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Impact of γ-Irradiation on Chemical Constituents, Antibacterial and Antioxidant Activity of Clove and Cinnamon Volatile Oils

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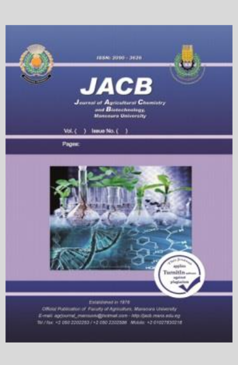

ABSTRACT

This study was carried out to investigate the effect of gamma-irradiation at doses of 0, 5, and 10 kGy on the essential oil constituents of clove and cinnamon; evaluate the efficacy of the extraction of essential oils from irradiated clove and cinnamon as an antimicrobial agent. Results showed that the main components of clove essential oils were eugenol (75.71%) and eugenol acetate (9.73%). The major components of cinnamon essential oils were cinnamaldehyde (75.55%). Gamma irradiation enhanced the antioxidant activity of essential oils. However, irradiation at a dose of 10 kGy was the best treatment for the antioxidant activity of clove and cinnamon essential oils. Essential oils of clove and cinnamon were tested for their antibacterial activity against four strains of *S. aureus, E. coli, B. cereus, and P. aeruginosa*. Results revealed that each oil tested had a growth-inhibiting impact on the microorganisms under study. Gamma irradiation at a dosage of 10 kGy was more effective against microorganisms.

Keywords: Clove oil; Cinnamon oil; Antioxidant activity; Antimicrobial activity; Gamma irradiation.

INTRODUCTION

Numerous foods must be protected during their shelf life against oxidative deterioration, microbiological spoilage, and contamination (WHO 2002). In the food business, antimicrobials and antioxidants are typically utilized as synthetic preservatives to preserve food items against microbial spoilage contamination, and oxidative deterioration. Antioxidants reduce or stop fatty acid auto-oxidation in food, whereas antimicrobials prevent the growth of harmful and spoilage microbes. Nevertheless, mounting evidence suggests these artificial food preservatives may be harmful and cancer-causing (Sharma 2015). Therefore, innovation should continue looking for novel, natural food preservatives that are safe and effective from alternative sources, particularly those derived from plants.

Essential oils (EOS) are important sources of novel phytochemicals that extend the shelf life of food products and enhance their physicochemical, organoleptic, and nutritional qualities. They are also safe for human consumption (Sadrizadeh *et al.,* 2018). Many different natural compounds, including terpenes, phenols, esters, hydrocarbons, and other substances, can be found in the formation of EOS, and their variety of characteristics may be relevant to their function in plant life (Probst, 2012). Due to phytochemicals, EOS had bioactive substances that permitted antioxidant and antibacterial activities (Pozzatti *et al.*, 2009). EOS are generally considered safe (GRAS) (Burt, 2004).

Cinnamon essential oil (CIEO) is isolated from cinnamon and mostly composed of various active substances such as cinnamaldehyde, eugenol, and cinnamic acid, with cinnamaldehyde being the most abundant (Xing *et al.,* 2014). Han *et al.* (2018) reported that CIEO and cinnamic aldehyde are most frequently utilized as antioxidant and antimicrobial agents in food preservation.

Clove essential oil (CLEO) is a common oil with powerful antioxidant and antibacterial effects. CLEO includes bioactive components such as eugenol, caryophyllene, sesquiterpenes, and triterpenes (Hasheminejad *et al.*, 2019). CLEO is also used in the food preservation, cosmetic, sanitary, pharmaceutical, biomedical, and active packaging sectors (Chen *et al.*, 2017).

Food irradiation has evolved as a method of food protection since the beginning of the 20th century. Many problems with the food supply, including microbial growth, potato sprouting, rapid fruit ripening, and insect infestation of grains, can be successfully treated by irradiation (Afify *et al.,* 2013). Douar-Latreche et al. (2018) demonstrated that gamma irradiation is a secure and efficient phytosanitary procedure for enhancing the hygienic quality of a variety of foods and herbal materials and lengthening their shelf life. Alloun et al. (2019) stated that γ-irradiation with a dose of 10 kGy is approved to disinfect dried herbs, spices, and vegetable flavorings. However, the Food and Drug Administration (FDA) has extended this restriction for the decontamination of spices and dry foods by up to 30 kGy.

The present work has been conducted to study the impact of γ-irradiation on chemical constituents, antioxidant, and antimicrobial activities of essential oils isolated from commercial clove buds and cinnamon powdered bark.

MATERIALS AND METHODS

Spices:

Clove buds *(Syzygium aromaticum)* and cinnamon bark *(Cinnamomum zeylanicum)* were obtained from a local market in Cairo, Egypt. Butylated hydroxyl toluene (BHT),

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anhydrous sodium sulfate, gallic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and quercetin were purchased from Sigma (St. Louis, MO, USA).

Microorganisms:

Two gram-positive strains, *S. aureus* (ATCC 20231) and *B. cereus* (ATCC 33018), and two gram-negative strains, *P. aeruginosa* (ATCC 9027) and *E. coli* (ATCC 35218), were obtained from the Microbiological Resources Center, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. These microorganisms were checked for purity and always generated to obtain active microorganisms. The cultures were stored in the refrigerator at 4ºC and reactivated monthly on a suitable medium.

Irradiation Treatments:

Clove and cinnamon samples were divided into three groups and exposed to 0, 5, and 10 kGy. Irradiation was performed using a ^{60}Co irradiator in Cyclotron Project, Nuclear Research Center, Atomic Energy Authority, Inshas, Egypt.

Extraction:

Around 100 g of dry spices were hydrodistilled for 4 hours using Clevenger-type apparatus, according to Senthilkumar et al. (2009). Oils were collected, dried over anhydrous Na2SO4 to remove traces of moisture, and stored in sealed vials at (4°C) until usage.

Essential oil percentages:

The essential oil percentages of clove and cinnamon were determined according to AOAC (2000).

Identification of essential oil constituents:

Analysis of essential oil constituents was carried out using GC/MS, according to Damjanovic et al. (2005) and Politeo et al. (2006).

Determination of Antioxidant activity

The electron-donating ability of the essential oil was determined by bleaching the purple-colored ofDPPH solution using the Gulcin et al. (2004) method. The free radical DPPH's antioxidant activity was calculated as follows:

$$(1 - [A_{sample} / A_{control}]) \times 100.$$

Where (A) $_{control}$ is the absorbance of the control reaction and (A) $_{sample}$ is the absorbance in the presence of plant extract.

FRAP method:

The reducing power of oils was measured by the method of Oyaizu (1986).

Determination of the antibacterial activity:

Aboaba et al. (2006) described the diffusion phase technique for assessing the antibacterial properties of the essential oil under study. Using a flamed corn borer, holes (1 cm diameter) were formed in the middle of Petri plates filled with nutrient agar media and previously seeded with a 1 mL suspension of the tested microorganisms. Each hole was filled with 50 μL of tested oil. For bacteria, the plates were incubated at 37°C for 24 h. The zone of inhibition was determined by measuring the underside of the plate in two planes with a millimeter-calibrated ruler.

Statistical analysis:

The data were subjected to the one-way ANOVA and Duncan's multiple range tests using the SPSS software (2009). Results were shown as mean ± SE (n = 3) (P <0.05).

RESULTS AND DISCUSSION

Essential oils percentage (%)

Results in Table (1) illustrate the influence of γ-irradiation at doses (0, 5, and 10 kGy) on EOs content (%). Un-irradiated clove buds contained 17.10% EOs, while irradiated clove buds contained 17.52 and 17.75% EOs for 5 and 10 kGy, respectively. It is noticed that increasing the radiation dosage, particularly the 10 kGy dose, produced the largest percentage. Likewise, as the irradiation dose was increased to 10 kGy, the yield of cinnamon essential oil (2.5%) dramatically increased (2.67). Irradiation treatments induce disruption of the cell wall structure and result in greater oil extractability from plant parts. These findings are consistent with Nada et al. (2022), who found that γ-irradiation boosted the EOs of clove, with the maximum rise occurring at a dosage of 10 kGy. Moreover, Shahin et al. (2019) reported that at doses of 30 kGy, irradiation processing caused an increase in clove essential oil content from 16.33% to 18.03%.

Table 1. Effect of γ-irradiation on essential oils percentage (%):

γ-irradiation dose (kGy)	Essential oil Percentage	
	Clove	Cinnamon
0	17.10±0.03^{c}	2.5±0.01^{c}
5	17.52±0.014^{b}	2.57±0.02^{b}
10	17.75±0.017^{a}	2.67±0.02^{a}

Essential oil constituents of clove:

Essential oil constituents of clove were identified using GC/MS and presented in Table (2) and Fig (1,2,3). Eugenol was identified as the main constituent in clove EO, accounting for 75.71%. The second most abundant component was eugenol acetate, which accounted for 9.73%. These results are in harmony with Alshawi (2016), who reported that clove EO contained 73.5% eugenol and 10.81% eugenol acetate, respectively. β-caryophyllene and caryophyllene were considered moderate components, accounting for 5.74% and 3.32%. Additional components fractioned were α-pinene, linalool, Copaene, α-Humulene, γ-cadinene, β-cadinene, and Δ-cadinene, which comprised 0.51, 0.67, 0.20, 1.21, 0.43, 0.51, and 0.47%, respectively. According to Amelia et al. (2017), clove EO includes 74.64% eugenol, 8.7% eugenol acetate, and additional compounds like caryophyllene, α-humulene, β-cadinene, γ-cadinene, and caryophyllene oxide in amounts of 12.79, 1.53, 0.039, 0.034 and 0.48%, respectively.

Clove samples underwent some modifications after being exposed to gamma radiation at 5 and 10 kGy. Due to a modest increase in their fractions, as demonstrated in (Table 2). Irradiation-induced decreases in these percentages relative to the control were commensurate to the doses given. Moreover, clove EO at a dose of 10 kGy had a higher concentration of the eugenol component. Irradiation at high doses weakens molecules' chemical bonds, leading to the creation of free radicals. Then it promotes free radicals to combine in order to prevent their reactivity with food ingredients, resulting in stable radiolytic products Woods and Pikaev (1994). According to Jo and Ahn (2000), numerous aldehydes produced in an oil emulsion that had been exposed to radiation and included amino acids increased in a dose-dependent manner to 10 kGy.

Table 2. Effect of γ-irradiation on essential oil constituents of clove.

RT (min)	Compounds	γ-irradiation dose (kGy)		
		0	5	10
5.96	α-pinene	0.51	0.49	0.48
8.61	Linalool	0.67	0.67	0.63
11.38	Copaene	0.20	0.27	0.33
15.90	Eugenol	75.31	75.75	75.86
17.53	β-caryophyllene	5.74	5.59	5.52
18.38	Caryophyllene	3.32	3.32	3.48
19.67	caryophyllene oxide	0.69	0.62	0.62
20.45	α-Humulene	1.21	0.92	0.66
22.35	Eugenyl acetate	9.73	9.57	9.37
22.89	γ-cadinene	0.43	0.43	0.43
23.71	β-cadinene	0.59	0.59	0.59
24.51	Δ-cadinene	0.47	0.47	0.45

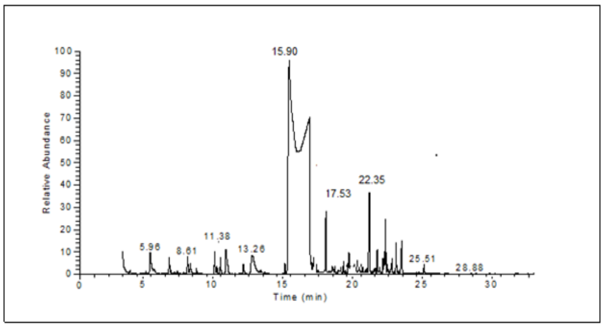

Fig. 1. Essential oil constituents of untreated (control) Clove buds

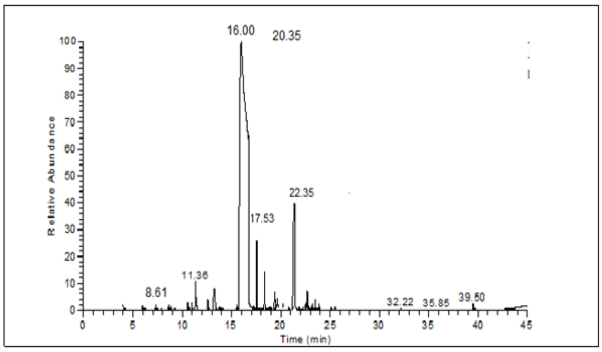

Fig. 2. Essential oil constituents of Clove buds irradiated at 5 KGy

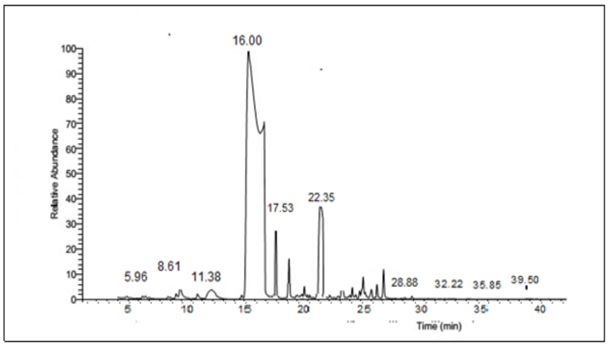

Fig. 3. Essential oil constituents of Clove buds irradiated at 10 KGy

Essential oil constituents of cinnamon:

The unirradiated and irradiated constituents of cinnamon EO analyzed by GC/MS are presented in Table (3) and Fig. (4,5,6). Cinnamaldehyde (75.55%) is the predominant component in cinnamon EO. The moderate compounds were benzaldehyde (3.18%), bornyl-acetate (4.30%), cinnamic acid (1.52%), Coumarin (1.14%), benzenepropanal (1.59) and cinnamyl acetate (2.86%). Other volatile compound area percentages were often less than 1%. Similar results were obtained by Raghavan (2007), who found the cinnamon EO comprises primarily cinnamaldehyde (65%-95%), cinnamyl acetate, cinnamic acid, benzaldehyde, and trace amounts of coumarin. Elgammal et al. (2020) discovered thirty compounds, with cinnamaldehyde (81.78%), bornyl acetate (5.33%), and cinnamyl acetate (2.82%) being the primary ingredients. In addition, Shahina et al. (2018) investigated cinnamon bark oil and discovered that the primary components were cinnamaldehyde (74%), α-caryophyllene (5.3%), linalool (3.9%) and E-cinnamyl acetate (3.8%). Limonene (2%), p-cymene (1.4%), eugenol acetate (0.6%), α-pinene (0.3%), and benzyl benzoate (0.6%) were minor components.

Concerning the impact of γ-irradiation on the constituents of cinnamon oil, data showed that gamma-radiation at different doses induced a slight fluctuation in their fractions (Table 3). The percent of cinnamaldehyde increased with increasing γ-irradiation doses from 75.88% to 85.80% at 10 kGy. Moreover, the other components were decreased or increased due to exposure to the different γ-irradiation doses. Abdelaleem (2013) and Helal (2000) confirmed these results and found that exposing fennel, peppermint, eucalyptus, and geranium essential oils to γ-irradiation at doses (10, 20, 30, and 40 kGy) induced remarkable changes in the individual fractions of the treated oils.

Table 3. Effect of γ-irradiation on the essential oil constituents of cinnamon:

RT (min)	Compounds	γ-irradiation dose (kGy)		
		0	5	10
4.71	α-pinene	0.21	0.83	---
0.50	Camphene	-----	0.50	----
5.43	Sabinene	0.51	0.67	---
6.95	Eucalyptol	0.52	1.94	-----
10.50	Benzaldehyde	3.18	2.03	5.07
10.59	Isoborneol	0.63	1.00	----
10.92	Terpinen-4-ol	0.25	0.64	----
11.29	Cinnamyl alcohol	0.75	1.49	0.31
12.07	Benzenepropanal	1.59	2.13	1.28
13.67	Cinnamaldehyde	75.55	77.28	85.60
13.93	Geranyl acetate	0.61	1.91	----
16.27	Copaene	5.62	0.47	-----
16.45	Bornyl acetate	4.30	0.56	0.53
16.88	cinnamic acid	1.52	2.45	3.27
17.76	Coumarin	1.14	1.36	2.43
18.04	Cinnamyl acetate	2.86	4.10	1.31
20.10	Caryophyllene oxide	0.44	0.27	----

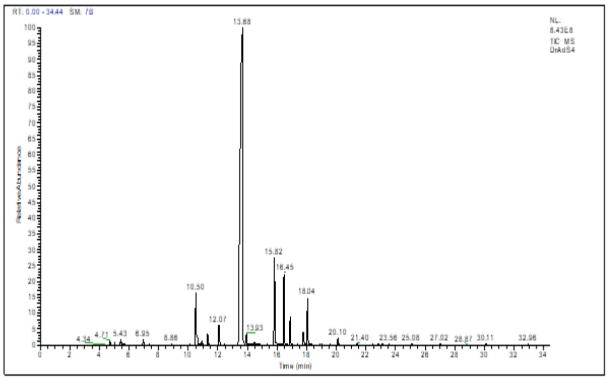

Fig. 4. Essential oil constituents of untreated (control) cinnamon barks

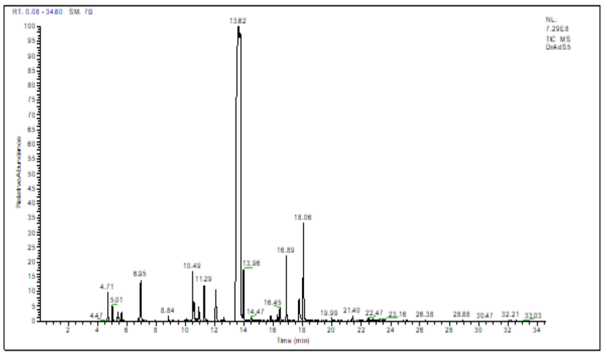

Fig. 5. Essential oil constituents of cinnamon barks irradiated at 5 KGy

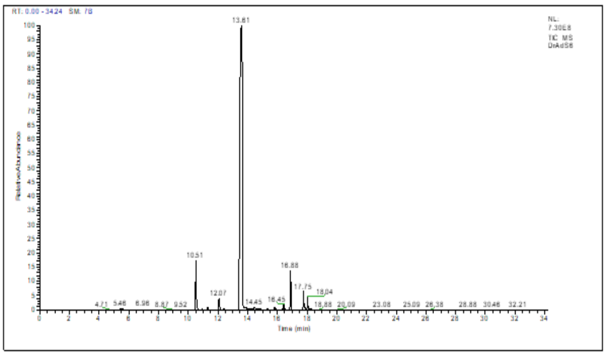

Fig. 6. Essential oil constituents of cinnamon barks irradiated at 10 KGy

Radical-scavenging Activity:

The scavenging activity (%) on the DPPH radical of non-irradiated and irradiated EOs is displayed in Table (4). paired with an increase in gamma radiation exposures and DPPH scavenging %. Clove essential oil in 10 kGy (after 30 min) showed the highest scavenging activity (86.39%) and the lowest scavenging activity (80.89%) in control (at zero time). According to Gulcin et al. (2012), clove oil had the highest scavenging activity when compared to BHT, trolox, BHA, and α-tocopherol, while Rojas-Cortes et al. (2014) that clove essential oil has a high concentration of antioxidant components when used as a food preservative. In addition, Ibrahium et al. (2013) studied the influence of clove EO on cake preservation to avoid synthetic antioxidants that have negative consequences. Regarding the identification of components by GC/MS, the antioxidant capacity of clove oil is caused by its major constituent, eugenol. The increased DPPH radical scavenging effect of irradiation clove EO could be attributed to a change in percentages or arrangement of specific component assessments (Siddhuraju, 2007).

In terms of cinnamon oil, all examined samples demonstrated varied degrees of excellent radical scavenging efficacy. The highest scavenging activity was shown at 10 kGy gamma irradiation (80.07%) after 30 min as compared to 0, 5 kGy, and BHT, respectively. El Baroty et al. (2010) found that cinnamon oil has a higher antioxidant capacity than synthetic antioxidants. These data suggest that CEO's antioxidant activity is mostly related to its primary components, which may act as chain-breaking antioxidants (Farag *et al.*, 1989; Abd El-Baky and El Baroty, 2008).

These outcomes are consistent with those reported by El-Beltagi et al. (2020) and Nada et al. (2022), who examined EOs isolated from celery seeds and clove buds that had undergone irradiation, respectively. They discovered that the amount of phenolic, flavonoid, and antioxidant content increased as the irradiation dose level rose, with the largest increase occurring at a dosage of 10.0 kGy. Likewise, Fatemi et al. (2011) found that DPPH radicals were greatly decreased by -irradiation (20.7%) in caraway essential oils, outperforming Trolox (12.75%). Finally, clove oil outperformed cinnamon oil in terms of antioxidant activity.

Table 4. DPPH radical scavenging activity of essential oils (Means ± SE).

γ –irradiation dose (kGy)	% Scavenging activity of DPPH			
	Clove oil		Cinnamon oil	
	zero time	after 30 min	zero time	after 30 min
0	80.89±0.04^{b}	83.74±0.13^{c}	72.17±0.04^{c}	78.05±0.06^{b}
5	82.44±0.2^{a}	85.05±0.1^{b}	73.06±0.07^{b}	78.12±0.07^{b}
10	82.99±0.13^{a}	86.39±0.06^{a}	78.91±0.05^{a}	80.07±0.06^{a}
BHT(200 mg/L)	27.21 ±0.22^{C}	49.44 ±0.07^{d}	27.21±0.22^{d}	49.44 ±0.07^{C}

Ferric reducing antioxid ant power (FRAP):

Table (5) shows the FRAP values of EOs. There is a noticeable increase in the FRAP value of the irradiated clove buds, and the highest value has resulted from the 10.0 kGy dose (2.88 relevant to the control samples of 2.66 and BHT 1.030). Data indicated that the essential oil of irradiated cinnamon at a dose of 10 kGy showed the best-reducing power than other oils with an absorbance value of 2.25, followed by 5 kGy and non-irradiated samples with values of 2.02 and 1.91, respectively. After irradiation, a plant's increased antioxidant capacity is primarily attributed to either an increase in enzyme activity (such as phenylalanine ammonia-lyase and peroxidase activity) or an increase in tissue extractability because of the irradiation's depolymerization and dissolution of cell wall polysaccharides (Alothman *et al.,* 2009). Moreover, there was a link between the FRAP and phenolic content in the EOs. This highlights the significance of phenolic content in the reducing power reported in this work, which may be owing to their powerful electron-donating capabilities (Bilto *et al.*, 2012).

From the results of DPPH and FRAP, clove EO is a more powerful antioxidant than cinnamon EO (Gotmare and Tambe, 2018).

Table 5. Ferric reducing antioxidant power (O.D) of essential oils (Means ± SE).

γ –irradiation dose (kGy)	Clove oil	Cinnamon oil
0	2.66±0.06^{b}	1.91±0.03^{c}
5	2.81±0.08^{a}	2.02±0.08^{b}
10	2.88±0.05^{a}	2.25±0.004^{a}
BHT(200 mg/L)	1.030±0.082^{c}	1.030±0.082^{d}

Antibacterial activity of essential oils:

EOs are high in lipophilic substances, which can dissolve in the microbe's biomembrane and interact with proteins and lipids, disrupting cells, releasing cell contents, and causing the death of the cell (Khalil *et al.,* 2018). This could explain the antibacterial activities of clove and cinnamon essential oils.

Results in Table (6) indicated that clove essential oil has antibacterial activity when exposed to gamma rays. The data showed that the inhibition zone varied depending on the irradiation dose, indicating that the treatments used had diverse impacts on the strains. Increased irradiation dose increased antibacterial efficacy, and the 10 kGy dose was more efficient at inhibiting bacterial growth. Since EOs target the outer membrane surrounding the cell, preventing hydrophobic substances from flowing through their lipopolysaccharide layer, they are more effective against Gram-positive bacteria than Gram-negative bacteria. Additionally, the chemical components, volatile molecule amounts, and interactions of EOs were connected to their antibacterial activities (Dhifi *et al.,* 2016). The current results are consistent with those of Aly et al. (2021), who found that 10 kGy was more efficient at suppressing Gram-negative and Gram-positive bacterial development.

Table 6. Inhibition zones (mm) of essential oil from clove buds at different irradiation doses .

γ –irradiation dose (kGy)	Inhibition zone (mm)			
	Tested organisms			
	E. Coli	P. aeruginosa	*B. cereus*	S. aureus
0	32±0.28	25±0.74	33±0.87	33±1.01
5	31.5±0.15	25±0.73	35±0.86	34±0.29
10	32±1.15	27.5±0.57	36±0.58	33±1.15

Results in Table (7) showed that gamma rays had an impact on the antibacterial activity of cinnamon essential oil. The antibacterial activity of cinnamon oil strongly suppressed all of the tested microorganisms. The results showed that there were significant differences in the inhibition of *B. cereus*, E. coli, and *S. aureus* growth between the control and irradiation of cinnamon oil at 10 kGy. The inhibitory zones of the previously studied microorganisms reached their highest values with a dose of 10 kGy, which were 40, 33, and 41 mm, respectively. Meanwhile, the extract of 5 kGy irradiated cinnamon showed the maximum inhibition of *P. aeruginosa,* which was 34 mm.

Table 7. Inhibition zones (mm) of essential oil from cinnamon barks at different irradiation doses.

γ –irradiation dose (kGy)	Inhibition zone (mm)			
	Tested organisms			
	E.Coli	P. aeruginosa	*B. cereus*	S. aureus
0	37±0.58	33±0.5	36±0.51	40±1.04
5	38.3±1.16	34±0.29	38±0.57	39.4±0.3
10	40±0.57	33±0.11	38.5±0.6	41±1.25

Through this study, it was observed that cinnamon oil was superior to clove oil against both kinds of bacteria. It is owing to the presence of cinnamaldehyde (which is extremely electronegative), an aromatic aldehyde that inhibits amino acid decarboxylase activity (Wendakoon and Sakaguchi, 1995; Gupta et al., 2011).

CONCLUSIONS

This study showed that cinnamon and clove oil could be used as effective natural alternatives to treat several food-borne disorders. Gamma-irradiation at 10 kGy was the most effective therapy for the antioxidant activity of cinnamon and clove essential oils, as well as having greater antimicrobial effects.

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تأثير أشعة جاما على التركيب الكيميائى والنشاط المضاد للبكتريا والمضاد للأكسدة لزيت القرنفل والقرفة

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الملخص

يهدف هذا البحث الى دراسة نأثير أشعة جاما المعامله بجرعات 5، 10 كيلو.جراى على التركيب الكيماوى للزيوت المستخلصة من كل من القرنفل والقرفة. بالإضافة إلى تقييم كفاءة هذه الزيوت المستخلصة من كل من القرنفل والقرفة كمضادات للميكروبات . أظهرت النتائج المتحصل عليها أن المكونات الرئيسية للزيوت المستخلصة من القرتفل هى ال Eugenolبنسبة 75.71% وEugenol acetate بنسبة 9.73 % بينما كانت المكونات الرئيسية للزيوت المستخلصة من القرفة هى Cinnamaldehyde بنسبة 75.55 % . كما أظهرت الدراسة أيضا أن الجرعة الإشعاعية 10 كيلو جراى ادت الى زيادة النشاط المضاد للأكسدة لهذه الزيوت. كما تم إختبار الزيوت المستخلصة من العينات المشععة وغير المشععة لكل من القرنفل والقرفة كمضادات للنشاط الميكروبى ل 4 سلالات من البكتريا هى : *S. aureus , B. cereus , P. aeruginosa, E. coli* وأظهرت النتائج المتحصل عليها أن هناك تأثيرا مثبطاً للزيوت المستخلصة ضد نمو هذه الميكروبات وان الجرعة الاشعاعية 10 كيلو جراى هى الاكثر فاعلية.