220 °C, respectively. The electron energy was set at 70 eV. The unknown components were identified using "National Institute of Standard and Technology (NIST, 2005) Library" and "WILEY, 2006, Library".

GC–MS analysis of standard fenchone

Around 2 µl of standard sample of fenchone was injected into the system with the split mode (split ratio 1:20). To perform the analysis, "Perkin Elmer GC–MS coupled with Clarus 600 T mass spectrometer (USA)" was used. The samples were separated on Elite 5 MS (30 m × 0.25 mm i.d., 0.25 µm film thickness) capillary CG column (Perkin Elmer, USA). Analyses were performed with the helium as a carrier gas at a constant pressure mode (65.2 kPa). The separation was carried out in a gradient temperature program. The oven temperature was maintained at 50 °C for 1 min, ramped to 200 °C at 5 °C/min for 1 min and again increased at a rate of 5 °C/min to 300 °C, ramped with the grade 5 °C/min and held for 2 min. The total run time was 14 min. The injector, ion source and interface temperature were set at 280 °C, 240 °C and 220 °C, respectively. The ionization voltage was 70 eV. Using same methodology, GC–MS response of different concentrations of fenchone was recorded and calibration curve was constructed between the concentration and GC–MS response. The concentration of fenchone in fennel extract and various commercial formulations was determined from the calibration curve plotted between concentration and GC–MS response. The experiments were performed in triplicates.

Results and discussion

The medicinal properties of *F. vulgare* have been utilized in our country (Saudi Arabia). Various phytoconstituents have been reported in *F. vulgare* which are responsible for different therapeutic activities of fennel fruits (Mojab et al. 2007; Renjie et al. 2010; Shahat et al. 2011; Bahmani et al. 2015). In this work, phytoconstituents of methanolic extract of *F. vulgare* were identified by GC–MS method. The proposed GC–MS method was also applied for the determination of fenchone contents in plant extract and various commercial formulations. The results of GC–MS profiling of methanolic extract of *F. vulgare* are listed in Table 1. Various identified compounds are presented in (%) along with their retention times (Table 1). By GC–MS analysis of unknown mixtures, various compounds are identified using NIST and WILEY library. These libraries provide information about various compounds based on their respective retention times and GC–MS response. Based on GC–MS response of various compounds, the amount of compounds can be calculated in % as reported in literature (Mojab et al. 2007; Cosge et al. 2009; Renjie et al. 2010; Acimovic et al. 2015). The calculation of these compounds in masses is not possible by NIST and WILEY libraries. Therefore, the amount of identified compounds is presented in (%) along with their retention times in Table 1. The representative GC–MS chromatogram of methanolic extract of *F. vulgare* is shown in Fig. 1. Results showed significant amount of the important phytoconstituents identified in the methanolic extract of *F. vulgare*.

Table 1 Components identified in methanolic extract of *F. vulgare seeds* by GC-MS technique

Component name	RT (Min)	Area	Area (%)
4-Hexen-2-one	4.54	111042	0.20
3-Hydroxytetrahydropyran	5.02	300106	0.55
2-Pentanone	5.56	13563417	25.01
Delta-3-carene	8.16	228785	0.42
Acetic acid	8.90	55015	0.10
Sabinene	9.34	91076	0.17
1,3,8-P-menthatriene	10.94	62420	0.12
DL-Limonene	11.08	954384	1.76
Fenchone	12.88	6333210	11.68
Tetradecane	13.26	490277	0.90
Trans-p-mentha-2,8-dienol	13.90	95814	0.18
Camphor	14.64	49474	Trace
1,1′-Bicyclohexyl	16.16	73771	0.14
1-Undecanol	16.32	80699	0.15
Benzene-1-methoxy-4-(2-propene)	16.54	825098	1.52
1-Methyl-2-methylene-4-isopropyl	17.58	30132	Trace
Fenchyl acetate	17.74	57226	0.11
Cyclohexane	18.06	167539	0.31
L-Histidine	18.20	38121	Trace
Benzaldehyde-4-methoxy	18.58	4344178	8.01
Trans-anethole	19.70	17075762	31.49
1-Hexyl-1-nitrocyclohexane	21.04	22706	Trace
4-Pentyloxy-2,3-dicyanophenyl	21.58	56719	0.10
4-Germacrane-1,7-dimethyl	22.08	151976	0.28
Cis-2,3-epoxy-2,3,4,5,6-pentam	22.52	379565	0.70
1-Tetradecene	22.92	347801	0.64
Tetradecane	23.16	111364	0.21
Hydroxymethylcyclododecane	23.28	72483	0.13
1-Methoxy-3-(ethenylcarbonyl)B	24.44	387111	0.71
P-methoxybenzamide	24.76	48233	Trace
11-Tricosene	26.38	49001	Trace
10-Oxoundecyl acetate	26.78	54200	0.10
1-Tetradecanol	27.16	37634	Trace
1-Heptadecanol	27.56	607788	1.12
E-15-heptadecenal	28.12	562459	1.04
Pentadecane	28.30	99652	0.18
Diphenyl methanone	28.94	359540	0.66
Menthol-1′-(butyn-3-one-1-yl)	29.46	75867	0.14
Cyclohexane eicosyl	29.62	105029	0.19
8-Pentadecanone	29.92	174330	0.32
1-Undecene-9-methyl	32.44	105299	0.19
8-Heptadecene	32.58	491381	0.91
Dotriacontane	32.74	63920	0.12
1,19-Eicosadiene	33.94	23330	Trace
1,2-Benzenedicarboxylic acid	35.76	268694	0.50
9-Octadecenoic acid	36.00	76572	0.14
3-Eicosene-(e)-(CAS)	36.56	283988	0.52
Hexadecanoic acid	37.10	60932	0.11
Octadecanal	38.06	53211	0.10
3-Eicosene	40.20	230501	0.43

Table 1 continued

Component name	RT (Min)	Area	Area (%)
Di(2-ethylhexyl)adipate	43.38	167431	0.31
Eicosyl acetate	46.66	51181	Trace
Decanal	48.00	89560	0.17
14-Heptadecenal	50.02	139144	0.26
Hexatriacontane	50.58	780581	1.44
1-Octadecanol	52.70	219018	0.40
10-Nonadecanone	53.26	1608688	2.97

The compound present in < 0.1% amount (trace)

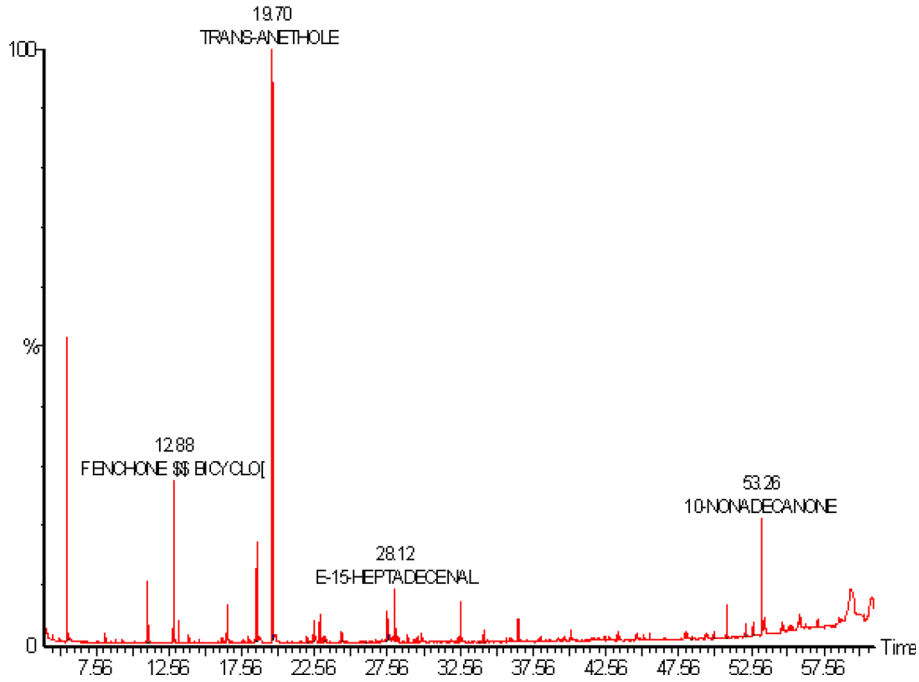

Fig. 1 Gas chromatogram of methanolic extract of *F. vulgare* seeds

Fifty seven different phytoconstituents were identified in methanolic extract of *F. vulgare* (Table 1). The most abundant includes trans-anethole (31.49%), 2-pentanone (25.01%), fenchone (11.68%) and benzaldehyde-4-methoxy (8.01%). Some other phytoconstituents such as 10-nonadecanone (2.97%), DL-limonene (1.76%), benzene-1-methoxy-4-(2-propene) (1.52%), hexatriacontane (1.44%), 1-heptadecanol (1.12%) and E-15-heptadecenal (1.04%) were also present in good amounts. Some phytoconstituents such as tetradecane, 8-heptadecene, 4-hexen-2-one, 3-hydroxytetrahydropyran, delta-3-carene, acetic acid, sabinene, 1,3,8-p-menthatriene, trans-p-mentha-2,8-dienol, 1,1′-bicyclohexyl, 1-undecanol, fenchyl acetate, cyclohexane, 4-pentyloxy-2,3-dicyanophenyl, 4-germacrane-1,7-dimethyl, cis-2,3-epoxy-2,3,4,5,6-pentam, 1-tetradecene, tetradecane, hydroxymethylcyclododecane, 1-methoxy-3-(ethenylcarbonyl)B, 10-oxoundecyl acetate, pentadecane, diphenyl methanone, menthol-1′-(butyn-3-one-1-yl), cyclohexane eicosyl, 8-pentadecanone, 1-undecene-9-methyl, dotriacontane, 1,2-benzenedicarboxylic acid, 9-octadecenoic acid, 3-Eicosene-(e)-(CAS), hexadecanoic acid, octadecanal, 3-eicosene, di(2-ethylhexyl)adipate, decanal, 14-heptadecenal and 1-octadecanol were present in moderate to low amounts. On the other hands, the phytoconstituents such as camphor, 1-methyl-2-methylene-4-isopropyl, L-histidine, 1-hexyl-1-nitrocyclohexane, p-methoxybenzamide, 11-tricosene, 1-tetradecanol, 1,19-eicosadiene and eicosyl acetate were identified in trace amounts (less than 0.1%). The variety of compounds was identified in the methanolic extracts of *F. vulgare* which could be responsible for different therapeutic activities of fennel fruit. Hence, methanolic of *F. vulgare* could be used for the treatment of various diseases.

GC–MS method was applied as standard method for the identification of extract. The contents of extract may vary from region to region depending upon seasonal and environmental conditions. The objective of this work was to identify the contents of fennel extract from the sample available in Saudi Arabia. The novelty of this method includes the application of proposed method in determination of standard fenchone in various commercial formulations. Many of the phytoconstituents identified in this work have been reported previously in literature (Diaz-Maroto et al. 2006; Ozcan et al. 2006; Mojab et al. 2007; Cosge et al. 2009; Renjie et al. 2010; Shahat et al. 2011). However, the amount of each compound could be different with those reported in literature. The variation in amounts of each compound depends upon various factors such as temperature, humidity, climate conditions and collection time for plant (Shahat et al. 2011). Several phytoconstituents such as trans-anethole, fenchone, sabinine, camphor, fenchyl acetate, bicyclohexyl and tetradecane etc. have been reported very well in literature (Ozcan et al. 2006; Renjie et al. 2010; Shahat et al. 2011; Acimovic et al. 2015; Bahmani et al. 2015). However, many phytoconstituents such as 4-hexen-2-one, 3-hydroxytetrahydropyran, 2-pentanone, 1,3,8-p-menthatriene, tetradecane, 1,1′-bicyclohexyl, benzene-1-methoxy-4-(2-propene), 1-methyl-2-methylene-4-isopropyl, L-histidine, 1-hexyl-1-nitrocyclohexane, 4-pentyloxy-2,3-dicyanophenyl, 4-germacrane-1,7-dimethyl, cis-2,3-epoxy-2,3,4,5,6-pentam, 1-tetradecene, tetradecane, hydroxymethylcyclododecane, 1-methoxy-3-(ethenylcarbonyl)B, p-methoxybenzamide, 11-tricosene, 10-oxoundecyl acetate, 1-tetradecanol, 1-heptadecanol, E-15-heptadecenal, pentadecane, diphenyl methanone, 8-pentadecanone, 1-undecene-9-methyl, 8-heptadecene, dotriacontane, 1,2-benzenedicarboxylic acid, 3-eicosene-(e)-(CAS), octadecanal and 10-nonadecanone have been reported for the first time in this work. Different species/varieties of extract were not investigated in this work. The most abundant phytoconstituents such as trans-anethole, 2-pentanone, fenchone and benzaldehyde-4-methoxy recorded in this study have great therapeutic applications (Anwar et al. 2009; Shahat et al. 2011; Mazaheri et al. 2013; Badgujar et al. 2014; Walker and Mills 2014). Trans-anethole has been investigated as anti-oxidant, antimicrobial, estrogenic and anti-inflammatory agent (Shahat et al. 2011; Mazaheri et al. 2013; Badgujar et al. 2014). 2-Pentanone, also known as methyl propyl ketone is used as a metabolic product of *Penicillium* mold growth (Walker and Mills 2014). Fenchone is found in almost all species of *Foeniculum* and reported as anti-oxidant, antimicrobial and anti-inflammatory agent (Anwar et al. 2009; Shahat et al. 2011; Mazaheri et al. 2013). Benzaldehyde-4-methoxy, also known as p-anisaldehyde has been investigated as anti-oxidant agent (Anwar et al. 2009).

The proposed GC–MS method was also applied for the analysis of fenchone in methanolic extract of *F. vulgare* and various commercial formulations. For this application, the calibration curve of standard fenchone was plotted between the concentration of standard fenchone and GC–MS response. The calibration curve of fenchone was found to be linear in the range of 1-100 µg/g with correlation coefficient of 0.9955. The regression equation for calibration curve was obtained as $y = 4842.8x + 26887$; in which x is the concentration of fenchone and y is the GC–MS response for fenchone. The overlaid GC–MS chromatograms of different concentrations of fenchone are shown in Fig. 2 which suggested uniformity in GC–MS response of fenchone at each concentration evaluated. From calibration curve of standard fenchone plotted between GC–MS response and concentration, the amount of fenchone (mg/g) in extract and various commercial formulations was determined. The mass of extract and formulations (A-D and H) used for the analysis of fenchone was 10 g. However, the mass of formulation E–G (soft gelatin capsules) was 100 mg. Soft gelatin capsules are not available in larger doses in the market. Therefore, the mass of formulations E–G was 100 mg for soft gelatin capsules. The results of fenchone contents in extract and various commercial formulations are presented in Table 2. The overlaid GC–MS chromatograms of various commercial formulations are presented in Fig. 3. The maximum amount of fenchone was recorded in fennel extract (9.789 mg/g) in comparison with various commercial formulations investigated. Among various commercial formulations investigated, the maximum amount of fenchone was obtained in formulation F (1.164 mg/g). The amount of fenchone in commercial formulations F, G and H were obtained in similar magnitude and were not statistically different ($P > 0.05$). However, the amount of fenchone in commercial formulations A, B, C, D was recorded as negligible (less than 0.1 mg/g). Overall, the proposed GC–MS method was found to be suitable for the analysis of fenchone contents in extract and various commercial formulations.

Based on GC–MS profile of various phytoconstituents identified in the methanolic extract of *F. vulgare,* it can be concluded that this plant could be explored in the treatment of different diseases. Moreover, the proposed GC–MS method could be applied for the analysis of fenchone contents in the commercial formulations containing fenchone as an active ingredient.

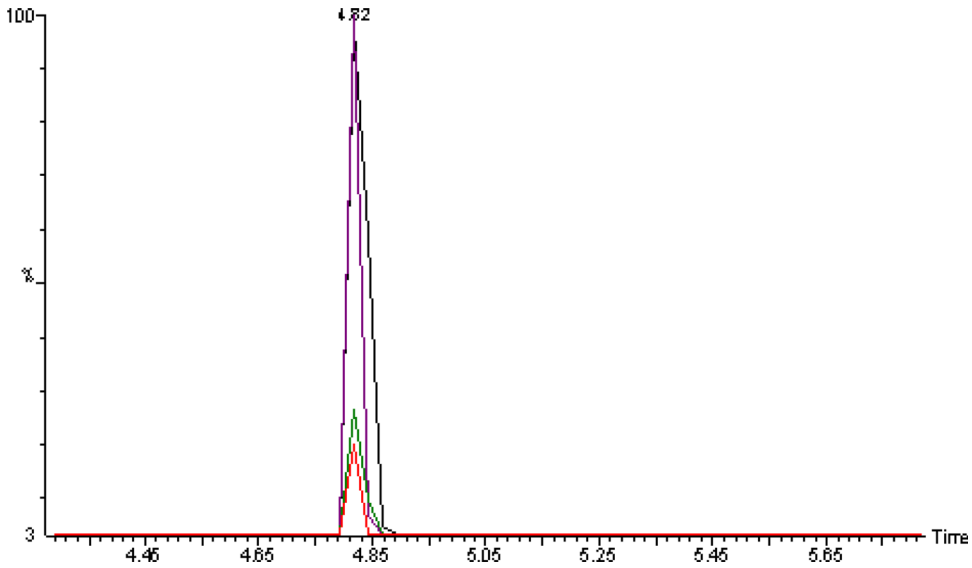

Fig. 2 Overlaid GC peaks of standard fenchone at different concentrations

Table 2 Analysis of fenchone contents in fennel extract and different commercial formulation by proposed GC–MS technique

Formulation code	Composition claimed	Fenchone found (mg/g) ± SD
Fennel extract*	Chamomile, fennel, anise, caraway	9.789 ± 0.250
A*	Chamomile, fennel, anise, caraway	0.006 ± 0.001
B*	Tilia, guava, verbascum, marjouram, peppermint, fennel, licorice	0.004 ± 0.000
C*	Thyme, fennel, salvia, anise, licorice, guava, melissa	0.002 ± 0.000
D*	–	0.004 ± 0.000
E#	α-Pinene, β-pinene, camphene, borneol, anethol, fenchone, cineol	0.002 ± 0.000
F#	α-Pinene, β-pinene, camphene, borneol, anethol, fenchone, cineol	1.164 ± 0.004
G#	Peppermint oil, fennel oil, ginger oil, caraway oil, chamomile oil	1.149 ± 0.003
H*	Fennel extract	1.103 ± 0.002

*The mass of extract and formulations A–D and H used for the analysis of fenchone content was 10 g

#The mass of formulations E–G (soft gelatin capsules) used for the analysis of fenchone content was 100 mg

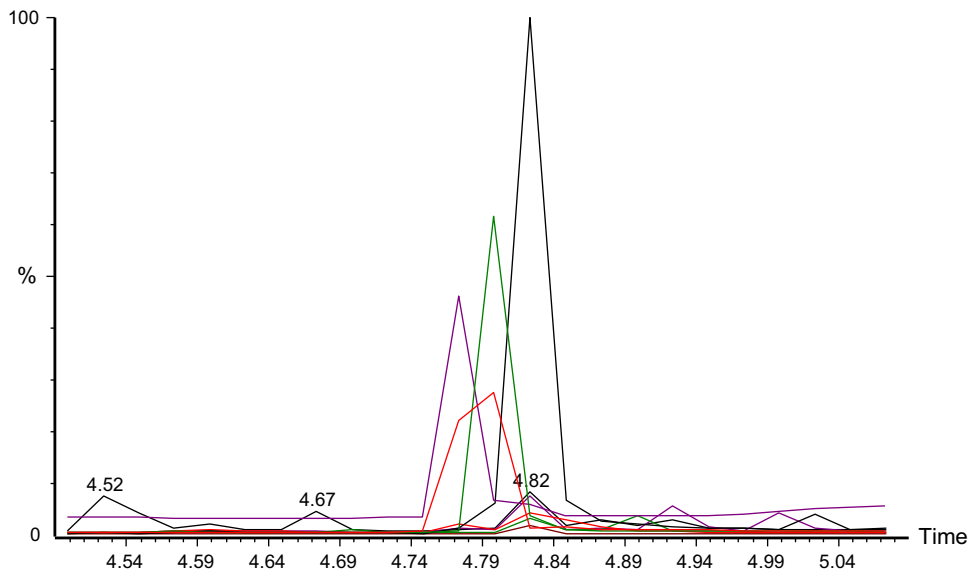

Fig. 3 Overlaid GC peaks of fenchone extract and various commercial formulations

Conclusion

In the present work, various phytoconstituents in the methanolic extract of *F. vulgare* were identified using GC–MS method. The proposed GC–MS technique was applied for the analysis of biomarker fenchone in extract and eight different commercial formulations. The main compounds identified in the methanolic extract of *F. vulgare* were trans-anethole, 2-pentanone, fenchone and benzaldehyde-4-methoxy. Several compounds were detected in higher amounts and some in trace amounts. Many compounds were identified for the first time in *F. vulgare* by GC–MS technique. The proposed method was applied in the analysis of fenchone contents in extract and various commercial formulations. The maximum amount of fenchone was obtained in extract in comparison with commercial formulations. Many phytoconstituents of methnolic extract of *F. vulgare* have been reported for the first time in this work. The results obtained in this work could be useful in standardization of commercial formulations containing fennel extract or fenchone.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest associated with this manuscript.

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