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Gas Chromatography/Mass Spectroscopy (GC/MS) analysis, antioxidant, and antimicrobial activity of essential oils based on γ-irradiated clove and cinnamon

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Abstract

This study was aimed to evaluate the extracted essential oils constituents of gamma irradiated clove buds and cinnamon barks as antioxidant and antimicrobial agents. Three doses of gamma irradiation i.e. 4, 8, and 12 kGy were done on clove and cinnamon and essential oil were extracted. The total phenolics content (TPC) and total flavonoids content (TFC) were investigated. Results from GC/MS analysis demonstrated that eugenol and trans-cinnamaldehyde were the main components of clove and cinnamon essential oils, respectively. At the dose 12 kGy, the TPC were 91.57 and 52.89 mg GAE /100g oil and TFC were 23.25 and 19.71mg QE/100g oil for clove and cinnamon essential oils, respectively. Moreover, DPPH radical scavenging assay values were 96.11 and 95.61% while, FRAP were 1332.94 and 1312.94 µM TE/mg oil for each clove and cinnamon essential oils, respectively. In addition the antimicrobial activity of essential oils against gram-positive bacteria (*Bacillus cereus*, *Bacillus Subtilis*, and *Staphylococcus aureus*), gram-negative bacteria (*Salmonella* Typhimurium*, Pseudomonas aeruginosa, and Escherichia coli*) and three fungi strains (*Aspergillus niger, Aspergillus flavus***,** and *Penicillium digitatum*) revealed that each tested oils had a growth inhibition impact on the microorganism under study, while 12 kGy was more effective against the majority of microorganisms. Consequently, the current study recommends that essential oils derived from clove buds and cinnamon barks could be used as natural, safe and efficient substitutes of synthetic (preservatives, antioxidants, and antimicrobials) in food products and pharmaceutical formulations due to their exceptional biological activities.

Keywords: Gamma irradiation, clove essential oil, cinnamon essential oil, GC-MS, antioxidant activity, antimicrobial activity.

1. Introduction

Essential oils (EOs), characterized by their distinct aromatic profiles, are complex matrices of volatile organic compounds synthesized as secondary metabolites within aromatic flora **[1]**. These natural extracts serve as a valuable reservoir of bioactive compounds, finding applications across food science, preservation, and therapeutic interventions. EOs are known for their diverse biological activities, encompassing antimicrobial (bactericidal, antiviral, fungicidal), antioxidant, and insecticidal properties, alongside their established aromatic and therapeutic attributes. Their potential extends to the substitution of synthetic additives in food preservation, and they exhibit analgesic, sedative, anti-inflammatory, spasmolytic, and local anesthetic effects **[2]**.

Cloves (*Syzygium aromaticum* L.) belong to the family Myrtaceae. Distilling the buds, leaves, or stems yields clove oil, with various properties, which is white or pale yellow and has the unique smell and aroma of cloves **[3]**. Essential oils derived from clove buds are widely recognized for their pharmacological and biological qualities, including antimicrobial, antioxidant, anticancer, anti-inflammatory, insecticidal, and antibiofilm properties, due to their high phenolic and flavonoid content **[4].**

Cinnamon (*Cinnamomum zeylanicum*) is classified within the Lauraceae family. The extraction of cinnamon essential oil is commonly achieved through fragrance extraction methodologies, such as distillation, utilizing diverse anatomical components of the plant, including bark, leaves, twigs, calyces, and seeds **[5]**. The bark is recognized as the primary commercial source of raw materials due to its elevated concentrations of essential oils and trans-cinnamaldehyde, a crucial bioactive compound in cinnamon oils **[6]**. Beyond its antimicrobial properties, cinnamon has demonstrated potential in the management of diabetes and glucose intolerance, inhibition of certain cancer cell lines, and alleviation of common cold symptoms **[7].** Furthermore, the antioxidant and preservative attributes of cinnamon are attributed to the presence of phenolic compounds.

It is normal practice to use food irradiation to increase the safety and shelf life of foods, including most herbs and spices. Gamma irradiation is a phytosanitary treatment according to **Douar-Latreche et al**. **[8]** that has been proven to be safe and effective in improving the hygienic quality of a range of foods and herbal materials in order to extend their shelf life. The FDA has raised this restriction to 30 kGy for decontaminating dry food and spices, although the gamma irradiation method still allows a maximum total absorbed dose of 10 kGy for decontaminating dehydrated aromatic herbs, spices, and vegetable flavorings **[9].**

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However, both qualitative and quantitative changes in plant phytochemicals can result from irradiation. Although the safety of irradiated food is well established, little is known about how irradiation affects the antioxidant and antibacterial capabilities as well as other chemical components of herbs and their seeds. Since some studies have shown that irradiation treatment can increase the contents of certain phytochemicals and enhance the antioxidant and antimicrobial activities and biological value of various materials, it can be assumed that gamma irradiation may not only be a useful decontamination viewpoint but also an enhancement factor for some important botanical properties **[10].**

Many previous studies have investigated the effect of gamma irradiation on the chemical composition and bioactivity of essential oils (EOs) from various herbs and spices. **Al-Kuraieef and Alshawi [11]** reported that gamma irradiation (5, 10, and 15 kGy) increased total phenols, flavonoids, and antioxidants in thyme, with moderate EO alterations at 5 and 10 kGy and high thymol sensitivity at 15 kGy. DPPH radical-scavenging activity decreased, while thiobarbituric acid (TBA) values increased at 10 and 15 kGy. **El-Beltagi et al. [12]** observed that gamma irradiation (2.5, 5, and 10 kGy) of celery seeds enhanced EO yield (maximum at 10 kGy), altered compound profiles (GC-MS). New compounds were detected in the irradiated samples, while other compounds increased or decreased in concentration. Antimicrobial activity varied, with optimal inhibition of *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsiella pneumoniae* at 5 kGy, and *Escherichia coli* and *Candida albicans* at 10 kGy. **Abd El Mageed et al. [13]** investigated the impact of gamma irradiation (6, 8, and 10 kGy) and thermal treatments on fennel EO composition and antioxidant properties. Roasting, including gamma irradiation, significantly increased phenylpropanoid content (especially estragole), with 10 kGy yielding the highest levels. Monoterpene content decreased with roasting, except for a slight increase in electric oven-treated samples. Oxygenated terpenoids decreased across all treatments. Microwave-heated samples showed the highest DPPH radical-scavenging activity (58.65%), followed by 10 kGy irradiated samples (49.27%), compared to BHT (98%).

While extensive research has explored the biological activities of clove and cinnamon essential oils and the effects of gamma irradiation on pathogenic bacteria, investigations into the chemical composition, antioxidant capacity, and antimicrobial efficacy of essential oils extracted specifically from gamma-irradiated clove buds and cinnamon bark remain limited. Therefore the present investigation aimed to evaluate the impact of some doses of gamma irradiation on clove and cinnamon seasonings, with a specific focus on assessing potential enhancements in their biological activity. To this end, the study examined the chemical composition, total phenolic content, total flavonoid content, antioxidant capacity, and antimicrobial efficacy of essential oils extracted from gamma-irradiated clove buds and cinnamon bark.

2. Material and methods

2.1. Plant Materials

Clove buds (*Syzygium aromaticum* L.) and cinnamon barks (*Cinnamomum zeylanicum*) were purchased from a local market, Cairo, Egypt. The samples were ground into a fine powder using electric grinder (Moulinex, France). The ground samples were packed in polyethylene bags, each bag contain 100 g, and kept at -18±1^{o} C to undergo gamma irradiation treatment before the oil was extracted.

2.2. Chemicals and reagents

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH), trolox, gallic acid, quercetin, folin- Ciocalteu reagent were purchased from Sigma (St. Louis, MO, USA). Sodium carbonate, aluminium chloride, Butylated hydroxyl toluene (BHT), sodium acetate, glacial acetic acid and ferric chloride were purchased from El-Gomhoria Company for Chemicals and Drugs, located in Cairo, Egypt. All chemicals are analytical grade.

2.3. Microbial strains

Three gram-positive strains (*Bacillus cereus* (ATCC 33018), *Bacillus Subtilis* (ATCC 9372) and *Staphylococcus aureus* (ATCC 20231)), three gram-negative bacteria (*Salmonella* Typhimurium (ATCC 98031), *Pseudomonas aeruginosa* (ATCC 9027) and *Escherichia coli* (ATCC 35218)) were obtained from the microbiological resources center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Pathogenic fungi strains (*Aspergillus niger* (ATCC 16404), *Aspergillus flavus* (ATCC 15548) and *Penicillium digitatum* (ATCC 10036)) were obtained from the Regional Center for Mycology and Biotechnology, Faculty of Science, El Azhar University, Cairo, Egypt.

2.4. Methods

2.4.1. Irradiation treatment

The irradiation treatment were performed at dose level of 0, 4, 8, and 12 kGy for packed clove buds and cinnamon bark samples using a ^{60}Co Russian gamma chamber (dose rate 0.39 kGy/h), Cyclotron Project, Nuclear Research Center, Atomic Energy Authority, Inchas, Egypt. The irradiated samples were kept at 4±1°C further the following step.

2.4.2. Extraction of essential oils

The essential oils were extracted from the ground samples (clove and cinnamon) by using the hydro-distillation method **[14]** via the Clevenger device (2 L round-bottom flask connected to a condenser with a heating mantle). We charged the apparatus with distilled water after introducing 100 grams of the plant powder. The hydro-distillation was conducted at a constant temperature of 100 °C (boiling point of water), maintained by a heating mantle with a temperature controller. Following the completion of the extraction procedure (4 hours), the extracted essential oils were gathered, dried over anhydrous sodium sulfate to eliminate any residual water, and kept in brown glass vials at 4±1°C until they were used **[15]**.

2.4.3. Gas chromatography/Mass spectroscopy (GC/MS) analysis of clove and cinnamon essential oils

Gas chromatography (Agilent 8890) with a mass spectrometer detector (Agilent 5977B) series and capillary column DB-5MS, 60 m x 250 µm id x 0.25 µm film thicknesses, were used to investigate the volatile component composition of clove and cinnamon essential oils. The carrier gas was helium gas, which was kept at a steady 65 kPa. 0.001 mL of the essential oil was injected in a split ratio of 1:50 and a solvent delay of 4 minutes. The program oven temperature was set to rise from 50 to 240°C in increments of 5°C per minute until it reached 240°C. Comparing the attained mass spectra and retention time (RT) with those of genuine standards and the National Institute of Standards and Technology (NIST) MS Library allowed for the identification of volatile components **[16]**.

2.4.4. Total phenolics content of clove and cinnamon essential oils (TPC)

According to a protocol presented by **Singleton and Rossi [17]**, with some modifications the Folin–Ciocalteu reagent-based method was used to ascertain the total phenol content of the essential oils of clove and cinnamon. 0.2 mL of each essential oil was mixed with 1 mL of newly made (1:10 *v/v*) Folin-Ciocalteu reagent, agitated for 30 seconds, and then allowed to sit at room temperature in the dark for 5 minutes. After adding 0.8 mL of sodium carbonate (7.5% *w/v*) to the mixture, it was agitated for 30 seconds and left to stand for 15 minutes at room temperature. The absorbance was measured at 765 nm using a spectrophotometer. Gallic acid was used as standard for the calibration curve. The total phenol content was reported as mg Gallic acid equivalent (GAE)/100g essential oil.

2.4.5. Total flavonoids content of clove and cinnamon essential oils (TFC)

Aluminium chloride method according to **Marinova et al. [18]** with some modifications was utilized. By combining 0.5 mL of each essential oil with 0.5 mL of 2% $AlCl_3$ (w/v) and leaving the mixture in the dark for 15 min. The absorbance was then measured at 415 nm was then measured as a blank value in comparison with water using a spectrophotometer. The results were reported as Quercetin equivalents (QE)/100g essential oil using a standard Quercetin calibration curve.

2.4.6. Antioxidant activity of clove and cinnamon essential oils

2.4.6.1. DPPH radical scavenging assay

The capacity of clove and cinnamon essential oils was assessed using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) **[19]** with a few minor adjustments. In short, 0.1 mL of each essential oil was combined with 3 mL of 0.1 mM DPPH that had been prepared in methanol. A UV spectrophotometer was then used to determine the optical density at 517 nm after the mixes had been left in the dark for 30 minutes at room temperature. As a control positive, butylated hydroxyl toluene (BHT) was employed. The following formula was used to determine the antioxidant activity:

$$\text{Antioxidant activity (Inhibition)}\% = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Where A sample is the absorbance in the presence of each essential oil and A control is the absorbance of the control response.

2.4.6.2. Ferric Reducing Antioxidant Power (FRAP)

The clove and cinnamon essential oils samples antioxidant capacity was determined using spectrophotometry in accordance with the FRAP method, which was detailed by **Benzie and Strain [20]** with minor modifications. Every time, FRAP solution was freshly prepared using the stock solutions. For the acetate buffer stock solution, 400 mL of water with 8 mL of glacial acetic acid was combined with 1.6 g of sodium acetate trihydrate. Using 1 M NaOH, the mixture's pH was adjusted to 3.6. The mixture was completed to 500 mL with water. 156 mg of TPTZ were dissolved in 50 mL of 40 mM HCl to create the TPTZ stock solution. 50 mL of a 20 mM ferric chloride solution was made as the final stock solution. 2.2 mL of FRAP reagent containing acetate buffer, TPTZ solution, and ferric chloride solution at a ratio of 10:1:1 (*v/v/v*), respectively, was pipetted into tubes. The FRAP reagent was then filled with 0.02 mL of each oil sample. For four minutes, the mixes were left at room temperature in the dark. A UV spectrophotometer was used to measure absorbance at 593 nm as the reaction time is reached. Trolox (TE) (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid) was used as the standard in the plotting of a calibration curve. µmol of Trolox per mg of the sample (µmol TE/mg sample) was used to express the results.

2.4.7. Antimicrobial properties of clove and cinnamon essential oils

2.4.7.1. Preparation of the Microbial inoculum

Bacterial strains under investigation were propagated on nutrient agar plates and incubated at 37 °C for 24 hours. Conversely, mold isolates were cultured on yeast and mold extract agar (YM, Difco) for 5–7 days at 25 °C. To achieve a turbidity level that matched the 0.5 McFarland standards (approximately 10^8 colony-forming units/mL), colonies from these plates were suspended in nutrient broth as well as broth containing yeast and mold extract. Antimicrobial assays were performed using Mueller-Hinton agar for bacteria and potato dextrose agar for fungi. Incubation conditions for each organism were implemented according to established protocols **[21]**.

2.4.7.2. Agar well diffusion assay for determine the antimicrobial activity

The agar well diffusion experiment, according to **Kowalska-Krochmal and Dudek-Wicher [22]**, was used to measure antimicrobial activity of clove and cinnamon essential oils. Using this procedure, flasks containing 25 mL of sterile Muller-Hinton agar (for bacteria) and Potatoes Dextrose Agar (for fungi) at 42–45^o C were filled with 0.25 mL of inoculum

suspensions of each test microorganism (bacteria or fungi), which were then transferred into Petri plates. Using a sterile cork-borer, 8 mm diameter wells were created in the media, and 0.07 mL of essential oil samples were then added to each well. The plates were incubated at 37 C for 24 hours and 30 C for 3–5 days for bacteria and fungus, respectively, after being allowed to sit at room temperature for 1 hour to allow the oil to diffuse into the media. For every sample, triplicates were made. Clear zones formed as a result of the essential oils antibacterial properties inhibiting microbiological growth. Millimeters were used to measure the zone of inhibition.

2.5. Statistical analysis

SPSS **[23]** analysis software version 18.0 (SPSS Inc., Illinois, and USA) was used to analyze the data. Each analysis was conducted in three replicates, and the mean ± SE was used to report the findings. The data were subjected to a one-way analysis of variance (ANOVA), and the means were compared using a Duncan test as a post-hoc test. A 95% confidence level ($p < 0.05$) was used to assess significance levels.

3. Results and Discussion

3.1. GC/MS analysis of clove and cinnamon essential oils

The GC-MS analysis of clove buds oil of revealed roughly sixteen components in the control sample and the eugenol (67.55%), eugenol acetate (13.52%), caryophyllene (13.41%), and humulenen (2.06%) were the principal constituents were observed. Less than 1% of the essential oil is composed of other substances **(Table 1 and Figure 1)**. These results are similar with previous studies **[24]**, who found that the primary compound responsible for 81.69% of clove essential oil was eugenol, eugenyl acetate was found to be the second largest component (8.31% of the total contents), and Caryophyllene was considered a moderate component, making up 4.32%. According to **He et al. [25]**, eugenol (86.07%) was the most common and the main volatile constituent among the 14 chemical components of clove essential oil, followed by β-trans-caryophyllene (10.51%). Even while these oils' primary constituents are largely the same, the concentrations of particular components can vary depending on a variety of circumstances, such as the environment, genetics, cultivation methods, and the essential oil extraction technology **[26]**.

The essential oil recovered from irradiated clove buds were impacted by the irradiation treatment where the Eugenol percentage rising from 67.55% in the control sample to 69.02%, 67.75, and 70.07% in samples exposed to dose levels of 4, 8, and 12 kGy, respectively. On the other hand, the irradiation treatment was decreased the amount of certain chemicals, including Caryophyllene, Humulene, and Eugenol acetate; Epicubebol and γ-Muurolene vanish; while, the Cubebol and Isogermacrene D were generated. These results are consistent with **[24]**, who found that the irradiation treatment increases the percentage of eugenol from 81.69% in the control sample to 81.76%, 81.8%, 81.9%, and 82.1% in samples exposed to dosage levels of 15, 20, 25, and 30 kGy, respectively. Additionally, the irradiation treatment results in a decrease in the amounts of α-pinene, β-caryophyllene, β-Humulene, and eugenyl acetate and an increase in the percentage of caryophyllene.

Table 1: GC/MS analysis of clove essential oil

Peak	RT	Compounds	Area Sum %			
			0 KGy	4 KGy	8 KGy	12 KGy
1	30.149	Chavicol	0.15	0.13	0.12	0.12
2	31.763	trans-Cinnamaldehyde	0.29	0.62	1.18	1.46
3	35.288	Eugenol	67.55	69.02	67.75	70.07
4	36.678	Copaene	0.7	0.77	0.81	0.79
5	38.904	Caryophyllene	13.41	12.64	11.99	11.19
6	40.466	Humulene	2.06	2.04	1.91	1.77
7	40.93	γ-Cadinene	0.19	0.22	0.19	0.19
8	41.536	α-Farnesene	0.19	0.21	0.23	0.19
9	42.08	Eugenol acetate	13.52	12.35	13.47	12.17
10	42.761	Cadina-1(10),4-diene	0.77	0.88	0.76	0.71
11	43.047	cis-Calamenene	0.15	0.18	0.16	0.16
12	43.51	Cubenene	0.19	0.23	0.21	0.18
13	46.051	Caryophyllene oxide	0.33	0.27	0.53	0.41
14	47.636	Epicubebol	0.04	-	-	-

15	48.174	Cubebol	-	-	0.17	0.14
16	48.18	γ-Muurolene	0.1	-	-	-
17	48.191	Isogermacrene D	-	0.11	-	-
18	49.604	2,4,6-Trimethoxyacetophenone	0.31	0.33	0.52	0.44
Total			99.95	100	100	99.99

(-) = Not Detected

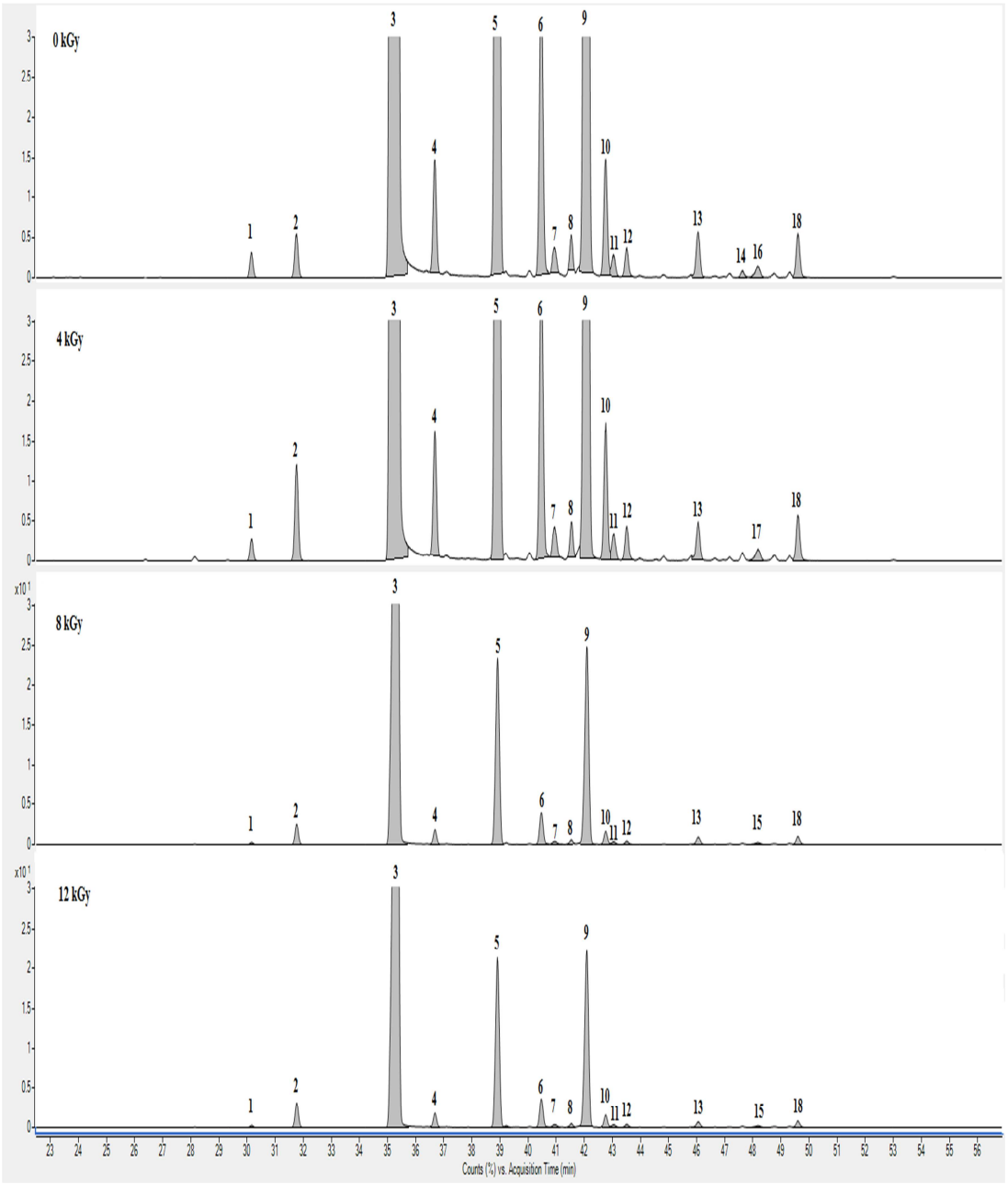

Figure 1: . Chromatogram of GC-MS of clove essential oil

Table 2 and Figure 2 refers to that the GC-MS analysis of cinnamon bark oil showed the control sample included about 18 different components. According to **Table (2)** and **Fig. (2)**, the main components of cinnamon essential oil are trans-cinnamaldehyde (82.64%), 2-Methoxycinnamaldehyde (2.30%), cinnamonyl acetate (2.24%), δ-cadinene (2.07%), and coumarin (2.06%). These results are agree with **[27]**, which found that the main and most common ingredient in cinnamon essential oil is (E)-cinnamaldehyde (82.85%). In another study, the primary constituents of cinnamon essential oil were (E)-cinnamaldehyde (71.50%), linalool (7.00%), -caryophyllene (6.40%), eucalyptol (5.40%), and eugenol (4.60%), according to **Behbahani et al. [15]**. Furthermore, a number of further investigations have verified that the primary chemical component of cinnamon essential oil is trans-cinnamaldehyde **[28, 29]**.

Table 2: GC/MS analysis of cinnamon essential oil

Peak	RT	Compounds	Area Sum %			
			0 KGy	4 KGy	8 KGy	12 KGy
1	26.648	Dihydrocinnamaldehyde	0.3	0.37	0.56	0.37
2	29.309	cis-Cinnamaldehyde	0.79	0.7	1.57	1.1
3	32.101	trans-Cinnamaldehyde	82.64	79.16	79.25	80.9
4	33.114	Cinnamyl alcohol	0.25	0.29	0.2	0.21
5	35.134	Eugenol	0.7	1	1.75	1.3
6	36.673	Copaene	1.85	2.19	2.06	2.17
7	38.882	β-Caryophyllen	0.26	0.4	0.59	0.3
8	39.173	Cinnamyl acetate	2.24	1.71	1.72	0.94
9	39.608	Coumarin	2.06	2.45	1.71	1.85
10	40.455	α-Humulene	-	-	0.17	-
11	40.981	γ-Muurolene	0.38	0.53	0.5	0.52
12	41.949	α-Muurolene	1.97	2.68	2.37	2.55
13	42.761	δ-Cadinene	2.07	2.68	2.6	2.64
14	43.03	2-Methoxycinnamaldehyde	2.3	2.77	2.1	2.3
15	43.511	Cubenene	0.63	0.87	0.79	0.74
16	43.968	α-Calacorene	0.19	0.26	0.24	0.23
17	45.765	Caryophyllen alcohol	-	0.19	0.2	0.2
18	46.789	Cubenol	0.26	0.25	-	0.21
19	47.613	1,10-Diepicubenol	0.33	0.39	0.44	0.43
20	48.231	T-MuuroloI	0.77	1.11	1.17	1.04
Total			99.99	100	99.99	100

(-) = Not Detected

From the same table, gamma radiation at dose levels of 4, 8, and 12 kGy, decreased the percentage of trans-cinnamaldehyde and cinnamyl acetate in the control sample from 82.64% and 2.24% to 79.16% and 1.71%, 79.25 and 1.72%, and 80.9% and 0.94%, respectively. According to comparable findings by **Alloun et al. [9], Al-Kuraieef and Alshawi [11], Nada et al. [10] and Ali et al. [30]**, the irradiation treatment significantly decreased the primary components of Thymus pallescens oil (carvacrol), thyme oil (thymol), clove oil (eugenol) and lemon peel oil (Limonene). Conversely, **Abdelmoety et al. [31]** found that the percentage of cinnamaldehyde increased from 75.88% to 85.80% as a result of irradiation treatment at 10 kGy.

However, after irradiation treatment, the amount of several chemicals increases, including eugenol, copaene, α-muurolene, and δ-cadinene; cubenol disappears at dose 8 kGy, and α-humulene and caryophyllen alcohol were generated.

Various EOs have various reactional environments for volatile molecules, and when exposed to γ-radiation, each EO undergoes a different process of isomerization, oxidation, and hydroxylation to produce novel chemicals **[32]**.

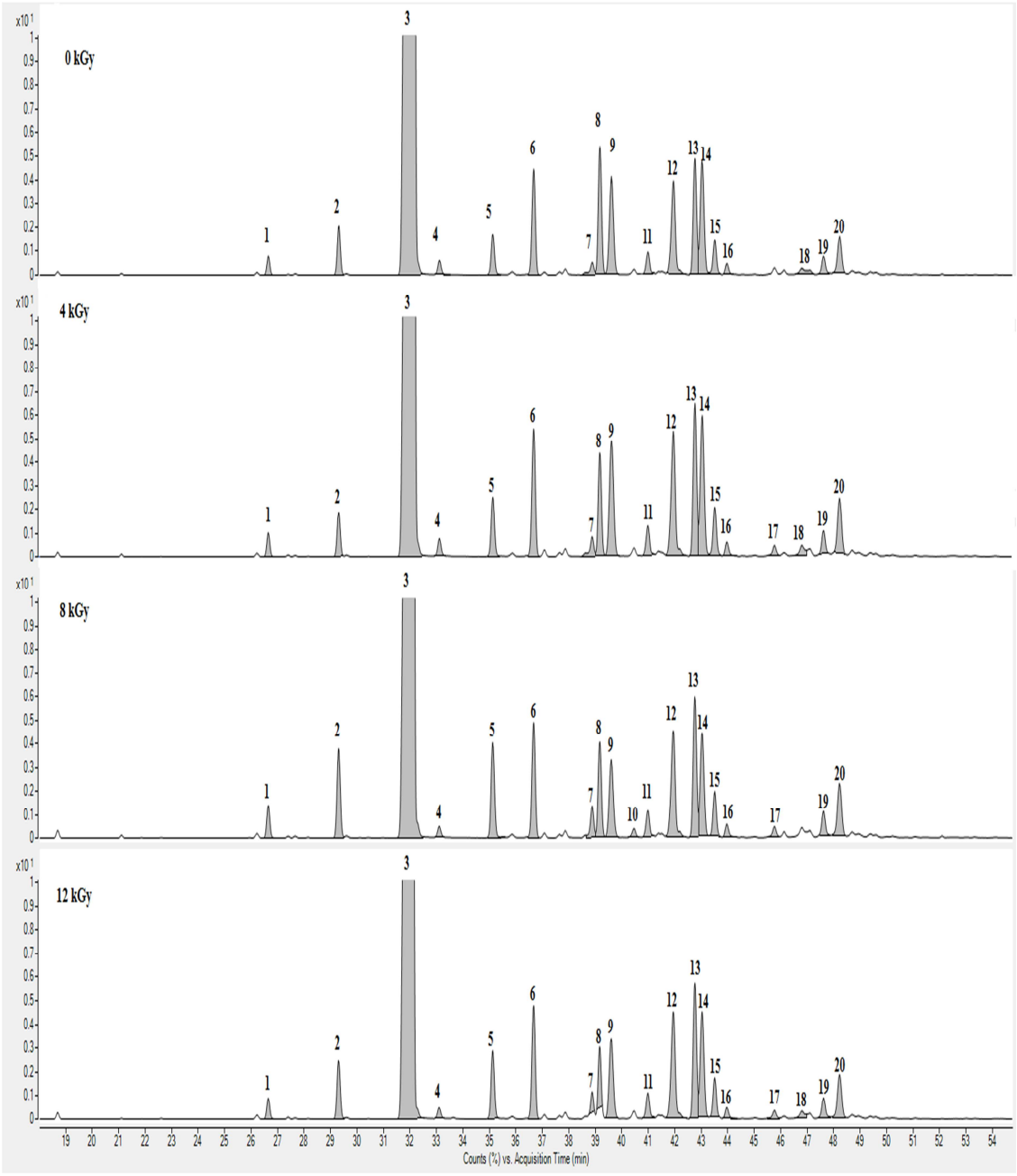

Figure 2: Chromatogram of GC-MS of cinnamon essential oil

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However, after irradiation treatment, the amount of several chemicals increases, including eugenol, copaene, α-muurolene, and δ-cadinene; cubenol disappears at dose 8 kGy, and α-humulene and caryophyllen alcohol were generated. Various EOs have various reactional environments for volatile molecules, and when exposed to γ-radiation, each EO undergoes a different process of isomerization, oxidation, and hydroxylation to produce novel chemicals **[32]**.

3.2. Total phenolic contents (TPC) and total flavonoid contents (TFC) of clove and cinnamon essential oils

The TPC and TFC of oils extracted from non-irradiated and irradiated (0, 4, 8 and 12 kGy) clove buds and cinnamon barks were determined and recorded in **Table (3)**. Where the TPC in clove bud and cinnamon bark oils were (85.86 mg GAE/100 g oil and 50.53 mg GAE/100 g oil, respectively), while, the TFC were (and 19.94 mg QE/100 g oil and 19.11 mg QE/100 g oil respectively) in the control samples, these results are close to which obtained by **Omar et al. [28]** who found that clove oil and cinnamon oil contents of TPC were 36.6 mg GAE /100 g oil and 22.5 mg GAE /100 g oil, respectively but, the TFC were 15.4 mg QE /100 g oil and 11.1mg QE /100 g oil, respectively.

On the other hand, the total phenolic and flavonoid contents of irradiated clove buds were significantly ($p > 0.05$) higher in the samples irradiated at the studied doses than in the control sample. Whereas, the sample irradiated at dose 12 kGy had the highest value of TPC and TFC (91.57 mg GAE/100 g oil and 23.25 mg QE/100 g oil, respectively), followed by the samples irradiated at doses 4 then 8 kGy.

Similar patterns were noted by **Nada et al. [10]**, who found that the levels of TPC and TFC in clove buds oil were preserved and increased at a dose level of 10 kGy also **Jamshidi et al. [33]**, who found that gamma irradiation treatment, raised the total phenolic content of cinnamon extract at dosage levels of 20 and 25 kGy.

The impact of gamma irradiation on the bioactive constituents of medicinal plants depends on several factors, but the main ones are the species and the dose of radiation applied **[34]**.

Khattak et al. [35] attributed the differential effects of gamma irradiation on phenolic and flavonoid compounds to variations in their extractability. Furthermore, the observed increase in phenolic and flavonoid content may be correlated with the radiation-induced degradation of tannins, with plant species exhibiting a higher proportion of hydrolysable tannins demonstrating greater sensitivity to irradiation compared to those predominantly containing condensed tannins.

3.3. Antioxidant activity of clove and cinnamon essential oils

The study examined the antioxidant activities of essential oils from both irradiated and non-irradiated clove buds and cinnamon barks using the two distinct methods of DPPH radical-scavenging activity and FRAP.

3.3.1. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity

The results in **Table 3** showed that the essential oils of all examined samples showed strong antioxidant capacities. The control samples of clove bud and cinnamon bark oils had DPPH radical scavenging activities of 94.45% and 94.73%, respectively.

It is evident that, in comparison to control samples, the irradiation treatment at a dose level of 12 kGy increased the DPPH radical scavenging potency of both clove bud essential oil to reach 96.11%, and cinnamon bark essential oil to become 95.61%. Similar patterns were noted by **Nada et al. [10]** in the essential oil of clove buds and **Fatemi et al. [36]** in the essential oil of peppermint when the irradiation treatment was administered at a dose level of 10 kGy.

Moreover, there were a remarkable stronger ability to neutralize DPPH radicals of irradiated clove buds (94.45 %, 95.045 %, 94.92 % and 96.11 %) and cinnamon barks (94.73 %, 92.60 %, 93.41 % and 95.61 %) at dose levels of 0, 4, 8 and 12 kGy, respectively than the reference standard butylated hydroxytoluene (BHT) which showed the lowest scavenging activity with a value equal to 91.5%. The antioxidant activity of clove and cinnamon essential oils is primarily due to the eugenol and cinnmaldahyde content, in addition a number of studies demonstrated a clear linear correlation between the (quantity of phenolic and flavonoid compounds) and antioxidant activity **[37]**. And the fragmentation of the sample's hydroxyl group as a result of irradiation process may have contributed to the increase in antioxidant activity; hydrogen atoms reacted with the free radical to produce a more stable product **[38]**.

The breakdown of larger phenolic substances into smaller ones by gamma irradiation or the activity of phenylalanine ammonia-lyase, the essential enzyme for the metabolism of phenolic compounds, could be the cause of the higher TPC and TFC in oils extracted from irradiated clove buds and cinnamon barks as opposed to those extracted from non-irradiated. Similarly, irradiation-induced damage may be the cause of rising flavonoid concentration **[39].** Consequently, the antioxidant effectiveness of EOs increases as their phenolic and flavonoid concentration rises.

Table 3: Total phenolics content (TPC), total flavonoids content (TFC) and antioxidant activity of clove and cinnamon essential oils

	γ-Irradiation doses (kGy)	TPC (mg GAE/100 g oil)	TFC (mg QE/100 g oil)	Antioxidant Activity	
				DPPH (%)	FRAP (µM TE/mg oil)
Clove E. O.	0	85.86^{D} ±0.124	19.94^{D} ±0.198	94.45^{A} ±1.032	1308.56^{A} ±3.22
	4	89.97^{B} ±0.149	22.03^{B} ±0.187	95.045^{A} ±0.385	1326.10^{A} ±2.12
	8	87.34^{C} ±0.086	20.90^{C} ±0.170	94.92^{A} ±0.376	1319.43^{A} ±2.43
	12	91.57^{A} ±0.372	23.25^{A} ±0.099	96.11^{A} ±0.654	1332.94^{A} ±9.11
	BHT	--	--	91.5^{B} ±1.053	941.72^{B} ±2.84
Cinnamon E. O.	0	50.53^{B} ±0.312	19.11^{A} ±0.350	94.73^{AB} ±0.626	1294.35^{A} ±1.67
	4	46.89^{C} ±0.238	15.94^{B} ±0.360	92.60^{C} ±0.687	1262.94^{B} ±5.16
	8	47.37^{C} ±0.194	16.27^{B} ±0.138	93.41^{BC} ±1.048	1216.28^{C} ±2.13
	12	52.89^{A} ±0.293	19.71^{A} ±0.155	95.61^{A} ±0.557	1312.94^{A} ±6.06
	BHT	--	--	91.5^{D} ±1.053	941.72^{D} ±2.84

Values are expressed as mean ± standard error; means with the same capital letter in the same columns (for each individual plant essential oil) are not significantly different ($p < 0.05$), BHT: butylated hydroxytoluene.

3.3.2. Ferric Reducing Antioxidant Power (FRAP)

It is evident from **Table 3** that the antioxidant capacity of the clove bud oil control sample was 1308.56 µmol Trolox/mg oil, but the cinnamon bark oil control sample had 1294.35 µmol Trolox/mg oil as a result of the FRAP technique. The same behavior was observed in clove essential oil, which **Karsli et al. [40]** reported had a significant ferric reducing ability (1734.5 ± 110.7 µmol Trolox/g oil). Eugenol, which is known to have antioxidant activity, may be the cause of clove EO's strong activity **[41]**. On the other hand, **Olszowy and Dawidowicz [42]** discovered that the ferric reducing power value of ceylon cinnamon bark oil is twice that of thyme oil, As well as, **Martiniaková et al. [43]** reported that ceylon cinnamon bark oil has a higher ferric reducing power value (1.64 mmol/L of Trolox Eq.) than oregano, tea tree, clove, thyme, and ylang-ylang oils, claim.

The oils derived from clove bud and cinnamon bark samples exposed to dose levels of 12 kGy showed the same trend as ferric reducing power, as did the DPPH method, when compared to the control samples and doses 4, 8 kGy, which were 1332.94 µM TE/mg oil and 1312.94 µM TE/mg oil, respectively.

Moreover, the synthetic antioxidant of butylated hydroxytoluene (BHT) had the lowest ferric reducing power value (941.72 µM TE/mg oil) as compared to both essential oils extracted from all clove buds (1308.56, 1326.10, 1319.43 and 1332.94 µM TE/mg oil) and cinnamon bark (1294.35, 1262.94, 1216.28 and 1312.94 µM TE/mg oil) samples treated with 0, 4, 8 and 12 kGy of gamma irradiation. Numerous studies have found a linear correlation between antioxidant activity and bioactive substances' electron donation capability, or reducing power **[44]**.

3.4. Antimicrobial activity of clove and cinnamon oils

By measuring the diameters of the inhibition zones, the antimicrobial activity (antibacterial and antifungal activity) of oils derived from both non-irradiated and gamma-irradiated clove buds and cinnamon barks was assessed against some pathogenic microorganisms. As shown in **Table (4)** all of the oils under study exhibited considerable antimicrobial activity against all tested microorganisms, considering variations in gamma irradiation dosage, strain type, and oil type. This may be due to strain sensitivity and the effect of irradiation doses on oil content of active compounds.

3.4.1. Antibacterial activity

Regardless of the applied dosages, there were discernible variations in the extraction oils of clove buds' ability to suppress the growth of all examined bacterial strains (*Bacillus Cereus, Bacillus Subtillus, Staphylococcus Aureus, Salmonella* Typhimurium*, Pseudomonas aeruginosa, and Escherichia coli*). The inhibition zones of the previously studied

microorganisms range from 20.66 mm at the control sample toward Bacillus Cereus to 31 mm at zero kGy against *Bacillus Subtillus*. As many academics have shown, such as **Shahbazi [45]**, clove bud oils have broad-spectrum inhibitory effect against both gram-positive and gram-negative bacteria. Furthermore, **El amrani et al. [46]** found that the clove oil's bacterial activity was significant against mentioned bacteria, with inhibition zones for *S. aureus* ATCC and *P. aeruginosa* ATCC ranging from 21.3mm to 35.6mm but with inhibitory diameters of 14.6 mm for *P. putida*, 15 mm for *E. coli*, and 15.6 mm for *S. aureus*, it was mediocre against the food's microorganisms.

However, the oils derived from cinnamon bark shown exceptional efficacy against all tested bacteria. According to a comparison of the antibacterial potency of oils extracted from irradiated and nonirradiated cinnamon barks, the oils extracted from the control and irradiated samples at dose levels of 4, 8, and 12 kGy showed a significant difference in terms of the inhibition of *Pseudomonas aeruginosa* and *Escherichia coli* growth, with the control sample exhibiting the highest inhibition of the two strains at 58 and 69.33 mm, respectively. In contrast, the inhibitory zones of *Bacillus Cereus, Staphylococcus Aureus,* and *Salmonella* Typhimurium did not significantly alter between irradiated and non-irradiated samples. The inhibitory zones of the previously studied bacteria reached their maximum values of 59, 53.66, and 59.33 mm, respectively, at a treatment dose of 12 kGy. However, *Bacillus Subtillus* was most suppressed by the oil extracted from cinnamon barks exposed to 4 kGy, which measured 61.33 mm.

Numerous investigations have demonstrated the high level of activity of cinnamon bark oils against both Gram-positive and Gram-negative bacteria **[47]**and **[48]**.

When compared to clove buds oil, the cinnamon barks oil in this study showed higher antibacterial activity against all bacterial strains in both irradiated and non-irradiated samples. These findings support those of other authors who have observed that the essential oil of cinnamon is more active than that of cloves **[49].** Eugenol and cinnamon aldehyde may inhibit the production of a crucial enzyme by bacteria, according to **Di Pasqua et al. [50].** Additionally, they might be responsible for cell wall damage. Given that they are the primary constituents of clove and cinnamon oils, their potent antibacterial qualities may be due to their high concentrations **[51].**

3.4.2. Antifungal activity

The essential oils isolated from all samples (irradiated and nonirradiated) of clove buds and cinnamon barks exhibit high action against all fungal strains under study, as indicated in **Table (4)**. According to the findings, the sample exposed to 12 kGy had the highest activity against *Aspergillus niger* (65 mm) and *Aspergillus flavus* (59.33 mm) when compared to other samples. However, the sample that was subjected to 4 kGy had the highest activity (55.33mm) against *Penicillium digitatum.* While there were non-significant differences between oils extracted from all samples (irradiated and nonirradiated) with regard to growth inhibition of *Aspergillus niger* and *Penicillium digitatum*, there were significant differences between the oil extracted from the sample irradiated at 12 kGy and others. Different studies found that clove bud oils can inhibit growth of some fungi strains such as *Aspergillus* (*A. flavus, A. parasiticus, and A. ochraceus*), *Penicillium, C. albicans*, and yeast **[15, 45]**.

The sample treated by 12 kGy had the highest activity against *Aspergillus niger* (51.33mm) and *Aspergillus flavus* (54.67mm) in comparison to the other samples for cinnamon bark oil. On the other hand, the control sample achieved the highest *Penicillium digitatum* inhibition (54.67 mm). In terms of *Aspergillus niger* growth inhibition, there were significant differences between the oils extracted from (the control and irradiated samples at 12 kGy) and other samples; however, in terms of *Aspergillus flavus* and *Penicillium digitatum* growth inhibition, there were non-significant differences between all samples (irradiated and nonirradiated).

Table 4: Antimicrobial activity of clove and cinnamon essential oils.

Microbial Strains	Inhibition zone diameter (mm)							
	(Clove E.O.)				(Cinnamon E. O.)			
	γ-Irradiation doses (kGy)				γ-Irradiation doses (kGy)			
	0	4	8	12	0	4	8	12
Bacillus Cereus	20.66^{B} ±0.33	25.66^{A} ±0.33	26^{A} ±0.57	26.33^{A} ±0.88	55.66^{A} ±0.33	57.33^{A} ±1.34	57^{A} ±0.57	59^{A} ±2.08
Bacillus Subtillus	31^{A} ±1.00	30.33^{AB} ±0.88	28.66^{AB} ±0.65	27.66^{B} ±1.2	60.66^{A} ±0.34	61.33^{A} ±1.33	60.33^{A} ±0.33	55^{B} ±0.57
Staphylococcus Aureus	25.33^{C} ±0.88	25.66^{C} ±0.33	27.33^{B} ±0.34	30^{A} ±0.57	53^{A} ±0.57	51^{A} ±1.00	52^{A} ±0.58	53.66^{A} ±1.45
Salmonella Typhimurium	27^{B} ±0.58	28.33^{AB} ±0.88	29^{AB} ±1.53	30^{A} ±0.57	58.65^{A} ±0.33	57.66^{A} ±0.88	58.33^{A} ±1.66	59.33^{A} ±0.33

Pseudomonas aeruginosa	23.66^{B} ±0.33	26^{AB} ±1.52	24.66^{AB} ±0.33	27^{A} ±0.58	58^{A} ±0.57	54^{C} ±0.58	53^{C} ±0.57	55.66^{B} ±0.33
Escherichia Coli	27^{B} ±0.57	29.33^{A} ±0.33	28.66^{A} ±0.33	29.66^{A} ±0.33	69.33^{A} ±0.33	60^{B} ±2.08	62.33^{B} ±1.20	62^{B} ±1.00
Aspergillus niger	60.33^{A} ±0.88	63^{A} ±1.00	62.66^{A} ±2.40	65^{A} ±1.73	49.66^{A} ±0.33	46.66^{B} ±1.76	46^{B} ±0.58	51.33^{A} ±0.33
Aspergillus flavus	52.33^{C} ±0.88	56.66^{B} ±0.90	54^{C} ±1.00	59.33^{A} ±0.66	53^{A} ±1.00	52.66^{A} ±0.67	53.33^{A} ±0.66	54.67^{A} ±0.88
Penicillium digitatum	51.66^{A} ±1.20	55.33^{A} ±2.18	53.66^{A} ±1.45	54.33^{A} ±1.76	54.67^{A} ±0.88	53.33^{A} ±2.40	52.66^{A} ±1.20	54^{A} ±0.57

Values are expressed as mean ± standard error; means with the same capital letter in the same row (for each individual plant essential oil) are not significantly different ($p < 0.05$).

Conclusion

The present study indicates that clove bud and cinnamon bark essential oils could be applied as natural alternatives and effective substituents of synthetic (preservatives, antioxidants and antimicrobial) in food products due to their remarkable antioxidant and antimicrobial activities, with the improvement of their efficiency by using a radiation treatment, especially with a dose of 12 kGy. The results of this study showed that the irradiation treatment caused an increase in eugenol percentage and a decrease in Trans-Cinnamaldehyde. The 12 kGy dose significantly increased TPC, TFC, and antioxidant activity. All oils showed significant antimicrobial activity against bacterial and fungal strains and the irradiated samples at a dosage of 12 kGy were more effective against the majority of the microorganisms under study in both clove and cinnamon.

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