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Gamma-Irradiation Affects Volatile Oil Constituents, Fatty Acid Composition and Antimicrobial Activity of Fennel *(Foeniculum vulgare*) Seeds Extract.

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ABSTRACT

Foeniculum vulgare (Apiaceae) commonly known as fennel is a well-known and important aromatic plant widely used as carminative and gastrointestinal disorders. This investigation was initiated to study the effect of different gamma radiation doses on volatile oil constituents, fatty acid composition and antimicrobial activity of fennel (F. vulgare) seed extracts. Fennel seeds were irradiated with 0, 5, 10 and 20 kGy gamma radiation. Volatile oils were extracted and analyzed by gas chromatography-mass. The GC-MS analysis in the non-treated sample showed different phytoconstituents mainly, p-allylanisole (71.40%), D-limonene (12. 35%) and ç-terpinene (5.17%). Fatty acids of the extracted oils were separated by gas chromatography mass spectroscopy. The results demonstrated that, all radiation treatments had effect on the fatty acid composition, and some fatty acids did not seem to be affected by the radiation treatments. The antimicrobial activity of the volatile oil and hexane crude extract were assayed. The results showed that, fennel volatile oils exhibited strong and a variable degree of antimicrobial activity compared to the hexane crude extract. This study demonstrated that the treatment of F. vulgare seeds with gamma irradiation could be considered as a useful tool that stimulate and influence the volatile oil composition and the antimicrobial activity.

Keywords: Antimicrobial activity, volatile oil, fatty acids, *Foeniculum vulgare*, gamma-irradiation, GC-MS analysis.

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INTRODUCTION

There is an urgent need to search for new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new infections disease. Indeed, natural crude extracts and biologically active compounds from plant species used in traditional medicine may represent valuable sources for such new preservatives [1]. Recently, spices have also received attention in their useful physiological functions and antimicrobial activity. There are a lot of reports about antimicrobial activity of spice extracts and its volatile oils, and use of natural volatile oils as antimicrobial agents in food systems may be considered as additional intrinsic determinant to increase the safety and shelf life of foods [2, 3]. However, some biologically active compounds isolated from spices and herbs have been in use for the inhibition of growth of pathogenic microorganisms because of the resistance that microorganisms have built against antibiotics [4]. *Foeniculum vulgare* is a biennial medicinal and aromatic plant belonging to the family Umbelliferaceae. It is a hardy, perennial–umbelliferous herb with yellow flowers and feathery leaves. It is widely cultivated throughout the temperate and tropical regions of the world for its aromatic fruits, which are used as a culinary spice [5]. Fennel volatile oil is used in cosmetics, pharmaceuticals, and perfumery and as a food additive and flavoring agent in food products [6]. Recently there has been considerable interest in the antioxidant potential and antimicrobial activities of fennel seed extracts and essential oil [7]. The major components of *F. vulgare* seed volatile oil have been reported to be trans-anethole, fenchone, estragol (methyl chavicol), and a-phellandrene [8]. It is well known that, one accredited preservation method for dried food ingredients is treatment with ionizing radiation, to increase the hygienic quality of herbs and spices by reducing the pathogenic and spoilage microorganisms [9, 10]. However, the influence of irradiation on chemical composition of some spices has been examined. In this concern, Fan et al. [11] reported that γ-irradiation treatments at 0.5, 1,and 2 kGy may act as stress signals and thus leads to a rapid increase of stress-response compounds such as phenols, flavonoids, and other antioxidant compounds in fresh-cut iceberg lettuce (*Lactuca sativa*). Additionally, low doses of γ-irradiation (0.1 KGy) have been reported to stimulate the accumulation of reducing and non-reducing sugars in onion (*Allium cepa*) and potato (*Solanum tuberosum*) plants due to the degradation of oligosaccharides [12]. Irradiated seeds with gamma rays could induce biochemical, physiological and cytological changes, the biochemical contents, i.e, enzyme, protein and phytohormone were severely changed by exposing seeds to gamma rays. For example, low dose of γ-irradiation has been applied to enhance several secondary metabolites compounds at 40 Gy as a potential antioxidant in Culantro (*Eryngium foetidum* L.) plantlets [13]. Moreover, the effects of γ-irradiation on the volatile oil constituents of several spices have been reported in the literature [14, 15]. Also, chromatographic analysis of some herbal extracts indicated that changes in total yield and constituents of volatile oil following irradiation were ranged from none to slight depending upon dose-based irradiation in variety of herbs [16, 17]. The present study was undertaken to investigate the effect of different doses of γ-irradiation on volatile oil constituents, fatty acid composition and antimicrobial activity, and to provide valuable information on the utilization of beneficial effects of γ-radiation on *F. vulgare* seeds.

MATERIAL AND METHODS

Plant material

Foeniculum vulgare seeds were purchased from Harraz Herbs Company (http://www.harrazherbs.com-Cairo, Egypt).

Irradiation treatment

Irradiation was performed using a Gamma cell 200 apparatus equipped with a ^{6o}Co γ source with average dose rate of 0.7 kGy/min at National Center for Radiation Research and Technology, Cairo, Egypt. The given doses were 5, 10 and 20 kGy. Immediately after irradiation treatment the seeds were stored at 4^{o}C for further experimental use.

Extraction of volatile oils

Irradiated and non-irradiated seeds (250 g) underwent water distillation for 3 h using all-glass Clevenger apparatus [18]. The volatile oil was dried over anhydrous sodium sulphate. The oil was stored at 4 ^{o}C until further analysis.

GC–MS analysis

Chromatographic analysis was carried out using a Thermo Scientific capillary gas chromatography (model Trace GC ULTRA) directly coupled to ISQ Single Quadruple MS and equipped with TG-5MS non polar 5% phenyl methylpolysiloxane capillary column (30 m × 0.25 mm ID × 0.25 µm). The operating condition of GC oven temperature was maintained as: initial temperature 40^{o}C for 3 min, programmed rate 5^{o}C/min up to final temperature 280^{o}C with isotherm for 5 min. For GC–MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium was used as a carrier gas at a constant flow rate of 1.0 ml/min. One microliter of diluted oil (1:1, v/v, in diethyl ether) of both irradiated and non-irradiated sample was injected automatically in the splitless mode. Detection was performed in the full scan mode from 40 to 500 m/z. The quantification of the components was based on the total number of fragments (total ion count) of the metabolites as detected by the mass spectrometer. The identification of the chemical components was carried out based on the retention time of each component (R_t) compared with those of the Wiley9 and NIST08 mass spectra libraries [19].

Preparation of hexane extracts

The pulverized irradiated and non-irradiated seeds (20 g) were macerated in 100 ml hexane in glass bottles. The bottles were labeled and put in an orbital shaker (Heidolph – Unimax 2010- Germany) for 24 h at room temperature. The extracts were filtered through Whatman No. 4 filter paper. Residues were re-extracted twice with fresh aliquots of hexane. The pooled supernatants were evaporated under vacuum (Heidolph-Germany) at 40^{o}C. The resulting hexane extracts were stored at 4 ^{o}C until further analysis.

Preparation of fatty acid methyl esters (FAMEs)

Methylation is the most general method of converting non-volatile fatty acids into volatile fatty acids methyl esters (FAMEs) [20]. Hexane extract fatty acids were methylated using benzene: methanol: concentrated sulfuric acid (10:86:4) as derivatizing reagent for 1h at 80-90^{o}C according to Stahl [21].

Fatty acid analysis by gas chromatography (GC)

The fatty acid methyl esters obtained by methylation were analyzed on a Perkin Elmer Auto System XL gas chromatograph provided with a fused silica capillary column ZB-5 (60 m x 0.32 mm i.d) and flame ionization detector (FID). 20 µl of sample was injected in split mode (1:10). Helium was used as the carrier gas at a flow rate of 1 ml/ min, with an injector and detector temperature of 230 and 250 ^{o}C, respectively. The oven temperature was maintained initially at 150^{o}C and programmed from 150 to 240^{o}C at rate 3^{o}C/min, then mentioned at 240^{o}C for 15 min until all peaks had appeared [22]. Peak retention times and areas are calculated by integration of areas under the peaks. Fatty acids composition is calculated from the area of the peak (%). Fatty acid methyl esters were identified by comparing the retention times of samples with authentic references in the same column and the same conditions.

Antimicrobial activity

Microbial strains

The microorganisms used for antimicrobial activity evaluation were obtained from the American type culture collection (ATCC; Rockville-MD-USA) as well as the culture collection of the Agricultural Microbiology Dept., National Research Centre, Egypt. Two Gram-positive bacteria *Staphylococcus aureus* (ATCC- 47077), *Bacillus cereus* (ATCC- 12228), two Gram-negative bacteria *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* strain OS4 as well as one yeast *Candida albicans* (ATCC- 10231) and one fungi *Aspergillus niger* (ATCC- 16888) were used.

Culture medium and inoculums

The stock cultures of microorganisms used in this study were maintained on plate count agar slants at 4^{o}C. Inoculum was prepared by suspending a loop full of bacterial cultures into 10 ml of nutrient agar broth and was incubated at 37^{o}C for 24 h. About 60 µl of bacterial suspensions, adjusted to 10^{6}-10^{7} colony forming

units (CFU)/ml were taken and poured into Petri plates containing 6 ml sterilized nutrient agar medium. Bacterial suspensions were spread to get a uniform lawn culture.

Antimicrobial activity assay

The agar-well diffusion method was applied to detect antimicrobial activity [23]. Wells of 6 mm diameter were dug on the inoculated nutrient agar medium and 100 µl of both hexane extracts and essential oil, dissolved in hexane at concentrations (250, 500 and 1000 µg/ml), were added in each well. The wells introduced with 100 µl of hexane were used as a negative control. The plates were allowed to stand at 4°C for 2 h before incubation with the test microbial agents. The plates were incubated at 37°C overnight and examined for the zone of inhibition. The diameter of the inhibition zone was measured in mm. All the assays were performed in triplicate and expressed as average values ± SD.

Statistical Analysis

All the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD).

RESULTS AND DISCUSSION

Essential oil composition

The data presented in Table (1) showed that the different gamma ray doses treatments had a generally favorable effect on the composition of essential oil.

Table 1: Volatile compounds identified in irradiated and non-irradiated seeds of *F. vulgare*.

No.	R_t [a]	Compounds name	Relative area %[b]			
			5 kGy	10 kGy	20 kGy	Control
1	4.61	2-Thujene	0.22	0.21	0.13	0.19
2	4.81	α-Pinene	1.45	1.18	1.35	1.37
3	5.27	Camphene	0.06	0.05	0.05	0.05
4	5.85	Sabinene	0.40	0.33	0.36	0.36
5	6.02	2-α-Pinene	0.16	0.15	0.12	0.15
6	6.29	α-Myrcene	0.59	0.47	0.38	0.46
7	6.87	l-Phellandrene	0.22	0.20	0.18	0.20
8	7.19	α-Humulene	0.62	0.65	0.37	0.55
9	7.50	o-Cymene	1.01	1.08	0.58	0.88
10	7.60	D-Limonene	12.20	11.00	12.29	12.35
11	7.73	Cineole	0.50	0.52	0.56	0.56
12	7.80	Cis-Ocimene	0.21	0.15	0.20	0.17
13	8.61	ç-Terpinene	5.90	6.12	3.74	5.17
14	9.15	Trans-Sabinene hydrate	0.05	0.04	-	0.02
15	9.59	p-Mentha-1,4-(8)-diene	0.04	0.03	0.03	0.03
16	9.88	L-Fenchone	5.46	5.36	5.34	5.55
17	10.37	α-Linalool	-	0.02	-	0.02
18	11.28	D-Fenchyl alcohol	0.04	0.04	0.04	0.04
19	11.76	Trans-Limonene oxide	0.04	0.04	0.03	0.03
20	12.28	(-)-Alcanfor	0.11	0.11	0.11	0.11
21	13.57	4-Terpineol	0.10	0.08	0.06	0.07
22	14.43	p-Allylanisole	69.89	71.62	73.60	71.40
23	15.53	Estragole	0.07	0.07	0.07	0.07
24	16.63	Anethole	0.04	-	-	-
25	18.86	Carvacrol	0.42	-	-	-
26	19.04	Thymol	-	0.32	-	-
27	19.35	Durenol	-	-	0.12	-
28	19.42	Phenol, 2-methyl-5-(1-methylethyl)-(CAS)	-	-	0.11	-
29	19.61	Phenol, 5-methyl-2-(1-methylethyl)-(CAS)	-	-	0.08	-
30	20.51	α-Terpinenyl acetate	-	-	-	0.08
31	23.30	Trans-Caryophyllene	0.21	0.15	0.12	0.12

[a] R_t: retention time (min).
[b] The percentage composition was computed from the gas chromatography peak areas.

Thirty one compounds constituting about 100% of the volatile oil were identified in both irradiated and non-irradiated (control) seeds of *F. vulgare* by GC-MS analysis. Data presented in Table (1) revealed that volatile oil of *F. vulgare* seed at control treatment contained mainly p-allylanisole (71.40%), D-limonene (12.35%), L-fenchone (5.55%) and ç-terpinene (5.17%) as predominant compounds. The chemical composition of essential oils of *F. vulgare* seeds was investigated previously by different authors and the results were; trans-anethole (65.4%), fenchone (8.26%), estragole (5.2%) and limonene (4.2%) represented the main components [24]; trans-anethole (69.87%), fenchone (10.23%), estragole (5.45%) and limonene (5.10%) as the major constituents [25]; trans-anethole (62.0%), fenchone (20.3%), estragole (4.90%) and limonene (3.15%) as the main components [26]. However, concerning the effect of irradiation in the volatile oil composition of *F. vulgare* in the present study, p-allylanisole, D-limonene, L-fenchone and ç-terpinene represented the major compounds in both irradiated and non-irradiated (control) *F. vulgare* seeds (Table 1). Different doses of γ-radiation showed diverse effect on the number and relative area % of volatile oil compounds of *F. vulgare*. Gamma irradiation resulted in the appearance of anethole and carvacrol at 5 kGy dose; thymol at 10 kGy dose as well as durenol, phenol-2-methyl-5-(1-methylethyl)-(CAS) and phenol-5-methyl-2-(1-methylethyl)-(CAS) at 20 kGy dose as compared to disappearance of these compounds in the control seeds (Table 1). Generally, treatment of *F. vulgare* seeds with higher radiation doses (20 kGy) had been suggested to have a dose-dependent reduction effect on the relative area % of volatile oil compounds of *F. vulgare* volatile oil content (Table 1).

It caused a decline in some compounds such as ç-terpinene which declined from 5.17 % at control treatment to 3.74 % at 20 kGy. Contrarily, there was a gradual increase in p-allylanisole at 20 kGy in addition to minor fluctuations were observed in D-limonene and L-fenchone. The diverse effects of irradiation in the volatile oil content in the present study are in consensus with the results of Seo et al. [27] who reported the variable response of volatile compounds of *Angelica gigas* Nakai for irradiation, where β-eudesmol, α-eudesmol, and verbenone were increased after irradiation, however, α-pinene and 2,4,6-trimethyl heptane were decreased after irradiation. Similarly, the variable effects of irradiation on the volatile contents of *Curcuma longa, Coriandrum sativum* L and *Allium fistulosum* L were reported [17, 28, 29].

The variable percentage of some volatile compounds as a result of irradiation in the present study might be attributed to the sensitivity of these compounds to the used radiation doses. Similar results have been reported formerly on the volatile constituents of irradiated herbs [27, 29].

Fatty acids content

Table 2: Fatty acid compositions (% of total fatty acids) of irradiated and non-irradiated seeds of *F. vulgare*.

Fatty acids	% of total fatty acids			
	5 kGy	10 kGy	20 kGy	Control
Myristic acid (C14:0)	1.04	ND	1.16	1.13
Palmitic acid (C16:0)	12.91	11.82	12.18	11.68
Margaric acid (C17:0)	2.84	11.62	8.05	5.45
Stearic acid (C18:0)	3.09	10.11	2.98	2.89
Arachidic acid (C20:0)	5.12	ND	5.07	4.83
∑SFA	**25**	**33.55**	**29.44**	**25.98**
Palmitoleic acid (C16:1)	ND	19.92	0.73	0.61
Oleic acid (C18:1)	62.11	40.68	59.18	61.74
∑MUFA	**62.11**	**60.6**	**59.91**	**62.35**
Linoleic acid (C18:2)	12.89	5.85	10.65	11.68
∑PUFA	**12.89**	**5.85**	**10.65**	**11.68**

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; and ND, not detected.

The constituents of *F. vulgare* fatty acids as influenced by gamma radiation are presented in Table (2) and expressed as the mean percentage value of each fatty acid with respect to the total fat content. The predominant fatty acids in non-irradiated seeds (control) were oleic, palmitic and linoleic acids, while margaric and arachidic acids were found in moderate ratio. The fatty acids compositions reported in this study are in line with the results from prior studies [30, 31, 32]. As for the effect of gamma radiation, fatty acids profile was the same in irradiated and non-irradiated seeds except the disappearance of palmitoleic acid at 5 kGy dose in addition to disappearance of both myristic and arachidic acids at 10 kGy dose (Table 2). Compared to the non-irradiated seeds (control), the 10 and 20 kGy doses increased the saturated fatty acids and decreased the

mono and poly-unsaturated fatty acids, while the 5 kGy dose increased polyunsaturated fatty acids. Moreover, oleic acid content fluctuated due to different radiation doses under study. In addition, linoleic acid content increased due to exposure to gamma radiation doses at 5 KGy (Table 2). It is well known that, linoleic acid is an essential fatty acid and originates the omega-6 fatty acids series. The dietary ω-6 fatty acids play a role in lowering both of hypertension and systolic blood pressure [33]. Also, the antitumor properties of dietary ω -6 fatty acids against different cell lines were reported [34, 35]. Therefore, the 5 kGy dose in the present study seems to be the most effective dose for the fennel radiation because it increased the linoleic and oleic acids that will increase the potential health benefits of fennel due to their valuable nutritional composition in essential fatty acids. In the present study, the different doses of gamma irradiation showed variable effect on fatty acids compositions of fennel.

The present findings agree with previous studies of Afify et al. [36] who found that gamma irradiation reduced and increased some fatty acids in soybean, peanut and sesame seed oils. Alternatively, the non-significant effects of irradiation on the fatty acids compositions were reported [37, 38, 39].

Antimicrobial activity

The antimicrobial activity of the volatile oils and hexane crude extracts of irradiated and non-irradiated seeds of *F. vulgare* were examined by agar well diffusion method against the tested microorganisms selected. The data expressed as diameter of growth inhibition zone (mm). The results presented in Tables (3), revealed that volatile oils of irradiated and non-irradiated seeds of *F. vulgare* exhibited variable antimicrobial activity against *B. cereus, S. aureus, E. coli* and *C. albicans* but it was ineffective against *P. aeruginosa* and *A. niger* at concentration levels up to 1000 µg/ml. The antimicrobial activity of volatile oil of *F. vulgare* may be due to its main components of p-allylanisole, D-limonene and L-fenchone or any other components presented in Table (1). The antifungal activity of *F. vulgare* volatile oil, which is rich in trans-anethole was reported [40]. Also, antimicrobial activity of limonene, α-pinene and fenchone individually was reported [41]. Table (4) shows that hexane extracts of irradiated and non-irradiated seeds of *F. vulgare* can inhibit the growth of *B. cereus, S. aureus* and *E. coli*. However, the hexane extracts were inactive against *C. albicans* and have limited activity against *P. aeruginosa* and *A. niger* at concentration levels up to 1000 µg/ml. The antimicrobial activity of *F. vulgare* hexane extract in this study could partly be accounted for by the presence of the free fatty acids, presented in Table (2), the antimicrobial activity of different types of free fatty acids (FFAs) has been reported [42]. FFAs may further affect the expression of bacterial virulence factors that are important or essential for the establishment of infection, probably by disrupting cell-to-cell signalling. Thus, saturated and unsaturated FFAs can prevent initial bacterial adhesion and subsequent biofilm formation [43, 44]. In addition to FFAs, some other components that cannot be detected by Gas Chromatography (GC) may also contribute to the antimicrobial activity of the hexane extract. In general, the diameters of growth inhibition zones (mm) were depend on the concentration and 1000 µg/ml was the most effective concentration for the antimicrobial activity in the present study. Also, *B. cereus, S. aureus* and *E. coli* were the most sensitive strains to volatile oil and hexane extracts, presenting the largest inhibition zones especially at 1000 µg/ml concentration. The present results are in agreement with the previous literatures reported the antimicrobial activity of fennel volatile oil and extracts [24, 25, 45].

Regarding the effect of gamma irradiation on the antimicrobial activity of volatile oil and hexane extracts of *F. vulgare,* the common results (Tables 3 and 4) showed that gamma irradiation increased the antimicrobial activity against *B. cereus* and *S. aureus* strains and decreased the activity against *E. coli* as compared to the control. Also, volatile oil of irradiated *F. vulgare* at 10 and 20 kGy doses showed growth inhibition zones (17.11±1.8 and 18.33±1.6 mm), respectively, against *C. albicans* at 1000 µg/ml concentration. However, the volatile oil of non-irradiated *F. vulgare* has no detectable growth inhibition zone against *C. albicans* at the same concentration (Table 3). Furthermore, the hexane extract at 5 kGy irradiation dose showed growth inhibition zone (10.33±0.57 mm) against *A. niger* at 1000 µg/ml concentration as compared to no inhibition zone at non-irradiated hexane extract at the same concentration (table 4). The gamma irradiation might be made some chemical modifications in volatile oil and hexane extract constituents, stimulating their activity against *C. albicans* and *A. niger*, respectively, similar conclusions were reported [46].

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Table 3: Antimicrobial activity of volatile oil of irradiated and non-irradiated seeds of *F. vulgare.*

Microbes	Inhibition zone (mm)											
	250 µg/ml				500 µg/ml				1000 µg/ml			
	5 kGy	10 kGy	20 kGy	Control	5 kGy	10 kGy	20 kGy	Control	5 kGy	10 kGy	20 kGy	Control
B. cereus	10.33± 0.58	10.33± 0.58	NI	9.67± 1.45	13.67± 1.16	13± 1.2	8± 1.1	12.33± 0.58	16± 1.7	15.33± 0.6	14± 1.4	13±1.3
S. aureus	20.33± 1.58	NI	12.33± 1.51	NI	21.33± 0.58	NI	14±1.5	9.67± 1.53	22± 2.5	NI	26± 2.2	13.67±2. 1
E. coli	10± 1.2	10.33± 0.58	NI	10±1.2	10.33± 0.55	11.33± 0.52	7.33± 1.53	11.33±0. 58	13.3 3±0. 6	12.33± 0.6	12± 1.1	15±1.7
P. aeruginosa	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
C. albicans	NI	NI	NI	NI	NI	10.33± 1.53	15.67± 2.01	NI	NI	17.11± 1.8	18.3 3±1. 6	NI
A. niger	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

The diameter of the well (6mm) is included. NI: No inhibition zone.
The values are expressed as means ±SD.

To our knowledge, no information in the literature is available on the effect of gamma irradiation on the antimicrobial activity of volatile oil and hexane extract of *F. vulgare.* However, the effect of gamma radiation on the antimicrobial activities of some other plants was reported. In this anxiety, Khattak and Simpson [47] showed that the radiation treatments up to a dose level of 20 kGy have no effect on the antibacterial activities of *Glycyrrhiza glabra* root; however the 25 kGy dose showed some enhancement in the antibacterial activity of *G. glabra* against *Micrococcus luteus* strain. Also, the results of An et al. [48] indicated that the irradiated green tea polyphenols at 40 kGy showed a higher anti-microbial activity than did the non-irradiated samples. Otherwise, the gamma-radiation up to 10 kGy dose showed no effect on the antimicrobial activity of the *Nigella sativa* seeds and *Camellia sinensis* [49, 50].

Table 4: Antimicrobial activity of hexane crude extracts of irradiated and non-irradiated seeds of *F. vulgare.*

Microbes	Inhibition zone (mm)											
	250 µg/ml				500 µg/ml				1000 µg/ml			
	5 kGy	10 kGy	20 kGy	Control	5 kGy	10 kGy	20 kGy	Control	5 kGy	10 kGy	20 kGy	Control
B. cereus	NI	NI	NI	NI	11.67 ±1.2	NI	8±.098	NI	12.67± 1.5	11.33± 0.58	14.33± 1.6	8±.61
S. aureus	NI	NI	NI	NI	8±1.1	10.33± 0.56	11±1.1	13±1.6	9.667± 0.58	11±1.1	13.33± 0.59	18±1.8
E. coli	NI	NI	NI	NI	10.33 ±0.57	10.67± 0.58	11.33± 0.58	12.67±0. 59	11.67± 2.11	11.33± 1.2	13±1.2	16±2.1
P. aeruginosa	NI	NI	NI	NI	NI	NI	9±1.4	NI	NI	11±1.3	14±1.4	14±1.9
C. albicans	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
A. niger	NI	NI	NI	NI	NI	NI	NI	NI	10.33± 0.57	NI	NI	NI

The diameter of the well (6mm) is included. NI: No inhibition zone.
The values are expressed as means ±SD.

CONCLUSIONS

This is the first report about the effect of gamma irradiation on the volatile oil constituents and free fatty acids content as well as on the antimicrobial activity of volatile oil and hexane extracts of seeds of *F. vulgare*. The main compounds of the volatile oil were p-allylanisole, D-limonene, L-fenchone and ç-terpinene in both irradiated and non-irradiated (control) seeds of *F. vulgare.* The treatment of *F. vulgare* with high radiation doses (20 kGy) showed a dose-dependent reduction effect on the relative area % of volatile oil compounds in addition to a gradual increase in p-allylanisole content. The 10 and 20 kGy doses increased the saturated fatty

acids and decreased the mono and poly-unsaturated fatty acids, while the 5 kGy dose increased polyunsaturated fatty acids in the hexane extracts of *F. vulgare*. The gamma irradiation increased the antimicrobial activity against *B. cereus* and *S. aureus* strains and decreased the activity against *E. coli* as compared to non-irradiated volatile oil and hexane extracts which used as control. The present results concluded that the gamma irradiation up to doses of 02 kGy can be considered as a food preservation method while keeping the chemical composition and antimicrobial properties of *F. vulgare* volatile oils and extracts.

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