

Chemical compositions and biological activities of the essential oils from gamma irradiated celery (*Apium graveolens* L.) seeds

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Abstract

Celery seed oils can help in detoxification processes of the body as it facilitates the elimination of toxins and other harmful substances out of the body. This study aimed to assess biological activity of essential oils extracted from un-irradiated and irradiated celery seeds by gamma rays. Celery seeds were irradiated with different dose levels of gamma radiation; control, 2.5, 5.0, and 10.0 kGy. The highest increase of essential oil extracted was registered with a dosage level of 10.0 kGy (2.42%). The GC-MS analysis showed that, there were new compounds detected in the irradiated samples and others compounds were increased or decreased. The FTIR spectra showed alterations in functional groups of essential oils extracted from celery seeds upon gamma-irradiation. Total phenols, total flavonoids and antioxidant activity increased proportional to the increase in the irradiation dose level and the maximum increase obtained in the irradiation dose level 10.0 kGy. This may be an evidence on the biological value, there were anticancer activity against 2 cell lines, breast cancer cell line (MCF-7) and lung cancer cell line (A549). The best IC₅₀ in A549 was 145 µg/ml in sample with irradiation dose level 5.0 kGy while the best IC₅₀ in MCF-7 was 124 µg/ml in irradiated sample with dose 5.0 kGy. Also, the highest inhibition zone of *S. aureus*, *B. subtilis* and *K. pneumoniae* were obtained with the oil extracted from seeds irradiated with 5 kGy dose level, where *E. coli* and *C. albicans* were more pronounced for the oil gotten from the seeds irradiated with 10.0 kGy. The irradiation process for celery seeds may be promising for biological value of celery oils.

Keywords: anticancer activity; antioxidant activity; *Apium graveolens*; gamma irradiation

Introduction

Spices and herbs have been used in several different ways, since the early times spices and herbs have been added to food to improve flavour and enhance their organoleptic properties (Ene-Obong *et al.*, 2018). Antioxidants from herbs are a great group of bioactive compounds that consist of phenolic compounds, flavonoids, sulphur-containing compounds, alkaloids, tannins, phenolic diterpenes, and vitamins

(McCormick, 2017). Celery (*A. graveolens* L.) is a plant of the Apiaceae family grown in tropical and subtropical regions of Africa, Asia, Central Europe, Western India and the Northwest Himalayas (Gauri *et al.*, 2015; Dolati *et al.*, 2018). It is one of the widespread aromatic vegetables and part of the daily regime around the world (Tashakori-Sabzevar *et al.*, 2016). The complete plant or its seeds have been used as medicine or for food purpose (Hassanen *et al.*, 2015). The celery seed extracts oils, ranged from 1.8 to 3.4% of volatile or essential oils and this oil contain (20% selinene and 60% limonene (Powanda *et al.*, 2015). Medical benefits of celery include prevention of coronary and vascular diseases; their phytochemical constituents consist of bergapten, flavonoids, glycosides, furanocoumarins, furocoumarin, limonene, psoralen, xanthotoxin, and selinene. Some of their pharmacological properties include anticancer, antioxidant, antimicrobial, antifungal, nematocidal, anti-rheumatism, antiasthma, anti-bronchitis, hepatoprotective, appetizer, anticonvulsant, antispasmodic, breast milk inducer, anti-jaundice, antihypertensive, anti-dysmenorrhea, prevention of cardiovascular diseases, and spermatogenesis induction (Salehi *et al.*, 2019). In the same concern, the celery oil has a most important bioactive components named phthalide that show beneficial effect to health because it shows protection against cholesterol level, cancer and high blood pressure. The most active compound of phthalide is sedanolide which reduce the tumours in the cancer patients. The 3-n-butyl phthalide and sedanolide are two main active components present in the seed oil of this plant and they have high activity to stimulate the detoxifying enzyme in the target tumour tissues called glutathione S transferase (GST) (Dąbrowska *et al.*, 2020).

Celery contains distinctive phytochemical compounds, in specific polyphenols (such as flavonoids, phenolic acids and tansioopropanoids) which collect free radicals and lead to high antioxidant activities (Nickavar *et al.*, 2007). Polyphenols provide biological effects, these effects are inducers for controlling free radicals and peroxidation, in particular the antioxidant activities. In general, polyphenols have identical chemical properties, meaning that one or more phenolic groups may interact with donors of hydrogen and neutralize free radicals (de Almeida *et al.*, 2005). There is information that various environmental stresses, including gamma irradiation, have altered the activities of substances involved in the reactive oxygen species (ROS) scavenging (Aly and El-Beltagi, 2010).

This activity is owing to the capability of these materials to decrease oxidative stress by scavenging or neutralizing of reactive species by hydrogen donation, before they attack biological components of cells (Kesba and El-Beltagi, 2012). In this meaning, there is excessive concern in discovery of natural antioxidants from plant materials, and many extracts and isolated compounds have been considered for their antioxidant activity, via different methods (El-Beltagi, 2011; Aly *et al.*, 2012; El-Beltagi *et al.*, 2012; El-Beltagi *et al.*, 2019). Many researches showed that certain herbs and medicinal plants have beneficial effects on liver disorders, renal diseases, anaemia, neurologic and mental disorders (Saki *et al.*, 2014; Wu *et al.*, 2014; Lone *et al.*, 2015; Mansouri *et al.*, 2015). The usage of *A. graveolens* was recommended as a nutraceutical or dietary supplement for the management of hyperuricemia (Dolati *et al.*, 2015). The metabolic profile of celery such as sugars, phenols polyphenols, amino acids, organic acids, sterols, fats, phathelides and flavonoids were detected at various concentrations in different parts have been found to reflect possible uses as nutraceutical sources (Ingallina *et al.*, 2019). A study has shown that the celery seed extract, can inhibit lipid peroxidation, decrease the level of plasma malondialdehyde and finally, act as a pulmonary fibrosis inhibitor (Javadi *et al.*, 2015).

Food irradiation becomes a way to protect food that has developed since earliest decades of the 20th century. If implemented properly, irradiation may treat a variety of food supply problems, such as infestation of insect grains, potato sprouting, rapid fruit maturation and microbial growth (Luckman *et al.*, 2002; Mahindru, 2005; Afify *et al.*, 2012; Afify *et al.*, 2013). Radiation therapy with gamma radiation or electron beam is an extraordinary function for decontamination of food after harvest between modern non-thermal methods. Gamma irradiation as a phytosanitary treatment has been proven to be safe and effective in improving the hygienic quality of various foods and herbal materials in order to extend their shelf life (Douar-Latreche *et al.*, 2018). Under normal conditions, γ -radiation by ^{60}Co is used to spices and herbs and around 50% of overall amount of irradiated food globally is dry herbs and vegetables (El-Beltagi *et al.*, 2018; Rezk *et al.*, 2019). Gamma

irradiation method is permitted to decontaminate dehydrated aromatic herbs, spices, and vegetable flavourings with a maximum total absorbed dose of 10.0 kGy, but the U.S. Food and Drug Administration (FDA) have increased this limitation for decontamination of dried food and spices by up to 30 kGy (Alloun *et al.*, 2019). Specific effects can be observed on the secondary metabolites in different essential oils, even though submitted to the same radiation conditions. The content of the constituents upon radiation is presumably due to its radiation sensitivity at different dose levels (Ameer *et al.*, 2020).

Aim of this research was to evaluate the impact of various dose levels of γ -irradiation on chemical composition of oil extracted from celery seeds and whether these treatments have an enhancement on the biological activities or not. The GC-MS, total phenolics, flavonoids, antioxidant activity, of the celery oils have been studied. Also, antimicrobial and anticancer effects of the celery oil extract were evaluated.

Materials and Methods

Source of samples

Celery (*A. graveolens*) seeds were obtained from the local market (traditional and folk medicine plants store) in Cairo, Egypt. *Apium graveolens* was botanically characterized by Dr. Samah Azooz from Botany Department, Faculty of Agriculture, Cairo University, Cairo-Egypt.

Chemical and solvents

Diphenyl-2-Picrylhydrazyl (DPPH), sodium carbonate, Folin-Ciocalteu's phenol reagent, gallic acid, aluminium chloride hexahydrate ($\text{AlCl}_3 \cdot 6 \text{H}_2\text{O}$), gallic acid, quercetin, penicillin G potassium + streptomycin, trypan blue, neutral red, glacial acetic acid, diphenyl and dimethyl poly siloxane were obtained from Sigma-Aldrich (Sigma-Aldrich, Milan, Italy). Phosphate buffer saline. calcium and magnesium free, trypsin-EDTA Mueller-Hinton agar Gibco (Thermo Fisher Scientific, USA). Complete media of DMEM supplemented with fetal bovine serum (SeraLab-Bio-Connect B.V., Begoniaaan, Netherlands). Sodium nitrite, gentamycin, nystatin, ethanol HPLC grade and sodium hydroxide were supplied by Roth company (Overland Park, KS, United States).

Irradiation treatment

Fresh celery seeds were packed in polypropylene bags, they were irradiated by a ^{60}Co source for irradiation at different dose levels; control, 2.5, 5.0 and 10.0 kGy (Seo *et al.*, 2007) using research irradiator (^{60}Co Gamma cell 220, Canada). Temperature and dose rate were 25 ± 1 °C and 1.2 kGy/h, respectively, for all samples. Irradiation treatments have been done at Atomic Energy Authority, National Center for Irradiation Research and Technology, Nasr City, Cairo- Egypt.

Essential oil extraction

Celery essential oil (CEO), the mature and healthy fruits of celery (100 g five times for each treatment) were crushed and used to extract the essential oil. By Hydro-distillation process separately using a Clevenger-type tool based on method described by British Pharmacopoeia (British Pharmacopoeia Commission, 1993). The EOs samples were dehydrated over anhydrous sodium sulphate and placed in dark glass vials in a refrigerator at 4 °C till they were used in various analyses. Yields of the celery EO have been estimated.

Separation and identification of oil constituents extracted from un-irradiated and irradiated celery seeds

Gas chromatography/mass spectrometry (GC-MS) analysis of celery seed oils extracts was performed using an Agilent 7000 Series Triple, Quad Gas Chromatograph interfaced with a Mass Spectrometer (GC/MS/ Santa, Clara, CA, USA) prepared capillary column (30 x 0,25 μm ID x 0,25 μm df) was fused with an Elite-5MS (5% diphenyl/ 95% dimethyl poly siloxane). An electron ionizing device with ionizing energy of

70eV was used for the GC-MS identification. Helium gas (99.99%) has been used as carrier gas at a 1.0 ml/min fixed flow rate and 2 µl injection volume (10:1 split ratio); injector temperature was 250 °C; ion-source temperature was 200 °C. Automated oven temperature from 110 °C (iso thermal for 2 min) varying from 10 °C/min to 200 °C, then 5 °C/min to 280 °C, finishing with 9 min isothermal at 280 °C, mass spectrum was taken at 70eV: scanning period of 0.5 seconds and remains of 45 to 450Da, total GC running time of 36 minutes. Relative percentage of every constituent was determined by associating their average peak area with the entire areas. Turbo mass was program approved for handling mass spectra and chromatograms. Mass spectrum GC-MS description was seen using National Institute of Standard and Technology (NIST) database that contains more than 62,000 patterns. Unknown spectrum of components kept in NIST library. Title, structure and molecular weight of test resource constituents is determined (Bagavathi and Ramasamy, 2012).

Fourier Transform Infrared (FTIR) spectroscopy

The FTIR analysis method uses infrared light to scan test samples and observe chemical properties. The infrared spectra were investigated by FTIR spectrometer, Spectrum One, Perkin Elmer, USA, over the range of 500-4000 cm⁻¹. A dry constant weighted sample was mixed with 3 mg of KBr and pressed to form a transparent disk (Hikima *et al.*, 2019).

Total phenol content

Folin Ciocalteu's method was used to assess the total content of phenols as defined by (Singleton and Rossi, 1965). One ml of ethanol containing 50 µg of oil sample was mixed with 0.5 ml of Folin-Denis and 1.0 ml of concentrated Na₂CO₃ solution, total volume was adjusted to 10 ml by distilled water. The absorbance was evaluated at 765 nm against the blank after an hour. Mean of three readings has been used to measure total phenol content, as gallic acid (GAE)/100 g oil extract equivalents.

Total flavonoid content

Aluminium trichloride method was used to determine total flavonoids content as mentioned (Zhishen *et al.*, 1999; Park *et al.*, 2006). Ethanol (0.5 ml containing 50 µg of oil) sample was combined with 0.3 ml of 5% sodium nitrite. Five minutes later, 0.3 ml of 10% aluminum chloride was added, 2 ml of 1.0 M sodium hydroxide was added after 6 min, and the complete volume with distilled water was made up to 5.0 ml. The absorption of the blend against the reagent blank was recorded at 510 nm using a spectrophotometer (Jasco V-530, Japan). The mean of three readings was used to calculate the total flavonoids content, which was expressed as g quercetin equivalents (QUE)/100g of oil.

DPPH free radical scavenging activity

The *A. graveolens* oils extract's antioxidant activity was measured using stable DPPH method in terms of hydrogen donating or radical-scavenging ability (Park *et al.*, 2006). Reaction mix containing 1.0 ml of extract at concentrations (10, 15 and 25 µg/ml) + 1.0 ml of DPPH (0.2 mM) was shaken and incubated at room temperature in the dark for 30 min. Absorbance was recorded at 517 nm utilizing UV-visible spectrophotometer (Jasco V-530, Japan). Radical scavenging activity was defined as the inhibition percentage and estimated utilizing equation below:

$$\%DPPH = [\text{Absorbance of Control} - \text{Absorbance of Sample} / \text{Absorbance of Control}] \times 100$$

Antibacterial activity

The agar-disc diffusion procedure measured the antimicrobial activity of the non-irradiated and irradiated seed oils extract of *A. graveolens*. The used bacterial strains *Staphylococcus aureus* and *Bacillus subtilis* as Gram-positive, *Escherichia coli* and *Klebsiella pneumoniae* as Gram-negative and fungi (*Candida albicans*) which were previously isolated and characterized at Microbiology Department, NCRRT, Atomic Energy Authority, Cairo, Egypt. Agar disc diffusion method has been used to assess antibacterial and antifungal

activities of the oil samples as defined (Bauer, 1966; Wistrieck, 1997). The strains were grown for 24 hours at 37 °C on Mueller-Hinton agar slants, and checked for purity. After incubation cells were washed off the agar surface, and then suspended in a sterile physiological solution. Volume of cells determined by the McFarland nefelometer in 1 ml of suspension for inoculation was 5~107 CFU/ml. One ml of such suspensions has been homogenized with 9 ml of melted Mueller-Hinton agar (45 °C) and dispensed into Petri dishes. Applied and impregnated with 15 µl of samples on the agar surface paper disks (HiMediaR, Mumbai, India), with a diameter of 5 mm. For each indicator strain plates were incubated at optimum temperature and evaluated after 24, 48 and 72 h. Growth inhibition has been scored positive and expressed in mm in existence of a clear zone (ZI) detectable across disk. Experiments were tripled car-outs, and reported an inhibition zone as mean replicate ± SE.

Anti-cancer assay

Lung cancer cell line (A549), and Breast cancer cell line (MCF-7) human cancer were bought from CURP, faculty of agriculture at Cairo University (Egypt). Cells were stored in (DMEM) append 10% fetal bovine serum heat-inactivated, 100 µg/ml streptomycin, and 100 unit/ml penicillin g potassium, 90% humidified and 5% (V/V) CO₂ at 37 °C. Neutral red (NR) assay was used to test the cytotoxicity of ethanolic extracts as mentioned before (Repetto *et al.*, 2008). Using 0.25 percent trypsin-EDTA, exponentially growing cells were collected and seeded at 20000 cells/well in 96-well plates. Extracts were added in concentrations (1000, 500 and 250 µg/ml) after incubation (overnight); 4 wells for every concentration. Following 24 h treatment with oil extracts, media were extracted and cells were exposed for 4 hours to a neutral red solution at 37 °C. Destination solution has been used to remove stained NR cells at 540 nm microplate reader (Biotek, ELX808, United States), and measure colour.

Statistical analysis

Values are analysed as means ± SE or SD. Statistical analysis was done utilizing “costat” statistic computer program. Statistical analysis was established on One-way analysis of variance ANOVA followed by Student–Newman–Keuls test, and least significant difference (LSD) at P < 0.05.

Results and Discussions

Essential oils (EO) yield

Yield of essential oils derived from un-irradiated and irradiated (2.5-10.0 kGy) celery seeds ranged from 1.90-2.42% as shown in (Figure 1). The highest increase was seen with a dosage level of 10.0 kGy (2.42%). The highest yield of EOs extracted from the irradiated samples was generally attributed to radiation-induced disruption of cell wall structure, giving higher extractability of oils from celery seeds. In addition, variations in the EOs extraction yield may be attributed to a time-recombination of radiolytic materials. Relevant effects may be observed on secondary metabolites on various essential oils, even though they are submitted under same radiation conditions. Content of irradiated sample constituent is possibly due to its susceptibility to specific doses of radiation (Araujo *et al.*, 2017). Effects of gamma radiation on constituents of EOs depend on various factors, such as exposure to radiation, dose rate, vegetable species, temperature and sample state. γ-radiation exposure will raise or reduce extraction yield of EO and its constituents (Yalcin *et al.*, 2011).

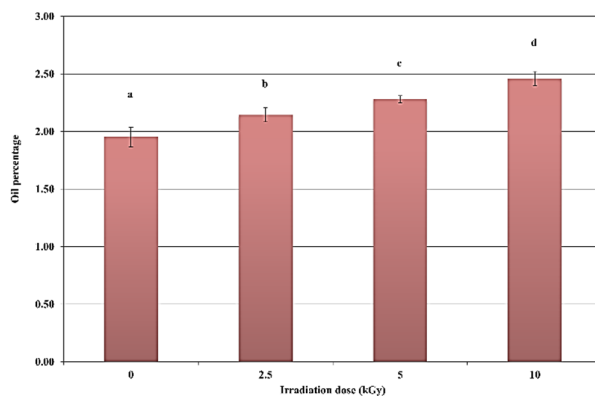


Figure 1. Yield percentage of essential oils extracted from un-irradiated and gamma irradiated *A. graveolens* seeds at different dose levels; control, 2.5, 5.0 and 10.0 kGy. Data are given as mean \pm SE (n = 3). Various letters above the bars denote significant differences among four treatments at the $p < 0.05$.

Identification of essential oil extracted from un-irradiated and irradiated celery seed oil constituents

Chromatographic analysis of essential oil extracted from un-irradiated and irradiated *A. graveolens* seeds revealed that there were qualitative and quantitative changes between un-irradiated and irradiated samples presence of many compounds, Table 1 and Figure 2. In addition, the analysis of *A. graveolens* oils highlighted four main components representing 39.02% to 58.1% of the total oil: 3-methylene-bicyclo [3.2.1] oct-6-en-8-ol (9.8-16.9%), isolongifolene (9.5-11.6%) and apole (8.6-13.7%). (E, Z, Z)-2,4,7-tridecatrienal (11.2-15.8%). It is worth noting that some compounds are increased by a dose level of 2.5 kGy; sabinene, 2-beta. -pinene, camphene, gamma terpinene, L-linalool, carveol, anisole and longifolenaldehyde. Other compounds increased till 5.0 kGy dose such as trans-*p*-mentha-2,8-dienol, myrtenol, β -citral, carveol, elemicin and 3 nbutyl phthalide, as well as there is some other increase till reached to the maximum dose use (10.0 kGy) such as, ethyl linoleate, apole and (E, Z, Z)-2,4,7-tridecatrienal. Otherwise, anithole (isomer of stragole), trans-caryophyllene (-)-spathulenol, and methyl palmitate were detected in irradiated samples only. On the other hand, there are other compounds decreased by irradiation such as cis-ocimene, 3-methylene-bicyclo [3.2.1] oct-6-en-8-ol, (+) limonene oxide and cis-carveol.

Anisole caryophyllene and trans-caryophyllene-cis-ocimene are monoterpene and used in perfumery for its sweet herbal aroma, and have anti-fungal properties so are thought to act as plant defense (Ehiabhi *et al.*, 2013). Camphene was shown to reduce triglycerides and plasma cholesterol in hyperlipidemia rats independent of the activity of HMGCoA reductase an also attributes to its antifungal properties, effectively in the treatment of fungal skin infections, displayed anti-inflammation and analgesic properties (Vallianou *et al.*, 2011; Marei *et al.*, 2012; Quintans-Júnior *et al.*, 2013). Myrtenol is a monoterpene alcohol, it was increased by increasing irradiation dose to 5.0 kGy. Myrtenol, was found to improve asthma by reducing inflammatory cell infiltration and preventing hyperplasia of goblet cells (Bejeshk *et al.*, 2019; Rajizadeh *et al.*, 2019). Isoeugenol also was detected only in the highest dose level 10.0 kGy it is a promising antidiabetic and it can work against Alzheimer disease because it works as α -glycosidase inhibitor and AChE inhibitor (Topal, 2019). Isolongifolene a tricyclic sesquiterpene which increased by increasing irradiation dose level can help in decreasing rotenone-induced mitochondrial dysfunction and cell apoptosis in SH-SY5Y cells. Which means it may attenuate Parkinson's disease (Balakrishnan *et al.*, 2018). The compound α -curcumene, have antimicrobial activity, Pozzatti *et al.* (2015) concluded that fungal strains are more sensitive for α -curcumene than the bacterial ones. Elemicin inhibited the bacterial growth of NCTC 11168 strain (Rossi *et al.*, 2007). Previous studies indicated that trans-caryophyllene is a natural sesquiterpene it has anti-inflammatory properties, reduced both chronic and acute pain in mice, which may be facilitated through the opioid and endocannabinoid systems (Fernandes *et al.*, 2007; Paula-Freire *et al.*, 2014). Caryophyllene oxide was detected

in the four samples, but it was highest in 10.0 kGy sample. It showed significant central and peripheral analgesic, along with anti-inflammatory activity (Chavan *et al.*, 2010). Methyl palmitate detected only in 2.5 and 5.0 kGy samples, a study demonstrated that methyl palmitate has anti-inflammatory effect (El-Demerdash, 2011). Methyl palmitate also was known for its effect on preventing silica-induced lung fibrosis. This effect can be attributed to methyl palmitate's capability to counteract the inflammatory cells' infiltration and therefore reactive oxygen species (ROS) generate and control cytokine effects (Kirkin *et al.*, 2014). Volatile compounds, such as terpenes and terpene derivatives, are usually majority components in EOs from plant material and contain different chemical functional groups. A volatile compound in different EOs is under specific reactional environments and the conditions of each EO submitted to different ways of isomerization, oxidation, and hydroxylation when exposed to γ -radiation provide new compounds (Sharawy *et al.*, 2013; Kizilay and Kahraman, 2018).

Table 1. GC-MS profile of chemical composition (area sum%) of *A. graveolens* seeds essential oils

Peak No.	Compound	RT	Gamma irradiation (kGy) dose levels			
			Area sum % Control	Area sum % 2.5	Area sum % 5.0	Area sum % 10.0
1	cis-Ocimene	6.60	2.4±0.20	1.6±0.20	1.5±0.30	1.6±0.30
3	Sabinene	7.377	0.23±0.01	0.29±0.01	ND	ND
5	2-Beta-pinene	7.438	2.0±0.10	2.68±0.03	1.75±0.02	1.5±0.10
6	Camphene	7.722	1.85±0.02	2.39±0.04	1.85±0.04	1.66±0.04
7	3-Methylene-bicyclo[3.2.1]oct-6-en 8-ol	8.557	16.9±0.50	12.4±0.40	9.8±0.30	11.9±0.42
8	GammaTerpinene	8.987	0.15±0.02	0.24±0.02	ND	ND
9	L-Linalool	9.703	0.30±0.04	0.43±0.01	0.39±0.01	0.39±0.10
10	trans-p-Mentha-2,8-dienol	10.084	0.37±0.03	0.34±0.02	0.42±0.2	ND
11	cis-p-Mentha-2,8-dien-1-ol	10.333	ND	ND	0.43±0.03	ND
12	(+) Limonene oxide	10.396	0.22±0.02	0.20±0.01	0.16±0.01	ND
13	cis-Verbenol	10.760	3.6±0.30	2.2±0.10	1.8±0.10	2.4±0.30
14	Myrtenol	11.431	0.32±0.02	0.36±0.03	0.38±0.01	ND
15	β -Citral	11.482	0.28±0.01	0.34±0.03	0.40±0.03	ND
16	cis-Carveol	11.486	1.98±0.06	0.94±0.03	0.88±0.04	ND
17	Carveol	11.892	0.66±0.02	0.84±0.04	0.96±0.02	0.43±0.02
18	Anisole	12.249	0.62±0.04	0.76±0.01	0.73±0.01	0.51±0.01
19	Anethole (isomer of stragole	12.984	ND	0.76±0.02	0.58±0.02	0.35±0.02
20	Isoeugenol	14.287	0.18±0.01	ND	ND	0.95±0.03
21	Caryophyllene	15.565	3.8±0.40	2.8±0.10	3.4±0.30	2.8±0.20
22	Longifolenaldehyde	16.456	2.4±0.50	3.1±0.10	2.9±0.10	2.9±0.30
23	α -Curcumene	16.702	0.57±0.03	0.99±0.03	0.96±0.20	0.59±0.02
24	Isolongifolene	16.886	11.6±0.60	10.1±0.50	11.5±0.40	9.5±0.40
25	Junipene	17.062	0.26±0.03	0.38±0.01	0.25±0.02	0.21±0.01
26	Isomyristicin	17.415	4.8±0.30	6.40±0.30	6.98±0.05	5.7±0.40
27	Elemicin	17.985	0.98±0.04	1.54±0.04	1.91±0.03	0.98±0.02
28	trans-Caryophyllene	18.129	0.20±0.01	0.26±0.02	0.23±0.02	ND
29	Caryophyllene oxide	18.625	1.1±0.10	1.7±0.10	1.6±0.30	0.85±0.03
30	(-)-Spathulenol	19.211	ND	ND	0.61±0.01	0.65±0.01
31	Ethyl linoleate	19.828	3.2±0.50	3.9±0.30	2.5±0.20	4.2±0.20
32	Apole	20.383	8.6±0.30	12.4±0.20	12.7±0.30	13.7±0.20
33	(E,Z,Z)-2,4,7-Tridecatrienal	21.373	11.5±0.40	11.2±0.40	13.2±0.40	15.8±0.30
34	3 N butyl phthalide	21.416	0.30±0.05	0.42±0.01	0.52±0.02	0.39±0.01
35	Methyl palmitate	24.432	ND	0.19±0.02	0.30±0.01	ND
	Total	-	81.37±1.61	82.15±0.09	86.59±1.98	79.96±2.73

RT=Retention time; Area sum %; Values were expressed as [area percentage] mean \pm SD (standard deviation) n=3; ND not detected

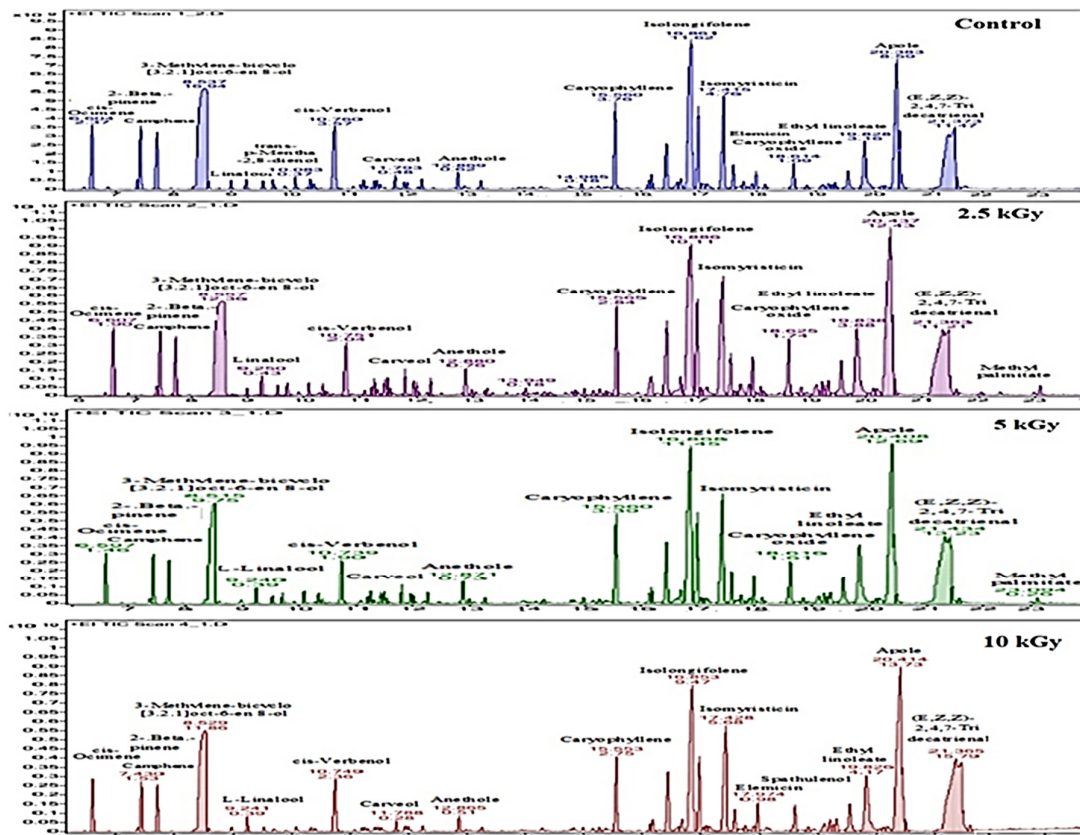


Figure 2. The gas chromatography/mass spectrometry chromatogram of the essential oil extracted from un-irradiated and gamma irradiated *A. graveolens* seeds at various dose levels; control, 2.5, 5.0 and 10.0 kGy

Fourier Transform Infrared (FTIR) spectroscopy

The technique of FTIR spectra has proved positive in measuring structural changes in oils and fats. It was performed to examine whether any structural changes occurred during gamma-irradiation proceed. The FTIR spectra are shown in Figure 3 and the wavenumbers of characteristic bands and corresponding assignments for the gamma-irradiated oil with different dose levels are listed in Table 2, the results detected that at certain wavenumbers, there were some functions group obtained of essential oil extracted from un-irradiated and irradiated celery seeds with different dose levels. The key bands of these treatments are at 3309, 2919, 1668, 1633, 1440, 1363, 1247, 1107, 889, 559 and 482 cm^{-1} . There was a broad absorption, band peaking at 3309 cm^{-1} in the control treatment and not appeared in the irradiated oil, this band is corresponding to OH stretching bands of alcohols and/or carboxylic acids vibrations. Followed by peaks ranged from 3100-2723 cm^{-1} which appeared in the treated seeds with all irradiation dose levels and assigned to vibration of the $-\text{CH}_3$ asymmetric stretching and symmetric stretching absorption band of the methylene group vibration, respectively. Other band in this spectrum is observed at 1636 cm^{-1} due to the bond vibrations of the asymmetrical carboxylic acid observed in the un-irradiated sample. Contrariwise, 1668 cm^{-1} which attributed to ester carbonyl $-\text{COOR}$ and carboxylate ion stretching ($-\text{COO}-$) groups were recorded in the irradiated samples with 2.5, 5.0 and 10.0 kGy dose levels and weren't recorded in the control. The bands C-O stretching vibration (amide) and C-C stretching from phenyl groups, COO symmetric stretching and CH_2 bending were observed at the wavenumbers ranged from 1600–1400 cm^{-1} in the extracted oils from seeds irradiated with 2.5, 5.0 and 10.0 kGy. In addition, stretching vibrations C-O of mono-, oligo-, carbohydrates, pyranoid ring were obtained in the IR spectra 1150-1000 cm^{-1} in the extracted oils from seeds irradiated all used dose levels.

Moreover, the IR spectra of 960-700 cm^{-1} corresponding assignments to α -glycoside bond, β -glycoside bond, C-H out-of-plane bending vibrations from isoprenoids detected in all the used irradiated samples. Otherwise, the 690-550 cm^{-1} wavenumbers detected in the un-irradiated and irradiated samples which was assigned to C-Br which indicates the presence of aliphatic bromo compound. In a previous work, it was found that FTIR spectroscopy discovered the chemical composition of the citrus lemon oil (Boughendjioua and Djeddi, 2014). The spectrum shows characteristic bands corresponding to CC at 1600-1680 cm^{-1} , the signals shown between 3100-3000 cm^{-1} and 3150-3050 cm^{-1} are provided by asymmetrical and symmetrical stretching vibrations of C-H groups. According to Elzey *et al.* (2016), FTIR spectra of clean essential oil of lemon, displaying expected characteristic C-H stretch ($\sim 2900 \text{ cm}^{-1}$), C=O stretch ($\sim 1700 \text{ cm}^{-1}$), broad O-H stretch ($\sim 3400 \text{ cm}^{-1}$), and C-O stretch ($\sim 1100 \text{ cm}^{-1}$) of terpenoid constituents. Constituents and compositions of essential oils can vary greatly and depend on geochemistry of soil in which it is produced. Overall, essential oils consist, for example, of terpenes, cineoles, terpineols, citronellals, and others. Gutiérrez *et al.* (2017) assessed physicochemical characteristics of oils extracted from gamma-irradiated *Plukenetia volubilis* seeds at four dose levels (control, 1.0, 5.0 and 8.0 kGy) exhibited that the FTIR spectroscopy can be related to exact functional groups like band at 3471 cm^{-1} is allocated to absorption of glyceride ester carbonyl, band at 3010 cm^{-1} ascribed to stretching vibration of cis olefinic CH double bands. The 2926 and 2854 cm^{-1} bands correspond to symmetrical and asymmetric methylene vibrations, while the 1746 cm^{-1} bands correspond to stretching vibration of triacylglycerol group C=O. Otherwise, Aly *et al.* (2019) cited that the attendance of metabolites screened such as amino acids, phenols, amines, carbohydrates, alkaloids, alkenes, carboxylic acid, lipids, sulphur compounds, and proteins in irradiated eggplant consistent to FTIR functional groups. On the other hand, Ameer *et al.* (2020) implied that no significant change in the absorption values of irradiated samples compared with those of controls. In addition, Rezanejad *et al.* (2019) showed that, the irradiated (30 kGy) and crude rosemary had identical phenol compound-type FTIR spectra patterns without any significant changes in the status of main bands and functional groups. In contrast 20 kGy dose caused the appearance of the specific absorption peak at 3385 cm^{-1} and disappearance of the absorption peak at 3419 cm^{-1} , generally corresponding to N-H stretching in amines and amides, in addition to the appearance of O-H peak of free hydroxyl in alcohols and phenols at 3683 cm^{-1} in *Foeniculum vulgare*. The 5 and 10 kGy are the most effective doses affected the total contents of phenolic and flavonoids in *Foeniculum vulgare* and *Carum carvi* seeds (Ali *et al.*, 2018).

Total phenols, flavonoids and antioxidant activity (DPPH)

The results shown in Table 3 revealed that there is a noticeable increase in the phenolic content of the irradiated samples and that highest phenolic content has resulted in the 10.0 kGy dose (1.54 g /100 g oil relevant to the control sample 0.68 g/100 g oil). Data in Table 3 indicates that there is a gradual increase in the flavonoids content in the celery oils by increasing irradiation dose level and the highest raise was found in dose level 10.0 kGy (0.59 g/100 g oil) compared to the control sample (0.22 g/100 g oil).

Table 2. Wavenumbers of characteristic bands and corresponding assignments for *A. graveolens* essential oils extracted from un-irradiated and gamma irradiated seeds at different dose levels)

Wavenumber cm^{-1}	Function groups assigned	Irradiation dose level (kGy)			
		0.0	2.5	5.0	10.0
3300-4000	Polymeric hydroxyl compound O-H stretching carbohydrate amino acids OH stretching bands of alcohols	3309	ND	ND	ND
3100-2723	O-H, N-H and C-H C-H stretching vibrations specific to CH ₃ and CH ₂	ND	3079 2962 2919 2723	3080 2962 2921	3079 2846 2919
1700-1630	C=O stretching vibration, C-N stretching, Lipids, Ester carbonyl – COOR and carboxylate ion stretching (-COO-)-	1636 ND	ND 1668	ND 1668	ND 1668

1600–1400	C-O stretching vibration (amide) and C-C stretching from phenyl groups, COO symmetric stretching, CH ₂ bending	ND	1511 1440 1363	1514 1440 1363	1513 1440 1363
1380-1200	P=O of phosphoryl, C-O stretching	ND	1363 1247	1363 1247	1363 1247
1150 -1000	Stretching vibrations C-O of mono-, oligo-, and carbohydrates, Pyranoid ring	ND	1150 1107	1147 1107	1149 1107
960-700	α-glycoside bond and β-glycoside bond C-H out-of-plane bending vibrations from isoprenoids	ND	957 889 765	958 889 766	956 889 767
690-550	Bromo compound	588 559	563 633 693	564 631 695	558 632 694
500-400	Iodo compound	ND	482 436	482 430	482 434

ND: Not detected

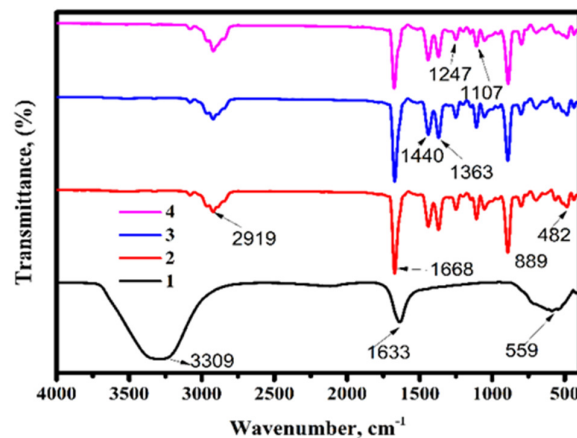


Figure 3. Spectra of FTIR of *A. graveolens* essential oils extracted from un-irradiated and gamma irradiated seeds (1: control kGy, 2: 2.5 kGy, 3: 5.0 kGy, and 4: 10.0 kGy)

Table 3. Total phenols and flavonoids content of *A. graveolens* essential oils extracted from the un-irradiated and gamma irradiated seeds at different dose levels

Irradiation dose level (kGy)	Phenols (g/100 g oil)	Flavonoids (g/100 g oil)
Control	0.68±0.00 ^d	0.22±0.01 ^d
2.5	1.00±0.07 ^c	0.33±0.00 ^c
5.0	1.20±0.03 ^b	0.42±0.00 ^b
10.0	1.54±0.01 ^a	0.59±0.04 ^a

Data are given as mean ± SE (n = 3). ^{a, b and c} Means within the same column with different letters are significantly differed (p < 0.05).

The results obtained in Table 4 indicates that there is an increase in the antioxidant activity of celery oil at different dose levels, while increasing the oil concentration used from each treatment (in µl) proved that the highest antioxidant activity is resulted in the irradiation dose level 10.0 kGy 88.30, 90.51 and 93.86% for the concentrations 10, 15 and 25 µg oil, respectively compared to the control samples 77.15, 81.35 and 84.09% at the same concentrations. Otherwise, the IC₅₀ for the essential oil ranged from 54.17 µg/g oil in control sample to 127.23 µg/g oil in the dose level of 5.0 kGy.

Table 4. Antioxidant activity of *A. graveolens* essential oil extracted from the non-irradiated and irradiated seeds at different dose levels against DPPH method

Irradiation dose level (kGy)	Oil concentration			IC ₅₀ µg/g oil
	10 µg	15 µg	25 µg	
Control	77.15±0.29 ^d	81.35±0.23 ^d	84.09±0.17 ^d	54.17
2.5	82.32±0.26 ^c	85.41±0.21 ^c	88.58±0.11 ^c	71.27
5.0	86.92±0.36 ^b	88.91±0.46 ^b	91.11±0.32 ^b	127.23
10.0	88.30±0.21 ^a	90.51±0.03 ^a	93.86±0.27 ^a	95.18

Data are given as mean ± SE (n = 3). ^{a, b and c} Means within the same column with different letters are significantly differed (p < 0.05).

It could be assumed that the rise in total phenolic and flavonoids is attributed to the irradiation that raises the celery oil's secondary compounds.

Phenolic components are considered to act as antioxidants not only because of their ability to contribute hydrogen atoms or electrons, but also because of their stable radical intermediates, which prevent the oxidation of different food ingredients, in especially fatty acids and oil. *Thymus algeriensis*, showed that essential oil total phenolic content was highly elevated at a dose level of 10.0 kGy after the irradiation. The findings obtained are in accordance with those of Aly *et al.* (2016) who found that phenolic compounds in *M. oleifera* oil increased by γ -irradiation up to 10.0 kGy. This rise in total phenolic content is due to the activity of phenylalanine ammonia-lyase (PAL), principal enzyme for phenolic metabolism. It catalyzes L-phenylalanine deamination to yield ammonia and trans-cinnamic acid which produces phenolic compounds. Quite an increase in total phenolic is attributable to release of phenolic compounds from glycosidic components and degradation of larger phenolic compounds into smaller ones, as indicated by gamma irradiation (Horváthov *et al.*, 2007).

Flavonoids are one of the best important secondary metabolites in celery, and most flavonoids occur with the formation of glycosides. Flavonoids, including apigenin, kaempferol, luteolin, quercetin and isorhamnetin, were isolated from celery (Nijveldt *et al.*, 2001; Romanova *et al.*, 2001). Such findings are in conjunction with those published on *M. oleifera* oil (Horváthov *et al.*, 2007). On the other hand, Fatemi *et al.* (2013), found that flavonoids content of essential oil extracted from cumin wasn't highly affected after being irradiated with 10.0 and 25.0 kGy doses, while in the current study it was indicated that flavonoids content of celery essential oil was noticeable changed as it was enhanced after being exposed to 10.0 kGy dose. In the concern El-Beltagi *et al.* (2019b) found that by raising irradiation dose level to 5.0 kGy, overall phenolic and flavonoid content had been substantially increased. 'Sakouti' cultivar displayed the highest degree of scavenging activity (DPPH %), with γ -irradiation dose level 5.0 kGy (82.85%).

The conversion of phenylalanine to cinnamic acid in the presence of PAL as a catalyst is flavonoid biosynthesis, which is an essential step in phenyl-propanoid pathway. The activity of PAL affects the synthesis of flavonoids in response to irradiation (gamma and UV-B stress) whereas flavonoids alleviate the damage caused by irradiation stress. Phenolic compounds serve as free radical terminators and the mechanism of flavonoid action is by process of scavenging or chelating.

Our findings are consistent with those recorded for *R. officinalis* essential oil for which antioxidant activity measured using DPPH increased 10.0 kGy with irradiation dose (Fatemi *et al.*, 2013). The radical DPPH in oil provided by un-irradiated and irradiated moringa seed oil revealed that scavenging activity raised progressively by raising dose level to 10.0 k Gy (Aly *et al.*, 2016). On the other hand, Horváthová *et al.* (2007) noticed the impact of oregano sample irradiation at 5.0-30.0 kGy doses on the radical-scavenging capability of DPPH and FRP to be negligible. In addition, Alloun *et al.* (2019) relived that the percentage of volatile oil yield from control and irradiated samples showed no substantial difference with irradiation level. The capabilities of scavenging DPPH radical of the essential oil of celery were studied. A study indicated that the maximum scavenging rates were 98.1% when 0.20 µl of the oil was used (Lu *et al.*, 2011). The extract of celery seed with concentrations 0.1-5.0 mg/ml gave 83%-93% inhibition to the DPPH radical (Lin *et al.*, 2011). The essential oil of celery seeds reduced the stable-free radical DPPH with an IC₅₀ of 89.11 g/l (Dąbrowska *et al.*, 2020).

Other investigation displayed that celery is effective as antioxidant compared to ascorbic acid (Ibrahim, 2016). The DPPH scavenging activity with irradiation dose dependent which may be attributed to the elevation inactive compounds. Essential oils contain monoterpene hydrocarbon which contains a phenolic ring as well as the oxygenated monoterpene which contains both the hydroxyl group and the phenolic ring, knowing that these groups play an important role in antioxidant activity (Hadjadj and Hazzit, 2020). The herbal phenols consider one of the most numerous classes of secondary biomolecules that have a common characteristic of an aromatic ring carrying one or more hydroxyl groups including functional derivatives as esters, glycosides, etc. Otherwise, flavonoids are found in the vacuoles, chloroplasts, and chloroplasts in the form of glycosides and the presence of OH groups linked to the carbon atoms of the benzene ring (Ameer, 2020).

Anticancer assay

Results obtained for the evaluation of cytotoxic characteristics of essential oils extracts obtained from non-irradiated and irradiated celery seeds toward two human cancer cell lines (Lung cancer cell line A549 and Breast cancer cell line MCF-7) for three concentrations (250, 500 and 1000 $\mu\text{g/ml}$) are given in Table 5. It is found that oil extracted from irradiated seeds by dose level 5.0 kGy was reduced the cell viability and anticancer activity enlarged (Table 5). Results revealed that the IC_{50} of oils extracted from seeds irradiated by dose level 5.0 kGy were 145 and 124 $\mu\text{g/ml}$ against Lung cancer cell line A549 and MCF-7 Breast cell lines, respectively. Some of the compounds in the seed of the *Apium* plant have collaborations in the molecular mechanisms and pathways of cellular targets that are considered to have a significant effect on the treatment of human cancers with its anti-inflammatory and analgesic effects (Salehi *et al.*, 2019). A study confirmed that celery seeds oil inhibits cell proliferation, down regulates inflammatory markers and up-regulates apoptosis and that might be, at least in part, the principal mechanisms associated with the tumour inhibition (Aprotosoie *et al.*, 2016). Anethole which irradiated up in 5.0 kGy samples has anticarcinogenic and chemo-preventive properties (Ahmedy, 2016). (-)-Spathulenol was identified in 5.0 and 10.0 kGy samples. As established previously, that antioxidant, anti-inflammatory, and anti-proliferative properties of spathulenol (do Nascimento *et al.*, 2018).

The constituents derived from *A. graveolens* have been shown to stop growth and to prompt apoptosis in several cancer tissues (Köken *et al.*, 2016). They induce apoptosis of Dalton's lymphoma ascites cells (Subhadradevi *et al.*, 2011), human colon cancer cell line HCT116 (Quassinti *et al.*, 2014), Breast cancer cell line MCF-7 (Park *et al.*, 2014), human cervical cancer cell line HeLa (Fu *et al.*, 2012). At the same time celery flavonoid had anticancer effect on HepG2 hepatic cancer, and MCF-7 breast cancer (Shakir, 2013). Regarding the impact of irradiation, the 2.0 and 5.0 kGy irradiated thyme samples showed lower toxicity than the control sample (0.0 kGy) on cell lines MCF-7, HeLa and HepG2, whereas thyme samples irradiated at 10.0 kGy increased their cytotoxicity in the assayed tumour cell lines compared with samples submitted to 2.0 and 5.0 kGy. They also identified the cytotoxicity of infusions that were prepared from non-irradiated and irradiated *Aloysia citrodora*, *Mentha piperita* L., also confirming that all cell lines tested displayed antiproliferative activity in those samples (Pereira *et al.*, 2018).

Table 5. Cytotoxicity of *A. graveolens* essential oils extracted from un-irradiated and gamma irradiated seeds toward Lung cancer cell line (A549) and Breast cancer cell line (MCF-7)

Concentrations ($\mu\text{g/ml}$)	Viability % and IC_{50} for tested cell lines							
	A549	MCF-7	A549	MCF-7	A549	MCF-7	A549	MCF-7
	Control		2.5 kGy		5.0 kGy		10.0 kGy	
1000	0	1	0	0	0	1.8	0	0
500	36.5	7	0	0	1.3	1.7	11	37.7
250	49.5	37.8	71.25	45.5	35.6	26	42.5	41.7
IC_{50} ($\mu\text{g/ml}$)	265	151	324.6	225	145	124	190.5	180.6

A549; Human Lung cancer cell line; MCF-7; Human Breast cancer cell line.

Antimicrobial activity

Antimicrobial activity of essential oils extracted from un-irradiated and irradiated celery seeds were evaluated using disc diffusion method (Table 6). There wasn't any resistant strain to the celery seed oil, the largest inhibition zones of *S. aureus*, *B. subtilis* and *K. pneumoniae* were recognized with the oils extracted from the irradiated seeds with 5.0 kGy dose level, where *E. coli* and *C. albicans* were more pronounced for the oils extracted from the irradiated seeds with 10.0 kGy. Maybe the antibacterial compounds were higher in 5.0 kGy sample, while antifungal compounds were higher in 10.0 kGy sample. For example, cis-verbenol which was detected in highest concentration in 5.0 kGy sample. Gamma irradiation is an effective method of breaking down dangerous microorganism, increasing shelf life and improving food safety. The decontaminating impact of gamma irradiation can be attributed to spontaneous and widespread destruction of microorganisms 'DNA molecules by treatment leading to irreversible inactivation of microbes' metabolic and cellular systems (Lima *et al.*, 2018). Meanwhile, Dąbrowska *et al.* (2020) cited that essential oils from celery seeds contains considerable amount of (R)-(+)-limonene, which is an attractive aroma compound used in the cosmetics and food industries. This monoterpene is known to exhibit numerous biological activities, including antimicrobial properties against Gram-positive bacteria. Also, found that the essential oils from celery seeds showed moderate activity against the tested microorganisms. The growth of Gram-negative bacteria *E. coli* and *P. aeruginosa* was inhibited at oils concentrations of 20 and 30 μ l/ml, respectively. It was found that the blend of the monoterpene cis-verbenol and the antibiotics amoxicillin and gentamicin displayed a synergic effect against a sensitive and a resistant strain of *S. aureus*, which is also resistant to methicillin (Bobadilla *et al.*, 2019). Also, aromadendrene was detected only in 5.0 kGy sample, it was indicated that might be responsible for antimicrobial properties (Mulyaningsih *et al.*, 2010). (-)-Spathulenol which was detected in 5.0 and 10.0 kGy samples only. It has been stated that some essential oil constituents interfere with the lipids of cell membranes, cause leakage of intracellular materials, and finally the cell lysis (Oyedemi *et al.*, 2009). It was reported that celery seed oils caused inhibition zone of 2 mm to *E. coli* (Teixeira *et al.*, 2013). The present results are in line with the previous study reported for rosemary irradiation (5.0, 10.0 and 15.0 kGy) (Abdeldaiem *et al.*, 2009). The antibacterial efficacy of essential oils, as reported by several research studies, is associated with the major components, including in particular the phenolic monoterpenes carvacrol and/or thymol reported to have antimicrobial activity toward a variety of bacteria, particularly foodborne pathogens (Hassan *et al.*, 2019).

Table 6. Antimicrobial activity (zone of inhibition in mm) of *A. graveolens* essential oils extracted from un-irradiated and irradiated seeds comparing to standard antibiotics

Microbial strains	Inhibition zone (mm)				Standard	
	Irradiation dose level (kGy)					
	Control	2.5	5.0	10.0	'CN	"NS
Gram positive						
<i>S. aureus</i>	14±0.13 ^d	20±0.34 ^b	25±0.46 ^a	20±0.21 ^b	15±0.12 ^c	NT
<i>B. subtilis</i>	17±0.21 ^d	20±0.25 ^b	23±0.33 ^a	19±0.15 ^c	13±0.09 ^c	NT
Gram negative						
<i>E. coli</i>	19±0.29 ^c	22±0.40 ^b	22±0.29 ^b	25±0.34 ^a	10±0.11 ^c	NT
<i>K. pneumoniae</i>	1±0.07 ^c	15±0.28 ^c	20±0.18 ^a	16±0.24 ^b	11±0.10 ^d	NT
<i>Candida albicans</i>	15±0.18 ^d	16±0.23 ^c	18±0.2 ^b	21±0.30 ^a	NT	12±0.14 ^c

Values are mean \pm SE, ^{a, b and c} Means within the same column with different letters are significantly differed ($p < 0.05$). *Gentamycin (CN 10 μ g), **Nystatin (NS 100 μ g), NT: not tested, R: resistant

Conclusions

Gamma radiation has emerged as the favoured methods of food irradiation in recent years. Irradiation induces certain alterations that can modify the chemical composition and nutritive value of the foods. Apparently, there is a huge benefit come out of irradiated *A. graveolens* seeds. The highest increase of essential oils extracted was registered with a dosage level of 10.0 kGy (2.42%). There were new compounds were detected in the irradiated samples and others compounds were increased. The total phenols and flavonoids increased by increasing γ -irradiation dose level, as well as there was a remarkable DPPH scavenging activity. Results revealed that the IC₅₀ of oils extracted from seeds irradiated by dose level 5.0 kGy were 145 and 124 $\mu\text{g/ml}$ against Lung cancer cell line A549 and MCF-7 Breast cell lines, respectively. The biggest inhibition zone of *S. aureus*, *B. subtilis* and *K. pneumoniae* were obtained with the oils extracted from seeds irradiated with 5.0 kGy dose level, where *E. coli* and *C. albicans* were more pronounced for the oils gotten from the seeds irradiated with 10.0 kGy. It could be assumed that gamma irradiation may not only be a valuable decontamination viewpoint but also as an enhancement factor for some properties through a preliminary study on celery seeds. Further studies are required to correlate the biological effects to specific compounds and to find out the best extraction method for exploiting these properties. But also, to highlight a possible interest for its by-products as nutraceutical sources for further develop.

Authors' Contributions

Conceptualization: H.S.E. and A.A.A.; Data curation: H.S.E., A.E.A. and A.A.A.; Formal analysis: H.S.E., A.E.A. and A.A.A.; Funding acquisition: H.S.E. and F.D.; Investigation: H.S.E., A.A.A. and F.D.; Methodology: H.S.E., A.E.A. and A.A.A.; Project administration: H.S.E., F.D. and A.A.A.; Resources: H.S.E., F.D., and A.A.A.; Software: H.S.E. and A.E.A.; Supervision: H.S.E. F.D. and A.A.A.; writing-original draft preparation: H.S.E., A.E.A. and A.A.A.; writing-review and editing, H.S.E.: F.D. and A.A.A. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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