Egypt. J. Chem. **Vol. 68** , No. 8 pp. 169 -179 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Effect of γ-irradiation on Chemical Composition and Antibacterial Activity of Celery Seeds Essential Oil

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Abstract

The rise of multidrug-resistant bacteria has exacerbated antibiotic resistance. New plant-derived antibacterial agents offer pharmaceutical industry new economic opportunities. This study evaluate the antibacterial activity of the celery (*Apium graveolens*) seeds extracts (Celery Seeds Essential Oil (CSEO), Celery Seeds Post-Distillation Extract (CSPDE), Celery Seeds Water Extract (CSWE), Celery Seeds Ethanolic Extract (CSEE), and Celery Seeds Hexane Extract (CSHE)) against five MDR clinical isolates (*Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Pseudomonas aeruginosa* and *Staphylococcus aureus*) by the minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC), and MIC/MBC ratios. the effect of gamma irradiation (5 and 10 kGy) on the chemical composition, and the antibacterial activity of CSEO (the most active extract) were investigated against the most resistant isolate, *Enterobacter cloacae*. All celery seeds extracts showed antibacterial activity to various degrees. The GC-MS analysis of the non-irradiated CSEO revealed that the main chemical constituents were -selinene (35.52 %), D-limonene (22.04 %), -cyclocitral (18.61 %), Kessane (8.16 %), -pinene (2.97 %), and α-guaiene (2.33 %), while irradiation of CSEO (5 and 10 kGy) showed a fluctuation in the proportions of most of these components along with the emergence and disappearance of specific minor components. CSEO irradiated at 5 kGy had significantly decreased the antibacterial activity against *Enterobacter cloacae*, with the MIC increasing to from 0.061 mL/mL to 0.245 mL/mL, while irradiation at 10 kGy couldn't affect the antibacterial activity of CSEO against *Enterobacter cloacae*. Our findings indicate that non-irradiated or irradiated (10 kGy) CSEO could potentially substitute traditional antibiotics as natural antibacterials. Also, suggest that irradiation improves essential oil chemical composition, minimizing physical, chemical, and microbiological contamination and boosting therapeutic efficacy.

Keywords: Gamma irradiation; Celery seeds Essential oil; Chemical composition; Antibacterial activity.

1. Introduction

Antibiotics are compounds that kill bacteria by impeding bacterial growth [1]. The emergence of antibiotic resistance is occurring rapidly due to the broad availability and improper or excessive use of antibiotics, which is facilitated by their easy accessibility without a prescription and through unregulated distribution channels [2]. Antibiotic resistance is a prominent source of morbidity and mortality worldwide and puts a considerable financial load on the healthcare system [3]. Although artificial compounds are more important for the prevention and therapy of several diseases, their usage against pathogenic disorders is restricted because of their high toxicity, carcinogenic level, and hazardous impact on the environment. Recently, the detrimental influences of synthetic drugs and increasing microbial resistance have directed researchers to search for novel alternatives from natural products [4 & 5]. Medicinal plants have been used in all cultures as a source of medicine since time immemorial. They serve as a significant reservoir of bioactive substances that vary in their modes of action and biological characteristics. Plants have been a great source of new drug candidates, as plant-derived medicines have made substantial contributions to human health and well-being [6].

Celery is used in the pharmaceutical, food, and ornamental plant industries, resulting in a high commercial value. All parts of the celery plant, such as seeds, leaves, stems, and roots can be used as medicinal plants **[7]**. Celery seeds essential oils (EOs) have been found to have significant nutritional and medicinal benefits, including the ability to scavenge free radicals, functions of anti-inflammatory, anticancer, and anticoagulation activity of blood plasma**,** prevention of cardiovascular diseases, suppression of different disease-causing microbes, treatment of rheumatoid arthritis, etc. **[8].**

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Received Date: 14 October 2024, Revised Date: 04 December 2024, Accepted Date: 17 December 2024
DOI: 10.21608/EJCHEM.2024.326690.10601

This discourse focuses on the application of gamma irradiation as a contemporary method for processing medicinal and aromatic plants. The gamma irradiation technique has been used to remove contaminants from dried aromatic herbs, spices, and vegetable seasonings. Initially, the highest average absorbed dose allowed was 10 kGy. However, the US Food and Drug Administration (FDA) has now increased this limit up to 30 kGy for these products **[9].** The capacity of γ-irradiation to penetrate materials, destroy chemical bonds, and eliminate germs has rendered it as a viable tool for sterilization, food preservation, and radiation therapy. γ-irradiation not only has disinfection benefits, but it can also improve the chemical composition of fragrant plants and their volatile oils, which results in improving their biological activity and guaranteeing their secure and efficient consumption **[10].**

The objective of this study is to develop a new generation of aromatherapy (celery seeds extracts). This new generation of phytomedicine has the potential to enhance the credibility of phytotherapy. Therefore, they can serve as a substitute for antibiotics with either no or minimal impact. Also, the current study is designed to investigate the antibacterial efficacy of celery seeds extracts against locally isolated microorganisms obtained from patients with infectious illnesses in our country. Then, study the effect of gamma irradiation at doses of 5 and 10 kGy on the chemical composition and antimicrobial activity of celery seeds essential oil.

2. Materials and methods

2.1. Plant Extract Preparation:

Celery seeds were purchased from a local market, Cairo, Egypt, and then extracted by three methods:

First, for the extraction by solvents: Celery seeds were extracted by solvents with different polarities (water, ethanol, and n-hexane) as described by **Anowi *et al.* [11]** with some modifications**.** Briefly, 50 g of celery seeds were soaked in 250 mL of the solvent for three days at 37 ℃. The samples were filtered and evaporated to dryness at 60°C. The obtained extracts were stored at - 4°C. An amount of 500 mg/mL stock of crude plant extract was prepared by dissolving 2.5 g of dried plant extract in 5 mL of 2.5% dimethyl sulfoxide (DMSO).

Secondly, for the extraction of volatile oil, Celery seeds were ground and hydro-distillated for 5 hours using a Clevenger-type apparatus to yield essential oil **[12]**. The oil was dried over anhydrous sodium sulfate and stored at a low temperature (-4°C) until used.

Third, Post-distillation Extract Preparation: After collecting the essential oil from celery seeds, the post-distillation wastes were recovered and then washed two times with methanol and water (8: 2; v/v) then the liquid residue was filtered. The filtrates were dried using a rotary evaporator at 60℃. The dried powders are stored at - 4°C for further testing. A stock solution of 1g/mL was prepared from dried powder by dissolving it in 2.5% dimethyl sulfoxide (DMSO) **[13].**

2.2. Bacterial isolates

The bacterial isolates were collected under sterile conditions from the Clinical Microbiology Laboratory of the Arab Contractors Medical Center, Cairo, Egypt. Subsequently, they were grown on nutrient agar slants and stored at 4 ℃. The bacterial isolates used in this study were: Escherichia *coli, Klebsiella pneumoniae, Enterobacter cloacae, Pseudomonas aeruginosa, and Staphylococcus aureus* according to the biochemical identification. The Kirby-Bauer disc diffusion technique was employed to test all clinical isolates for antibiotic resistance **[14]**. Various antibiotics that target cell walls (Penicillin G (P, 10U), Amoxicillin/Clavulanate (AMC, 30µg), Vancomycin (VA, 30µg), Amoxicillin (AX, 25 µg), Ampicillin/sulbactam (SAM, 20µg), Nitrofurantoin (F, 300µg), Aztreonam (ATM, 10µg), Cephradine (CE 30 µg) and Cefoperazone (CEP, 75 µg)), protein synthesis (Chloramphenicol (C, 30µg), Clindamycin (DA, 2µg), Gentamycin (CN, 10 µg), Tetracycline (TE, 30µg) and Erythromycin (E, 15µg)), and DNA (Ofloxacin (OFX, 5µg), Norfloxacin (NOR, 10µg), Ciprofloxacin (CIP, 5µg) and Trimethoprim/Sulfamethoxazole (SXT, 25µg)) were utilized in the screening process (results are not displayed). ***Enterobacter cloacae*** (which is considered the most resistant isolate) was selected to be genetically identified by using 16S rRNA gene. The genomic of the selected isolate was extracted using a commercial kit K0691 and purified using a commercial kit K0721 (GeneJet Genomic DNA Purification Kit, Thermo Fisher Scientific, USA). The 16S rRNA gene was amplified using universal bacterial primers 16S rRNA-F (5'-AGAGTTTSATCCTGGCTCAG-3') and16S rRNA-R (5'-ACGGMTACCTTGTTACGACTT -3'). The reaction mixture of PCR was performed in a total volume of 25 µl containing 10 pmol of forward and reverse primers. The amplification reaction was performed in PCR (Lapcycler Basic and Labcycler Gradient, SensoQuest Biomedical Electronic, German). The thermal cycle parameters were as follows: initial denaturation at 95 ℃ for 5 min, followed by 30 cycles for 35 s at 95 ℃, 53 ℃ for 40 s, 72 ℃ for 1 min, and finally an extension cycle at 72 ℃ for 10 min. Synthesized DNA fragments were detected on 1% agarose gels by ethidium bromide staining **[15]**. Gene ruler plus 1 kb DNA was used as the standard. Bands were screened using a UV-imaging system (UV transilluminator, Wealtec, USA).

The purified PCR product was sequenced by Colors Medical Labs (www.colors-labs.com) and compared to alignments available at the NCBI database (http://www.ncbi.nlm.nih.gov/ BLAST), and the nucleotide sequence was submitted to the Genbank to obtain accession number. Then the phylogenetic tree was drawn using MEGA-X software, version 11.

2.3. Antibacterial activity assay by determining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The Minimum Inhibitory Concentrations (MIC) of celery seeds different extracts against the five selected isolates (Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Pseudomonas aeruginosa, and Staphylococcus aureus) were determined by a resazurin based microtiter dilution assay **[16].** Under sterilized conditions, 96 well microtiter plates were used for the resazurin-based microtiter dilution assay. All the wells of microtiter plates were filled with 100 µL of nutrient broth.

The process of achieving two-fold serial dilutions includes adding 100 µL of stock plant extract to the first well and then transferring 100 µL to the subsequent wells in the next row of the same column. This resulted in each well containing 100 µL of plant extract with decreasing concentrations in a sequential manner. 10 µL of resazurin solution, used as an indicator, was added to each well. Ultimately, a 10 µL was taken from the bacterial solution and subsequently introduced into each well, resulting in a final concentration of 5×10^6 CFU/mL. In order to prevent the dehydration of the bacterial culture, each plate was loosely covered with cling film to guarantee that the bacteria did not become dehydrated. Each microtiter plate had a set of 3 controls: columns with all solutions except bacterial solution replaced by 10 µL of nutrient broth as a negative control, a column containe solutions except for the test extract as a positive control, and a column with ciprofloxacin antibiotics. The plates were incubated at 37°C for 24 h. Any visible transition in the well's color, from purple to pink, was considered a positive indication. The lowest concentration (high dilution) of plant extract with a color change represents the MIC value. All the experiments were performed in triplicate, and the average values were calculated. The Minimum Bactericidal Concentrations (MBC) were determined by subculturing the bacteria from the test wells produced from the MIC test (which showed no color changes) onto the sterile Mueller–Hinton agar (MHA) plates. Then the plates were incubated at 37℃ for 24 h. The concentration that showed no visible growth on the agar plates was considered the MBC value **[17]**. According to the ratio MBC/MIC, we described the antibacterial activity. If the ratio MBC/MIC ≤4, the effect was considered bactericidal but if the ratio MBC/MIC > 4, the effect was defined as bacteriostatic **[18].**

2.4. Effect of gamma irradiation on celery seeds essential oil.

Celery seeds were categorized into three groups, the first was not subjected to irradiation and was considered a control, while the second and third groups were exposed to gamma irradiation at doses of 5, and 10 kGy, respectively; the dose rate of the irradiator source at the time of irradiation was 0.766 kGy/hour. Gamma irradiation was conducted with a cobalt-60 irradiator source (Gamma Chamber 4000 India). This irradiator source is situated at the National Center for Radiation Research and Technology (NCRRT) in Nasr City, Cairo, Egypt. After irradiation, the essential oil was extracted by the previously mentioned method.

2.5. Gas chromatography–mass spectrometry analysis (GC-MS) of celery seeds essential oil

GC-MS analysis of non-irradiated and irradiated celery seeds essential oil (CSEO) has been performed using Gas chromatography **(Agilent 8890**) with a mass spectrometer detector (Agilent 5977B) series with capillary column DB-5MS, 60 m x 250 µm id x 0.25 µm film thicknesses. The carrier gas utilized was helium, maintained at a consistent pressure of 65 kPa. The essential oil was injected with a volume of 1 µL in a split ratio of 1:50 and a solvent delay of 4 minutes. The increasing oven temperature was programmed from 50 to 240°C with a step of 5°C per minute until reaching 240°C.

2.6. Antibacterial activity assay of essential oil extracted from irradiated celery seeds by determining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC):

CSEO (the most effective extract) was selected to study the effect of irradiation on the antibacterial activity. Also, Enterobacter cloacae was selected for additional inquiries as it was found to have higher MIC values against tested celery extracts. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of irradiated (5 and 10 kGy) celery seeds essential oils (CSEO) against Enterobacter cloacae were determined by a resazurin-based microtiter dilution assay as previously described **[16].**

3. Results and Discussion:

3.1. Antibacterial activity of Celery seeds extracts:

Antibiotics are currently in risk of becoming extinct, as they face a global increase in antibiotic resistance. the clinical bacterial isolates in this study showed resistance to multiple antibiotics (MDR clinical isolates). So, the in vitro antibacterial

activity of the Celery seeds Essential Oil (CSEO), Celery seeds post-distillation extract (CSPDE), Celery seeds water extract (CSWE), Celery seeds Ethanolic extract (CSEE) and Celery seeds hexane extract (CSHE) against Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Pseudomonas aeruginosa, and Staphylococcus aureus, were quantitatively assessed by the minimum inhibitory concentration (MIC) **(table 1)** and the minimum bactericidal concentration (MBC**)** (**table 2**). All celery seeds extracts showed antibacterial activity to various degrees. Other studies have confirmed the antimicrobial activity of celery seeds extracts [**19, 20, 21 & 22]**. The data indicated that S. aureus was the most sensitive isolate to all extracts, Gram-positive bacteria are more susceptible to tested celery extracts than Gram-negative bacteria, which was reported by many investigators **[23, 24, 25, 26 & 27].**

Table (1): Minimum Inhibitory Concentration (MIC) of different celery seeds extracts against selected clinical isolates.

Celery extract	Bacterial isolates (MIC in mL or mg/mL)				
	E. coli	***Enterobacter cloacae***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
CSEO	$0.031^{b}_{a}\pm0.01$	$0.061^{c}_{a}\pm0.01$	$0.061^{c}_{a}\pm0.002$	$0.031^{b}_{a}\pm0.00$	$0.0009^{a}_{a}\pm0.001$
CSPED	$125^{b}_{e}\pm0.4$	$250\ ^{c}_{e}\pm1.5$	$250^{c}_{e}\pm0.69$	$250^{c}_{d}\pm0.2$	$62.5^{a}_{e}\pm0.1$
CSW	$112^{b}_{d}\pm0.36$	$224^{c}_{d}\pm0.3$	$224^{c}_{d}\pm0.2$	$112^{b}_{c}\pm0.2$	$56^{a}_{d}\pm0.3$
CSEE	$14^{b}_{c}\pm0.17$	$56^{d}_{c}\pm0.3$	$28^{c}_{c}\pm0.5$	$112^{c}_{c}\pm0.2$	$3.5^{a}_{c}\pm0.3$
CSHE	$3.5^{b}_{b}\pm0.2$	$28^{d}_{b}\pm0.2$	$7^{c}_{b}\pm0.1$	$7^{c}_{b}\pm0.17$	$0.438^{a}_{b}\pm0.068$

CSEO: Celery seeds Essential Oil, CSPED: Celery seeds post-distillation extract, CSWE: Celery seeds water extract, CSEE: Celery seeds Ethanolic extract, CSHE: Celery seeds hexane extract. Mean values followed by different superscript (within rows) and different subscripts (within columns) are significantly different ($p < 0.05$).

As shown in **Table 1** the CSEO showed the strongest antibacterial effect against *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Pseudomonas aeruginosa, and Staphylococcus aureus*, with a MIC value of 0.0009 to 0.061 mL/mL. On the other hand, CSPED and CSWE showed low to moderate antibacterial effects against most of the tested bacteria, with MIC values ranging from 56 to 250 mg/mL. The CSHE also revealed a great potential of antibacterial activity against *S. aureus* (Gram-positive) and *E. coli, E. cloacae, K. pneumoniae,* and *P. aeruginosa* (Gram-negative bacteria), their respective MIC values were 0.438, 3.5, 28, 7, and 7 mg/mL, respectively. The CSEE displayed a moderate inhibitory effect against *E. cloacae and K. pneumoniae*, and low activity against *P. aeruginosa*. In this study, the CSEO exhibited higher antibacterial activity compared with that of CSHE, CSEE, CSWE, and CSPED against all Gram-negative bacteria and Gram-positive bacteria, it can be arranged as follows, CSEO > CSHE > CSEE >CSWE >CSPED. In this study, the minimum bactericidal concentration (MBC**)** values were equal to or higher than the minimum inhibitory concentration values for all celery seeds extracts (**Table 2**).

The ratios between MIC and MBC for the tested bacterial isolates were determined to clearly distinguish between the celery seeds extracts' killing and inhibiting capabilities (**Table 3**). It was accepted that an MBC/MIC ratio ≤4 indicated the bactericidal impact of the extract, while MBC/MIC proportion >4 a signified bacteriostatic one **[18]**. The results revealed that all celery extracts have a bactericidal effect against *E. coli* and *S. aureus*, with MBC/MIC ratios ranging from 1 to 2 in the cases of CSEO, CSPED, CSWE, and CSHE and 4 in the case of CSEE. CSEO, CSWE, CSEE, and CSHE were shown to have bactericidal effects against *E. cloacae* and *K. pneumoniae* with MBC/MIC ratios of 1 and 2. All extracts except CSEE were found to have a bactericidal effect against *Pseudomonas aeruginosa.*

Based on the MIC, MBC, and MBC/MIC values, celery seeds essential oil was selected for further studies as it showed more efficient antibacterial activity than other extracts. Also, *Enterobacter cloacae* was chosen for further studies because it was the least affected among the tested isolates by celery seeds extracts.

Table (2): Minimum bactericidal Concentration (MBC) of different Celery seeds extracts against selected clinical isolates.

Celery extract	Bacterial isolates (MBC in mL or mg/mL)				
	E. coli	***Enterobacter cloacae***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
CSEO	$0.061^{c}_{a}\pm0.002$	$0.061^{c}_{a}\pm0.01$	$0.061^{c}_{a}\pm0.005$	$0.031^{b}_{a}\pm0.004$	$0.0009^{a}_{a}\pm0.0$
CSPED	$250^{b}_{e}\pm0.36$	$>500^{c}_{d}\pm0.5$	$>500^{c}_{e}\pm0.2$	$500^{c}_{e}\pm1.0$	$62.5^{a}_{e}\pm0.26$
CSWE	$112^{b}_{d}\pm0.5$	$448^{d}_{e}\pm1.0$	$224^{c}_{d}\pm0.2$	$224^{c}_{c}\pm0.4$	$56^{a}_{d}\pm0.3$
CSEE	$56^{b}_{c}\pm0.9$	$56^{b}_{b}\pm0.46$	$56^{b}_{c}\pm1.0$	$>448^{c}_{d}\pm2.0$	$14^{a}_{c}\pm0.6$
CSHE	$7^{b}_{b}\pm0.25$	$56^{d}_{b}\pm0.15$	$7^{b}_{b}\pm0.36$	$14^{c}_{b}\pm0.35$	$0.875^{a}_{b}\pm0.1$

CSEO: Celery seeds Essential Oil, CSPED: Celery seeds post-distillation extract, CSWE: Celery seeds water extract, CSEE: Celery seeds Ethanolic extract, CSHE: Celery seeds hexane extract. Mean values followed by different superscript (within rows) and different subscripts (within columns) are significantly different ($p < 0.05$).

Table (3): bactericidal and bacteriostatic effects of different tested plant extracts against clinical isolates.

Plant extract	MBC/MIC ratio				
	E. coli	***Enterobacter cloacae***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
CSEO	**2**	**1**	**1**	**1**	**1**
CSPED	**2**	**ND**	**ND**	**2**	**1**
CSWE	**1**	**2**	**1**	**2**	**1**
CSEE	**4**	**1**	**2**	**ND**	**4**
CSHE	**2**	**2**	**1**	**2**	**2**

CSEO: Celery seeds Essential Oil, CSPED: Celery seeds post-distillation extract, CSWE: Celery seeds water extract, CSEE: Celery seeds Ethanolic extract, CSHE: Celery seeds hexane extract, ND: not detected (MBC values were higher than tested).

3.2. Bacterial isolate identification

For confirming the identification of the selected isolate (*Enterobacter cloacae*), the DNA was extracted, and the 16S rRNA gene was amplified using universal bacterial primers. After PCR running and agarose gel **(Fig. 1a),** the purified PCR product was sequenced, and the retrieved sequence was deposited on Genbank with accession number PP388966. BLAST searches were performed to investigate the homology of the tested isolate to other *Enterobacter cloacae* and phylogenetic tree was drawn **(Fig. 1b).**

3.1. Gas chromatography–mass spectrometry (GCMS) analysis of irradiated and non-irradiated celery seeds essential oil.

Essential oils contain several chemical compounds that affect their bioactivities; Therefore, it is crucial to analyze the chemical composition of the oils prior to assessing their bioactivities. The alterations in the volatile oil constituents of CSEO before and after γ-irradiation, at doses of 5 and 10 kGy, were analyzed using gas chromatography–mass spectrometry (GC–MS). For the purpose of comparison, the composition of the non-irradiated and irradiated CSEO has been provided in **Table (4)**. GC–MS analysis showed the presence of 24, 21 and 29 different constituents in the non-irradiated and irradiated (5 and 10 kGy) CSEO, respectively.

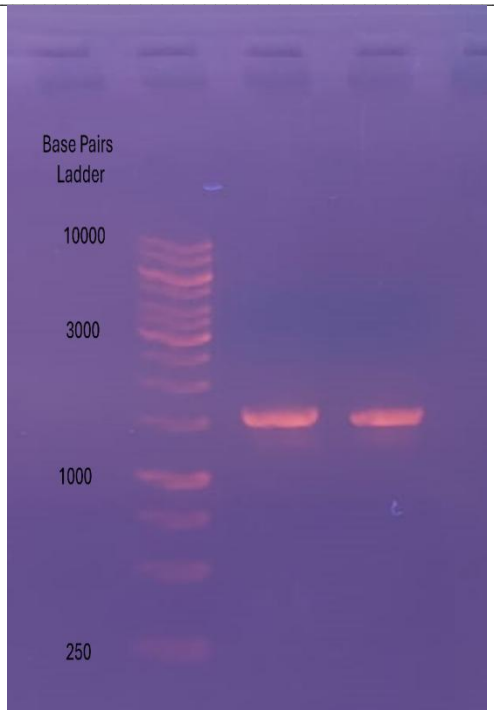

Fig (1a): Agarose gel electrophoresis of 16S rRNA genes. The first lane represents the ladder, lanes 2 & 3 represent the sample and its replicate.

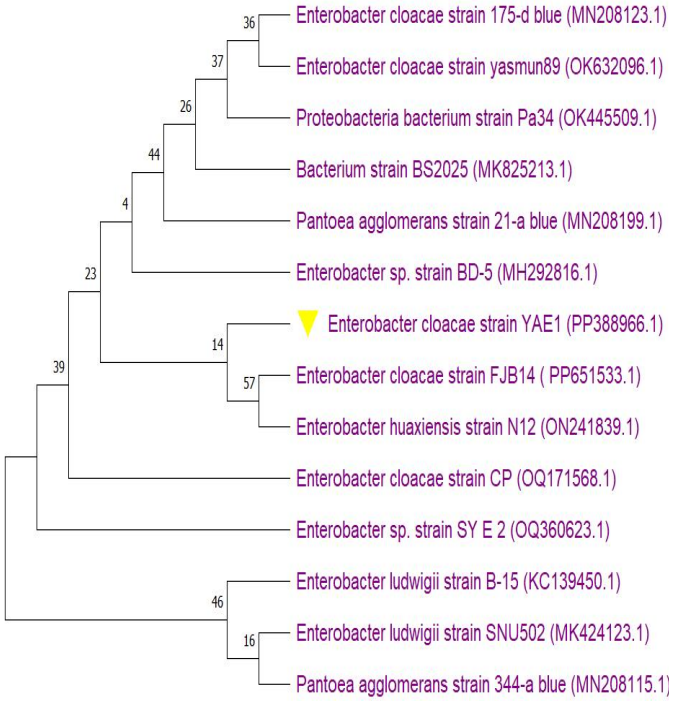

Fig (1b): phylogenetic tree analysis of *Enterobacter cloacae* PP388966

The results of the GC-MS analysis of the non-irradiated CSEO revealed that the principal chemical constituents were -selinene (35.52 %), D-limonene (22.04 %), -cyclocitral (18.61 %), Kessane (8.16 %), -pinene (2.97 %), and α-guaiene (2.33 %), which were somewhat in line with the data presented in the literature. To compare the Egyptian CSEO with other studies **[28, 29, 30, & 31]**, we have found over 24 components in our CSEO, and most of them were found in fluctuating ratios. D-limonene was found in the oil with a percentage of 22.04 % which was less than expected. However, the oil exhibits a greater percentage of -selinene and -cyclocitral, while displaying a reduced level of -pinene. These findings indicate that the essential oil analyzed had a composition that differed from what was anticipated based on prior studies.

The GC-MS analysis shows a composition that differs from what was predicted, with certain components being present in lower quantities than anticipated and others in higher quantities, The variations in component percentages can be ascribed to factors such as plant type, growth conditions, harvest season, geographic origin, and extraction procedure **[32],** Additionally, differences can be attributed to environmental factors like temperature alteration, photoperiod, and light intensity, which can impact the biosynthesis of volatile compounds and consequently alter their quality and chemical composition **[33].** The chemical composition of essential oils (EOs) can vary greatly, even among oils derived from the same species and obtained from the same place. These variations can significantly impact the medicinal and aromatic characteristics of essential oils, as well as their suitability for different applications **[34].**

The impact of γ-irradiation at doses of 5 and 10 kGy on the composition of CSEO revealed significant changes in the proportions of its constituents, including the appearance and disappearance of some minor constituents. Depending on the impact of irradiation, there is a notable reduction in the percentages of D-limonene from 22.04, to 17.51 % at 5 kGy. and 17.97 at 10 kGy, thymoquinol from 1.63 to 0.75 % at 10 kGy and an increase in -selinene from 35.52 to 40.49 % at 5 kGy, kessane from 8.16 to 11,13% at 5 kGy and 10.15% at 10 kGy, -cyclocitral from 18.61 to 23.11% at 10 kGy and 1,3,4-trimethyl-3-cyclohexene-1-carboxaldehyde from 2.6 8 to 3,05 % at 10 kGy. The reduction in certain components may be attributed to the breakdown or alteration of these substances caused by gamma irradiation. Alternatively, the rise in certain components may be attributed to the development of novel compounds or an escalation in the concentration of pre-existing compounds. The differences in chemical composition can be accredited to the influence of irradiation on the molecular

structure of the components of CSEO. The identified alterations may have implications for the potential utilization and implementation of the irradiated CSEO.

Tabel (4): Chemical composition of irradiated and non-irradiated Celery essential oil (5 & 10 kGy).

Peak	RT	Compounds	Control	5 kGy	10 kGy
			Area Sum %	Area Sum %	Area Sum %
1	19.158	3,5,5-Trimethylcyclohexene	0	0	0.04
2	22.391	α-Pinene	0.16	0.11	0.16
3	24.422	β-Pinene	2.97	1.97	2.64
4	26.448	D-Limonene	22.04	17.51	17.97
5	27.329	cis-3,3,5-Trimethylcyclohexanol	0.11	0.06	0.07
6	27.695	7-Methyl-3-octen-2-one	0.07	0	0
7	28.468	(-)-cis-Carane	1	0.43	0.81
8	29.435	cis-Chrysanthemyl alcohol	0.14	0	0.13
9	30.825	trans-Limonene oxide	0.28	0.26	0.54
10	31.409	1,3,4-Trimethyl-3-cyclohexene-1-carboxaldehyde	2.68	1.62	3.05
11	32.244	Piperitenone oxide	0.04	0	0
12	32.256	Menthol	0	0	0.08
13	32.433	cis-Isogeraniol	0.08	0	0
14	33.12	α-Cyclogeraniol	0.54	0.44	0.99
15	33.52	Thymoquinol	1.63	1.63	0.75
16	34.43	β-Cyclocitral	18.61	18.35	23.11
17	34.791	Carvone	0.05	0.2	0.06
18	35.426	Methyl (2E)-4-isopropyl-5-methyl-2,4-hexadienoate	0.25	0.22	0.26
19	36.404	2-Isopropyl-5,5-dimethyl-2-cyclohexen-1-one	0.67	0.6	0.8
20	36.85	Chrysanthemic acid	0	0	0.08
21	39.586	β-Elemene	0.57	0.45	0.48
22	40.845	β-cis-Caryophyllene	0.06	0	0
23	42.229	Aristolochen	1.73	1.66	1.51
24	42.458	α-Guaiene	2.33	2.26	2.12
25	42.87	β-Selinene	35.52	40.49	32.92
26	43.397	Seselin	0.19	0	0.45
27	44.04	Kessane	8.16	11.13	10.15
28	44.97	Rolipram	0	0	0.05
29	45.874	Dill apiole	0	0.08	0
30	46.395	Cubebol	0.12	0.07	0.12
31	46.412	4(15)-Selinene-11,12-diol, methyl ether	0	0	0.11
32	47.579	Selinenol	0	0.46	0.47
33	48.117	Epicubebol	0	0	0.08
		Total	100	100	100

In this study, before irradiation**,** Menthol, Chrysanthemic acid, Rolipram, 4(15)-Selinene-11,12-diol, methyl ether and Epicubebol were absent in the oil but appeared at a dose of 10 kGy. In contrast, Piperitenone oxide, cis-Isogeraniol, and β-cis-Caryophyllene disappeared from irradiated CSEO. The disappearance of some constituents from the irradiated oil may suggest the sensitivity of these constituents to irradiation. The results obtained were very similar to the results documented by **El-Beltagi *et al.* [35]**, who reported that there were new compounds detected in the GC-MS analysis of oil extracted from irradiated celery seeds, while other compounds increased or decreased. In another study, the chromatogram of the Moroccan *Lavandula dentata* L., essential oil irradiated at 5 kGy exhibits a reduction in the peak areas associated with non-aromatic monoterpene hydrocarbons, while an elevation in the percentages of sesquiterpene hydrocarbons, oxygenated monoterpenes, and diterpenes can be observed. Also, the chromatogram of the EO exposed to 10 kGy shows a greater reduction in non-aromatic monoterpene hydrocarbons, along with a noticeable elevation in the peak regions of oxygenated monoterpenes, sesquiterpene hydrocarbons, and diterpenes. This suggests that higher doses of irradiation have a more pronounced impact on the molecular composition of the EO components **[10]**.

The precise mechanism through which radiation causes alterations in essential oil constituents remains unclear. However, these changes may be attributed to the sensitivity of the essential oil components and the modifications in molecular configuration caused by radiation. The observed changes are caused by the oxidation and hydroxylation of the aromatic rings, possible degradation of certain components during gamma irradiation, as well as the radiolytic impact and potential formation of free radicals **[36].**

Our results give useful information regarding the composition of the non-irradiated and irradiated (10 kGy) CSEO and the quantity of its components, which may be used as a basis for further investigations into the potential applications of this EO in various industries (pharmaceutical, medicine, and foods).

3.2. Effect of γ-irradiation on antibacterial activity of CSEO against *Enterobacter cloacae*

This study indicates that the CSEO may exhibit an extensive range of biological effects due to its numerous active components. The distinctive chemical composition of the essential oil suggests that it could potentially serve as a natural source of antibacterial agents. **Fig 2** revealed the MICs and MBCs of non-irradiated and irradiated CSEO (5 and 10 kGy) against *Enterobacter cloacae*. Non-irradiated CSEO was potentially active against *E. cloacae* with MICs values of 0.061 mL/mL, and the MBC value was equal to the MIC value. On the other hand, using the 5 kGy irradiated CSEO showed a substantial decline in antibacterial efficacy, with the MIC increasing to 0.245 mL/mL from the initial 0.061 mL/mL before irradiation, while the antibacterial activity of CSEO against E. cloacae was unaffected by γ-irradiation at 10 kGy. A plant extract's antibacterial activity is deemed substantial when the MICs are less than 100 µg/mL, moderate when the MIC is between 100 and 625 µg/mL, and low when the MIC is greater than 625 µg/mL **[37]**. Therefore, the antibacterial activities reported by CSEO in this investigation can be primarily considered significant. Utilizing CSEO as a natural antibacterial agent has the potential to serve as a viable substitute for traditional antibiotics in the future.

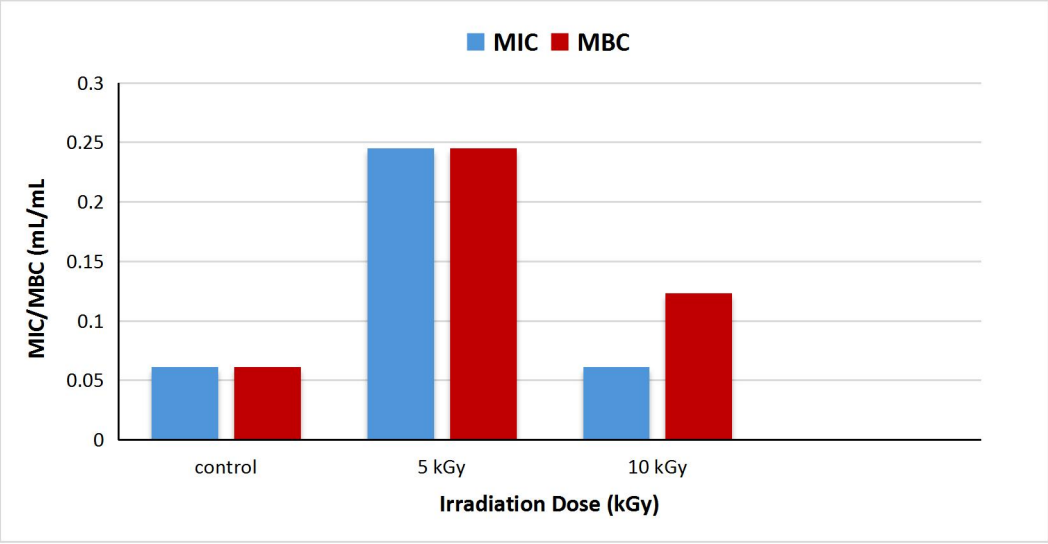

Fig (2): Minimum Inhibitory concentration (MIC) and Minimum Bactericidal concentration (MBC) of non-irradiated and irradiated (at doses of 5 and 10 kGy) celery seeds essential oil (CSEO) against *Enterobacter cloacae* PP388966.

The GC-MS analysis in this study showed that the effectiveness of the CSEO can be attributed to its elevated concentrations of D-limonene, -selinene, and -cyclocitral. Nevertheless, the oil's antibacterial properties may not be exclusively attributed to the existence of these particular molecules, but rather to the interplay between various aromatic structures. These compounds have the potential to work together in a synergistic manner, either individually within the essential oil or in combination with one another. Additionally, it should be emphasized that the presence of minor molecules in essential oils (EOs) might make a substantial contribution to their antibacterial effectiveness **[10].**

The GC-MS analysis in this study revealed disparities in the chemical composition of irradiated and non-irradiated oil. The observed decline in antibacterial activity after exposure to 5kGy can be attributed to changes in the chemical composition of the oil. This modification may have resulted in a decrease in the availability or structural modifications of the main constituents responsible for the antibacterial activity, resulting in a diminished antibacterial impact. The variations in the effect of radiation on antibacterial activity can be attributed to several factors that include microorganisms, plant species, geographical and environmental conditions, sample state (solid or dry), chemical composition, extraction methods, temperature, and gamma irradiation dosage, etc.

In another study, **El-Beltagi *et al.* [35]** reported that gamma irradiation can improve the antibacterial activity of the celery seeds essential oil against *Staphylococcus aureus* at irradiation dose of 5 kGy, while increased antibacterial efficiency was detected after irradiation at 10 kGy against *Escherichia coli* and *Candida albicans*. **Belcadi *et al.* [10]** suggested that exposing the essential oil of Tanacetum annuum L. to doses of 5 and 10 kGy did not significantly affect its ability to inhibit the growth of *E. coli*. Using the same oil at a dose of 5 kGy shown a significant improvement in antibacterial activity against *Staphylococcus aureus*, however, decreased inhibition zone was detected at 10 kGy irradiation dose. Some researchers reported that an irradiation dose of 10 kGy was more efficient in inhibiting tested bacteria **[38 & 39]**. It is difficult to determine the effect that radiation has on the chemical composition of plants because this effect is random and occurs in an unspecified and unknown way. However, it is necessary to determine doses that do not affect the biological properties of medicinal plants that are used for medicinal and nutritional purposes. Therefore, this study recommends that celery seeds could be sterilized at 10 kGy without affecting the antibacterial properties of the essential oil of this plant.

Additional investigation is required to ascertain the optimal irradiation conditions that will enhance antibacterial effectiveness while limiting any adverse effects on the chemical composition and quality of the oil. However, irradiated CSEO, used as a natural antibacterial agent, could potentially serve as a feasible substitute for traditional antibiotics in the future.

4. Conclusion

This study proved the importance of celery seeds essential oil to suppress the proliferation of drug-resistant bacteria, and it indicates that CSEO has the potential to emerge as a novel competitor to conventional antibiotics in underdeveloped nations, particularly Egypt. The presence of multiple active components in celery seeds essential oil could be related to its broad spectrum of biological activity. Overall, the chemical composition of *celery seeds'* essential oil indicates that it has the potential to be used as a source of innovative medicinal agents. Studying the impact of gamma-irradiation on the chemical composition and antibacterial properties of celery seeds essential oil demonstrated that gamma irradiation had a notable influence on the chemical composition of the oil. The detected alterations in the chemical composition may affect the therapeutic properties of the essential oil and should be considered when evaluating the impact of irradiation on the oil's quality. The study proved that irradiation at 10 kGy can preserve biological activity, especially the antibacterial activity of the volatile oil extracted from celery seeds, this is important for determining the irradiation doses used in sterilizing medicinal and aromatic plants that are used in medical, pharmaceutical, and food applications. The utilization of non-irradiated or irradiated (10 kGy) celery seeds essential oil as an initial basis for creating a novel resistance-modifying agent strategy appears to be promising. Using essential oil shows a minimal likelihood of promoting bacterial resistance to its effects. The essential oil comprises many active components which render bacterial adaptation compared to single-constituent antibiotics.

5. Conflict of interests

The authors declare no conflicts of interest.

6. Acknowledgements

The authors acknowledge the National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, for facilitating the completion of this work.

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