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Full Length Research Paper

GC-MS analysis of fennel essential oil and its effect on microbiology growth in rats' intestine

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The compositions of the essential oil of fennel have been analyzed by ion-trap GC-MS. The main components identified were: Benzene, 1-methoxy-4-(1-propenyl) - (82%), D-Limonene (6.55%), Estragole (3.53%), 3-Carene (1.12%) and 1,6-Octadien-3-ol, 3,7-dimethyl - (1.12%). The effect of the fennel on *Enterococcus*, *Clostridium perfringens* and *Bacillus bifidus* were tested. Fennel essential oils inhibited *Enterococcus* and *Clostridium perfringens* growth in rat's intestine. By contrast, the essential oil promoted *Bacillus bifidus* growth. The results of this study confirmed the possibility of using the fennel essential oils in maintaining useful bacteria colony on rat's intestine.

Key words: *Enterococcus*, *Clostridium perfringens, Bacillus bifidus,* fennel, essential oil, GC-MS.

INTRODUCTION

Fennel (*Foeniculum vulgare*) is a plant belonging to the *Umbelliferae* (*Apiaceae*) family that is known and used by humans since antiquity. It was cultivated in every country surrounding the Mediterranean Sea because of its flavour (Gross et al., 2009).

Ethnobotanical data currently available on wild useful plants in China highlight the importance of fennel' culinary and medicinal uses (Singh and Kale, 2008). Typically, fennel and its preparations are used to cure various disorders, acting as a carminative, digestive, lactogoge and diuretic agent (Javidnia et al., 2003). Moreover, fennel (*F. vulgare*) has been used for centuries in the Mediterranean area as an aromatic herb and also in folk medicine, due to the pharmacological properties of its essential oil. Fennel essential oil (FEO) possesses emmenagogue and galactagogue properties (Ostad et al., 2001; Ramesh Babu et al., 2010) and is also used in the pediatric colic and some respiratory disorders due to its antispasmodic effects (Ostad et al., 2004; Liu et al, 2010; Sajomsang et al., 2009)."

In this study, we analyzed constituents of fennel essential oil by employing GC-MS. Then anti-bacteria activity of the essential oil was evaluated in rat's intestines.

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MATERIALS AND METHODS

Material

Fennel was purchased from a local shop.

GC and GC–MS analysis

The GC analyses were accomplished using a HP-5890 Series II gas chromatography equipped with a FID and HP-WAX and HP-5 capillary columns (30 m × 0.25 mm, 0.25 µm film thickness) working with the following temperature program: 60℃ for 10 min, rising at 5℃/min to 220℃, injector and detector temperatures 250℃, carrier gas, nitrogen (2 ml/min), detector, dual FID and split ratio 1:30. The percentage composition was obtained from electronic integration measurements using flame ionization detection. Alkanes were used as reference points in the calculation of relative retention indices (RRI).

GS-MC analyses were performed under the same conditions with GC using a Hewlett Packard 5890 II gas chromatography equipped with a Hewlett Packard 5972 mass selective detector. Analytic conditions, injector and transfer line temperatures, 220℃ and 240℃, respectively, oven temperature programmed from 60℃ to 240℃ at 3℃/min, carrier gas, helium at 1 ml/min, injection of 0.2 µl (10% hexane solution) and split ratio 1:30. Identification of the constituents was based on the comparison of their retention times and mass spectra with those of authentic samples, NBS75K library

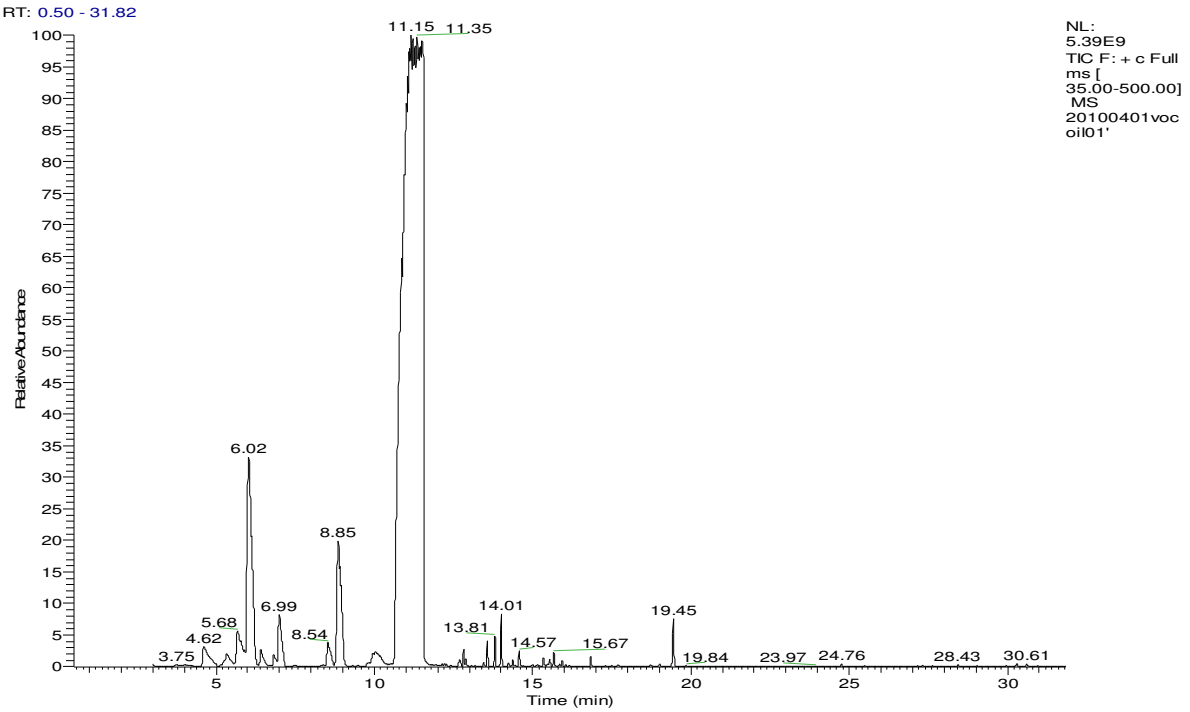

Figure 1. GC–MS analysis of composition of the essential oil of fennel.

data of the GC–MS system and literature data (Table 1).

Animal model design and treatment

A total of 24 Wistar rats (220 – 260 g) were allowed to adapt to our laboratory environment for 1 week before the onset of the experiment. The rats were kept under a constant temperature (23°C) and humidity with 12 h light/darkness cycles. They were allowed commercial chop diet and tap water ad libitum. The animals were randomized into three groups (n = 8): group I, group II and group III. The first group, as a control, was fed wth the rat chow diet only, the second group was fed with the rat chow diet with the addition of 0.2% essential oil and the third group was fed with the rat chow diet mixed with 0.5% essential oil. During the 1-week feeding period, feces produced were collected daily.

Microbiology culture

For experiments, cultures were initiated by inoculating 5 ml trypic soy broth (TSB) with a single colony of from the agar plates. The cultures were grown overnight (18 – 20 h) in a shaker at 35°C and were then expanded by diluting ten-fold into fresh TSB broth. After growth to late log phase (3 – 4 h), the bacteria were pelleted by centrifugation and washed twice in Hanks' balanced salt solution (HBSS, Gibco-BRL, Gaithersburg, MD). The final pellet was suspended in HBSS and the optical density of the culture was read at 600 nm. Using a conversion of an OD of 1.0 being equivalent to 1.25×10^9 bacteria/ml (confirmed for the cultures and spectrophotometer being used), the bacteria were diluted to a density of 2.5×10^8/ml. This final bacterial suspension was kept on ice until the start of the experiment. Control studies demonstrated that the viability of this suspension stored on ice remained constant over at least a 4 h period. The amount of the bacteria in the samples was assessed by the classic CFU assay.

RESULTS AND DISCUSSION

Chemical compounds of essential oil

Water-distilled essential oil from fennel was analyzed by GC–MS and resulted in the identification of 50 compounds representing 96.03% of the oil. The most main compounds of the essential oil were Benzene, 1-methoxy-4-(1-propenyl) - (82%), D-Limonene (6.55%), Estragole (3.53%), 3-Carene (1.12%) and 1,6-Octadien-3-ol, 3,7-dimethyl- (1.12%) (Figure 1 and Table 1).

Effect of fennel essential oil on *Bacillus bifidus* in rat's intestine

No marked change in amount of *Bacillus bifidus* in control rat's intestine was detected within 7 days. The amount of *B. bifidus* in rat's intestine was significantly increased from 1st to 7th day in case of the experimental groups fed diets with 0.2 and 0.5% essential oil, respectively.

Bifidobacteria were first isolated from a breast-fed infant by Henry Tissier who also worked at the Pasteur Institute. The isolated bacterium named *B. bifidus* communis (Killer et al., 2010) was later renamed to the genus *Bifidobacterium*. Experiments into the benefits of probiotic therapies suggest a range of potentially beneficial medicinal uses for probiotics. For many of the potential benefits, research is limited and only preliminary results are available. It should be noted that the effects described are not general effects of probiotics

Table 1. Composition of the essential oil of fennel (GC–MS analysis).

No.	components	RT	Percentage
1	1R-α-Pinene	4.62	0.82
3	β-Pinene	5.34	0.29
4	3-Carene	5.68	1.12
5	D-Limonene	6.02	6.55
6	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	6.41	0.32
7	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	6.82	0.20
8	1,6-Octadien-3-ol, 3,7-dimethyl-	6.99	1.12
9	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	8.54	0.60
10	Estragole	8.85	3.53
11	Benzene, 1-methoxy-4-(1-propenyl)-	10.03	0.78
12	Benzene, 1-methoxy-4-(1-propenyl)-	11.3	82.0
13	Thujopsene-(I2)	11.93	0.01
14	3-Cyclohexene-1-methanol, à,à,4-trimethyl-, propanoate	12.14	0.01
15	Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	12.21	0.01
16	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	12.27	0.01
17	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	12.68	0.08
18	Copaene	12.82	0.12
19	2-Propanone, 1-(4-methoxyphenyl)-	12.89	0.06
20	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]-	13.07	0.01
21	Tetradecane, 2,6,10-trimethyl-	13.19	0.01
22	Di-epi-à-cedrene	13.28	0.00
23	Isocaryophillene	13.46	0.03
24	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	13.58	0.18
25	Caryophyllene	13.81	0.21
26	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-	14.01	0.36
27	2-Propen-1-ol, 3-phenyl-, acetate, (E)-	14.24	0.03
28	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-	14.37	0.05
29	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)-	14.57	0.13
30	(-)-Tricyclo[6.2.1.0(4,11)]undec-5-ene, 1,5,9,9-tetramethyl-(isocaryophyllene-I1)	15.01	0.02
31	Benzene, 1,2-dimethoxy-4-(2-propenyl)-	15.34	0.07
32	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1à,7à,8aà)]-	15.46	0.03
33	à-Farnesene	15.54	0.05
34	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-	15.67	0.10
35	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1à,4aà,8aà)-	15.84	0.02
36	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	15.93	0.04
37	Cedrene	16.05	0.01
38	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1à,4aà,8aà)-	16.37	0.00
39	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	16.84	0.08
40	(-)-Spathulenol	17.29	0.01
41	(-)-Globulol	17.51	0.01
42	Octadecane, 1-chloro-	17.70	0.02
43	.tau.-Cadinol	18.71	0.02
44	à-Cadinol	19.01	0.02
45	1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene	19.45	0.37
46	Tetradecane, 2,6,10-trimethyl-	19.84	0.00

Table 1. Contd.

47	Phthalic acid, 6-ethyl-3-octyl isobutyl ester	20.97	0.00
48	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	24.76	0.02
49	Heptadecane, 2,6,10,15-tetramethyl-	24.86	0.00
50	1,2,3,4-Tetrahydroisoquinolin-6-ol, 1-[3-hydroxybenzyl]-	28.43	0.01

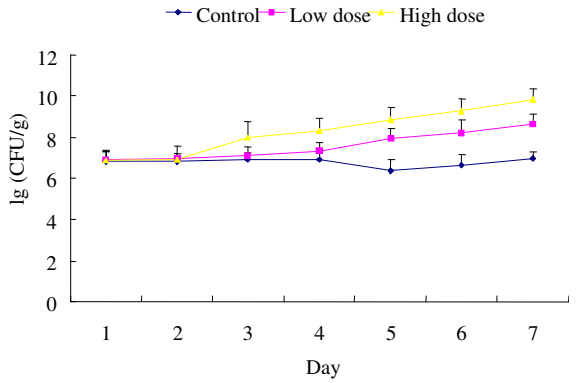

Figure 2. Effect of fennel essential oil on amount of (Lg(CFU/g)) *B. bifidus* in rat's intestine.

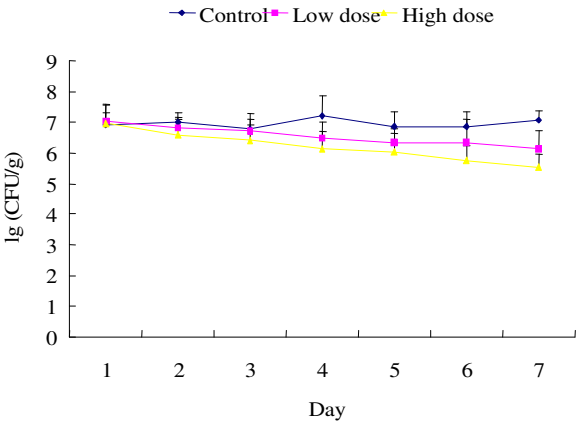

Figure 3. Effect of fennel essential oil on amount of (Lg(CFU/g)) *Enterococcus* in rat's intestine.

(Arunachalam, 1999). Our present study suggested that fennel essential oil could enhance the amount of B. bifidus in rat's intestine (Figure 2).

Effect of fennel essential oil on *Enterococcus* in rat's intestine

The genus *Enterococcus* belongs to a group of micro-organisms known as lactic acid bacteria (LAB). *Enterococci* are gram-positive, non-sporeforming, catalase-negative, oxidase-negative, facultative anaerobic cocci that occur singly, in pairs, or in chains (Klare et al., 2003). In this study, no marked change in amount of enterococcus in control rat's intestine was detected within 7 days. The amount of enterococcus in rat's intestine was significantly decreased from 1st to 7th day in case of the experimental groups fed diets with 0.2% and 0.5% essential oil, respectively (Figure 3).

The use of *enterococci* as probiotics remains a controversial issue. While the probiotic benefits of some strains are well established, the emergence and the increased association of *enterococci* with human disease and multiple antibiotic resistances (see below) have raised concern regarding their use as probiotics. The fear that antimicrobial resistance genes or genes encoding virulence factors can be transferred to other bacteria in the gastrointestinal tract contributes to this controversy (Van Horn and Rodney, 1998).

Effect of fennel essential oil on *Clostridium perfringens* in rat's intestine

Clostridium perfringens is a gram-positive spore forming anaerobic bacterium that is found in soil and in the gastrointestinal tract of humans and animals. It is also an opportunistic pathogen that is responsible for gas gangrene, necrotic enteritis and food poisoning in humans (Wnek and McClane, 1986). *C. perfringens* produces an arsenal of exotoxins, defined asmajor andminor, based on their lethality in mice. The pathogenic properties of the major toxins involve disruption of the target cell membrane or modification of host cell cytoskeleton integrity. In contrast, many minor toxins are carbohydrate-active glycoside hydrolases and their conspicuous role in infection appears to involve degradation of other complex glycans, such as the mucosal layer of the human gastrointestinal tract, which comprises a group of highly hydrated glycoproteins, glycosaminoglycans found throughout the body and other cellular glycans (Nakamura et al., 2004).

In this study, no marked change in amount of C. perfringens in control rat's intestine was detected within 7 days. The amount of C. perfringens in rat's intestine was significantly decreased from 1st to 7th day in case of the experimental groups fed diets with 0.2 and 0.5% essential oil, respectively (Figure 4).

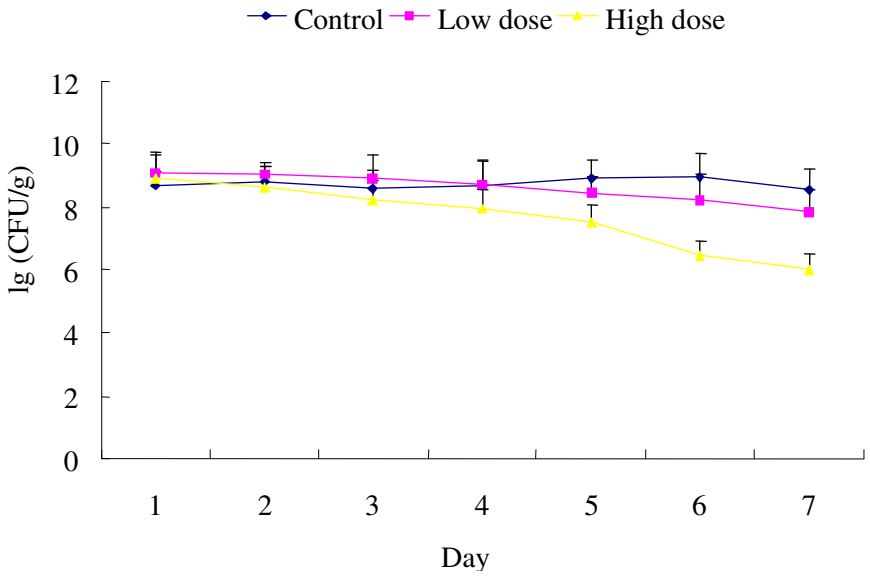

Figure 4. Effect of fennel essential oil on *C. perfringens* in rat's intestine.

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