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Gamma Irradiation-Mediated Alterations in Chemical Composition and Antimicrobial Efficacy of Zubaidi, a Wild Edible Desert Truffle (*Terfezia boudieri*), with Implications for Pharmaceutical Applications

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Abstract

In exploring the impact of γ-radiation on the biochemical makeup and antimicrobial attributes of Zubaidi truffle (*Terfezia boudieri*), this study delved into the nuanced interplay between irradiation doses, chemical components, and antimicrobial efficacy. This research scrutinized the influence of γ-radiation on the chemical constitutes and antimicrobial characteristics of Zubaidi truffle (*Terfezia boudieri*). By applying four distinct doses of γ-radiation (2.5, 5.0, 7.5, and 10 kGy), the study assesses various parameters, including phenols, flavonoids, total and reduced soluble sugars, crude and soluble protein, total amino acids, antioxidant activity, and the susceptibility of examined strains to irradiated and non-irradiated truffle extracts in comparison to standard antibiotics. The findings elucidate that γ-irradiation induces moderate adjustments in the chemical composition, coupled with a dose-dependent escalation in antioxidant activity. Remarkably, irradiated truffle extracts highlight antibiotic efficacy comparable to standard antibiotics, unveiling a nuanced correlation influenced by both radiation dosage and bacterial strain.

Keywords: gamma irradiation; zubaidi truffle; chemical composition; antimicrobial efficacy; pharmaceutical.

Practical Application: The impact of γ-irradiation on chemical and antimicrobial properties of Zubaidi truffle.

1 INTRODUCTION

The term "truffle" refers to the subterranean, edible fruiting bodies of mycorrhizal ascomycetes (Figure 1). Its etymology can be traced back to the Latin word "*tubera*" denoting a "swelling" or "lump." This term evolved into "tufer" and subsequently spawned various European counterparts such as "*trufa*," "*trufe*," "*trufel*," among others, in different languages (Thomas et al., 2019). The term "desert truffle" encompasses members of the genera Terfezia and Tirmania within the family Terfeziaceae, order Pezizales. Thriving in arid and semi-arid regions post-rainfall, these fungi are particularly prevalent in the eastern and northern desert areas of the Kingdom of Saudi Arabia, where they are referred to as "faga" or "Kamma" in classical Arabic (Shavit, 2013). Among the diverse desert truffle varieties, the Khalassi and Zubaidi stand out. Khalassi, characterized by its oval shape, dark skin, and light pink interior with a subtle nutty flavor, requires meticulous cleaning to remove sand particles. On the other hand, Zubaidi boasts cream-colored skin and a more delicate taste (Bradai et al., 2015; Kovács & Trappe, 2014; Morte et al., 2017).

Figure 1. Wild Edible Desert Truffle *(Terfezia boudieri)*.

Truffles, as non-flowering fungi, encompass numerous species and genera, with Africa and the Middle East recognized as rich truffle habitats (El Enshasy et al., 2013; Trappe, 1988). Beyond their culinary appeal, truffles are esteemed for their richness in protein, antioxidants, amino acids, and fatty acids. Historically, they have been utilized for their medicinal properties, contributing to infection treatment and immune system fortification (Janakat et al., 2005; Smith & Bonito, 2012).

Despite their nutritional richness, truffles are highly perishable and susceptible to post-harvest challenges such as browning, water loss, and microbial contamination (Akram & Kwon, 2010). As a response to these concerns, irradiation has emerged as a prominent preservation technique globally, renowned for its efficacy in enhancing food safety and prolonging shelf life (Akram et al., 2012; Fernandes et al., 2012). However, questions persist regarding the impact of irradiation on nutrient loss and microbial resistance, variables contingent on the dosage and raw materials employed. In recent years, γ-irradiation has emerged as a pivotal method for preserving and enhancing the safety of various food products (Odueke et al., 2018).

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Motivated by such background, this study delved into the transformative effects of γ-irradiation on the chemical constituents and antimicrobial efficacy of Zubaidi, a wild edible desert truffle belonging to the species Terfezia boudieri. With its unique flavor profile and nutritional richness, Zubaidi holds promise as a valuable dietary resource. Understanding how γ-irradiation influences its chemical composition and antimicrobial properties is not only essential for optimizing preservation methods but also holds significant implications for potential pharmaceutical applications. This research aimed to unravel the intricate interplay between gamma irradiation, the biochemical composition of Zubaidi, and its antimicrobial capabilities, shedding light on its pharmaceutical potential and contributing valuable insights to the intersection of food science and pharmacology.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant materials

Zubaidi truffle specimens were gathered in March 2022 from AlKhafji City in the Eastern Province of Saudi Arabia. Subsequently, these samples were conveyed to the laboratory, meticulously cleaned, peeled, sliced, and subjected to a thorough drying process at 40°C. Following this, the truffles were finely pulverized into a powder and stored in a dry, dark atmosphere at ambient temperature until additional use.

2.1.2 Chemicals

Folin-Ciocalteu's phenol reagent and gallic acid (GA) monohydrate were obtained from Fluka in Madrid, Spain, while 1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Aldrich in St. Louis, MO, USA. Tryptic soy broth or agar, supplemented, was acquired from Difco in Detroit, MI, USA. Additionally, various media, antibiotics, solvents, and anaerogen sachets for establishing anaerobic conditions were supplied by Oxoid Ltd in Basingstoke, Hampshire, England, and Biolife in Milano, Italy.

2.2 Methods

2.2.1 Irradiation process

The powdered samples were hermetically sealed in plastic bags and subjected to γ-radiation at varying dosage levels (0.0, 2.5, 5.0, 7.5, and 10.0 kGy) with a dosage ratio of 1.9 kGy/h. Subsequently, the γ-radiation samples were stored at 4°C until further analysis.

2.2.2 Crude protein

Total nitrogen content was estimated through the Kjeldahl assay as outlined in the Association of Official Analytical Chemists (AOAC, 1995) procedure. A conversion factor of 6.25 was smeared to adapt total nitrogen into crude protein.

2.2.3 Preparation of plant extract

In this step, 5.0 g of powder from each treatment was immersed in 15 mL of 80% EtOH and agitated at room temperature (RT) for 24 h. The resulting extracts were sieved thrice via Whatman filter paper No.1. The ethanolic extracts obtained were utilized for analyzing total phenolic and flavonoid content, sugars, total soluble protein, and antimicrobial properties.

2.2.4 Total soluble protein

The estimation of total soluble protein was conducted using the Coomassie Brilliant Blue G-250 assay in accordance with Bradford (1976), employing Bovine Serum Albumin (BSA) as a standard.

2.2.5 Total free amino acids

The determination of total free amino acids involved the ninhydrin test, following the procedure charted by Jayeraman (1981). Optical density at 570 nm was estimated, and the content of free amino acids was expressed as mg/g lysine based on the standard curve.

2.2.6 Amino acids composition

A 1.0 g sample of dehydrated and defatted material was meticulously assessed into screw-covered tubes, followed by the addition of HCl 6.0 N (5 mL). These hydrolysis tubes were affixed to a system designed to accommodate nitrogen and vacuum lines seamlessly without interrupting the sample. The tubes were subsequently positioned in an oven set at 110°C for 24 hours, in accordance with AOAC (1995) guidelines. After hydrolysis, the tubes were unsealed, and the contents of each tube were cleaned and subjected to evaporation for dehydration using a rotary evaporator. Subsequently, 2 mL of Na-citrate buffer (pH 2.2) were added to each dried-out film of the dissected sample. The samples underwent filtration using a 0.2 µm membrane filter, rendering them ready for analysis as per the method outlined by Baxter (1996). The High-Performance Amino Acid Analyzer was employed for the subsequent analysis.

2.2.7 Total sugars

Total sugars were quantified utilizing the Phenol-H_2SO_4 assay at 490 nm *versus* a blank, following the protocol described by Dubois et al. (1956). The outcomes were conveyed as mg/g of glucose corresponding, considering the dry weight of the samples.

2.2.8 Reducing sugars

Miller (1959) described the 3,5-dinitrosalicylic acid (DNS) colorimetric technique, which was used to quantify reducing sugars using glucose as the reference standard. In this process, 1 mL of DNS reagent and 1 mL of the sample were mixed and boiled for fifteen minutes in a water bath. After the mixture cooled to RT (25°C) in a cold-water bath, 10 mL of dH_2O were added. Using a Jasco V 530 spectrophotometer, the absorbance at 540 mm was measured. The interpolation was based on

predicted values for glucose solutions with known concentrations. dH_2O was used in place of the sample solution to create blank solutions. The results were expressed in mg of glucose equivalent (Glu) per g of the samples' dry weight (mg Glu/g d.w.), with each measurement being made in triplicate.

2.2.9 Total phenolic content

The process for determining the total phenolic content (TPC) content was adhered to by Singleton et al. (1999). This process involved adding 1.0 mL of a Na_2CO_3 saturated solution to an aliquot of the extract in methanol (0.5 mL), mixing it with 0.5 mL of the Folin-Ciocalteau reagent, and topping it off with water to make a volume of 10 mL. After 1h of dark storage at RT, the resultant mixture was examined using a UV-Vis spectrophotometer set to detect absorbance at 765 nm for each sample. The results were given as mg GAE/g d.w., or mg of gallic acid equivalent (GAE) per g of dry extract.

2.2.10 Determination of total flavonoid content

According to Marinova et al. (2005), the $AlCl_3$ technique was used to determine total flavonoid (TF) concentration. Using this procedure, 0.5 mL of the sample and 0.3 mL of 5% $NaNO_2$ were mixed together. 0.3 cc of 10% $AlCl_3$ was added after a 5-min interval. After 6 min, 2.0 mL of 1 M NaOH was added, and dH_2O was used to get the total volume down to 5.0 mL. At 510 nm, the absorbance of the resulting combination was measured against a blank for the reagent. TF was expressed in mg of quercetin equivalent (QUE) per 100 g of the samples' dry weight.

2.2.11 Determination of Antioxidant Activity via DPPH Assay

The radical scavenging effects of the sample extracts *versus* DPPH radical were evaluated following the method outlined by Gulluce et al. (2004) at 517 nm. The radical scavenging activity was determined via the Equation 1:

$$DPPH\ Scavenging\ Activity\ (\%) = \frac{AC - As}{AC}\ x\ 100$$

Where:

AS: the absorbance when the sample extract is added;

AC: the absorbance of the control reaction, which includes all reagents except the sample.

2.3 Antimicrobial effects

2.3.1 Determination of antibacterial activity

Stock cultures of pathogenic Gram+ and $Gram^-$ bacteria were used in this experiment. *Staphylococcus aureus, Bacillus cereus*, and *Streptococcus faecalis* were categorized as Gram+ strains, whereas *Escherichia coli, Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* were classified as $Gram^-$ strains. These bacterial cultures were maintained at 4°C with a 15-day interval among subcultures on Tryptone Glucose Yeast Extract Agar (TGY) slants. To reactivate every bacterial strain, the slants were kept and then transferred into TGY broth media and incubated at 37°C overnight before the antimicrobial experiment was performed. The agar well diffusion technique was used to evaluate the antibacterial activity of the extracts. In this method, 15 mL of melted Mueller-Hinton Agar was mixed with 1 mL of an overnight sterile nutritional broth (NB) culture media containing approximately (1.5 x 105 CFU/mL). The mixture was then put onto a sterile Petri dish and left to harden. Wells were made in the set agar with the aid of a sterile cork-borer (5 mm diameter), and 0.1 mL of the tested extract was added to each well. After that, the plates were incubated at 37°C for 24 h. Additionally, controls were included, such as EtOH (negative control) and gentamycin (positive control), a common antibiotic. The zone of growth inhibition around the well, which was larger than 5 mm, served as a proxy for antibacterial efficacy. Each experiment was conducted three times (Mishra & Padhy, 2013).

2.3.2 Assessment of antifungal effects

Aspergillus flavus, Aspergillus niger, Rhizopus sp., and *Candida albicans* were the 4 fungi examined in this study. Before each test, they were cultivated on potato dextrose agar (PDA, Oxoid) plates that were incubated at 28°C. The fungi were kept in Sabouraud dextrose (SD) broth (Oxoid) with 20% glycerol at 28°C. The cultures used for inoculation were 5-7 days old. Diffusion assaying was the method used to screen for antifungal activity against the tested fungi. The antifungal activity of the studied extracts was assessed using Sabouraud agar medium containing 1.5% agar, 1% peptone, and 4% dextrose. After the medium was ready, it was autoclaved for 15 min for sanitization and then transferred into Petri plates aseptically. Following a 2-h incubation period, fungal suspensions were turbidity-corrected to 1.0×10^6 CFU/mL using a sterile saline solution (0.85% NaCl) and individually injected onto Petri plate surfaces. A sterile cup borer was used to create aseptic cups with a diameter of about 5 mm in the Sabouraud agar medium. Next, each extract was applied to the cups in 0.1 mL increments. Following a 72-h incubation period at 28°C, inoculated plates were observed for growth as well as zones of inhibition (measured in mL). The identical solvents used to dissolve the tested extracts were also utilized to generate a negative control. Nystatin was used as the conventional antifungal (positive control). The clear inhibition zones surrounding the wells demonstrated antibacterial action. To ensure the accuracy of the findings, each method was reiterated 3 times.

2.4 Statistical analysis

One-way ANOVA was used to statistically analyze all the data at a significance threshold of $p < 0.05$. Using IBM SPSS software version 24 as the statistical tool, Duncan's multiple range tests were run to evaluate variations between means.

3 RESULTS AND DISCUSSION

3.1 Impact of γ-irradiation on the chemical attributes of truffles

3.1.1 Phenols, flavonoids, total soluble sugars and reducing sugars

The data presented in Table 1 provides insights into the chemical composition of wild edible desert truffle (Zubaidi)

from Saudi Arabia when exposed to γ-irradiation at different dosage levels. Measured parameters include phenols, flavonoids, total soluble sugars, and reducing sugars. The phenol content of the truffle shows a decreasing trend with increasing irradiation dose levels. The control has the highest phenol content (2.789 mg/g DW), while the lowest content is observed at 10.0 kGy (2.366 mg/g DW). The Least Significant Difference (LSD) value for phenols is 0.171, indicating that differences in phenol content between the irradiation dose levels are statistically significant. Similarly, the flavonoid content tends to decrease with higher irradiation doses. The control has the highest flavonoid content (6.060 mg/g DW), and the lowest is observed at 5.0 kGy (6.170 mg/g DW). The LSD value for flavonoids is 0.826, suggesting significant differences in flavonoid content among the irradiation dose levels. The total soluble sugar content shows variations across irradiation dose levels. The highest content is observed at 10.0 kGy (0.621 g/100g DW), while the lowest is at 7.5 kGy (0.414 g/100g DW). The LSD value for total soluble sugars is 0.035, indicating statistically noteworthy alterations in total soluble sugar content among the irradiation dose levels. The reducing sugar content increases with irradiation dose levels. The control has the lowest reducing sugar content (0.8307 g/100g DW), while the highest is observed at 10.0 kGy (2.344 g/100g DW). The LSD value for reducing sugars is 0.6436, suggesting significant differences in reducing sugar content among the irradiation dose levels.

γ-irradiation appears to influence the chemical composition of the truffle, with varying effects on phenols, flavonoids, total soluble sugars, and reducing sugars. The decrease in phenols and flavonoids might be ascribed to the impact of irradiation on the truffle's bioactive components. Total soluble sugars show a fluctuating pattern, with the highest content at 10.0 kGy, indicating a potential response to irradiation stress. The increase in reducing sugars at higher irradiation doses might be indicative of changes in carbohydrate metabolism in response to irradiation. The irradiation does not have a substantial impact on the irradiated fungal mycelium (Duan et al., 2010). This increase can be associated with the radiation dose, as it was found that high doses of radiation led to an expansion in TPC and TF in pumpkin (Abdul Azeem et al., 2022).

3.1.2 Crude protein, soluble protein, and total free amino acids

Table 2 provides information on the crude protein, soluble protein, and total free amino acids of truffles exposed to γ-irradiation at different dose levels. The crude protein content shows a decreasing trend with increasing irradiation dose levels. The control has the highest crude protein content (18.59 g/100g DW), while the lowest content is observed at 10.0 kGy (15.73 g/100g DW). The LSD value for crude protein is 2.40, indicating statistically significant differences in crude protein content between the irradiation dose levels. Similarly, the soluble protein content tends to decrease with higher irradiation doses. The control has the highest soluble protein content (2.688 g/100 g DW), and the lowest is observed at 5.0 kGy (2.273 g/100 g DW). The LSD value for soluble protein is 0.3666, suggesting significant differences in soluble protein content among the irradiation dose levels. The total free amino acid content varies across irradiation dose levels. The highest content is observed at 7.5 kGy (0.394 g/100 g DW), while the lowest is at 5.0 kGy (0.2713 g/100 g DW). The LSD value for total free amino acids is 0.06567, indicating statistically noteworthy variances in total free amino acid content among the irradiation dose levels.

Table 1. Phenols, flavonoids, total soluble sugars, and reducing sugars of truffle exposed to gamma irradiation.

Irradiation dose level (kGy)	Phenols mg/g DW	Flavonoids mg/g DW	Total Soluble sugars g/100g DW	Reducing Sugars g/100g DW
control	$2.789^{a} \pm 0.049$	$6.060^{a} \pm 0.110$	$0.495^{b} \pm 0.060$	$0.8307^{c} \pm 0.0745$
2.5	$2.575^{b} \pm 0.133$	$5.180^{b} \pm 0.180$	$0.455^{c} \pm 0.001$	$1.584^{b} \pm 0.149$
5.0	$2.537^{b} \pm 0.0711$	$6.170^{a} \pm 0.071$	$0.459^{c} \pm 0.002$	$1.584^{b} \pm 0.149$
7.5	$2.461^{bc} \pm 0.002$	$6.006^{a} \pm 0.002$	$0.414^{d} \pm 0.014$	$1.474^{b} \pm 0.187$
10.0	$2.366^{c} \pm 0.0403$	$6.200^{a} \pm 0.040$	$0.621^{a} \pm 0.260$	$2.344^{a} \pm 0.175$
LSD	0.171	0.826	0.035	0.6436

The outcomes are presented as the mean of triplicate measurements along with the corresponding standard errors (±). Different letters (a, b, c, d) specify statistically noteworthy variances among means at a significance level of $p < 0.05$.

Table 2. Crude protein, soluble protein, and total free amino acids of truffle exposed to γ-irradiation.

Irradiation dose level (kGy)	Crude protein g/100g DW	Soluble protein /100g DW	Free amino acids g/100g DW
control	$18.59^{a} \pm 1.31$	$2.688^{a} \pm 0.305$	$0.262^{d} \pm 0.006$
2.5	$17.61^{ab} \pm 1.21$	$2.300^{b} \pm 0.220$	$0.3277^{bc} \pm 0.001$
5.0	$17.05^{ab \pm} 1.33$	$2.273^{b} \pm 0.351$	$0.2713^{cd} \pm 0.0515$
7.5	$16.19^{b} \pm 0.44$	$2.500^{ab} \pm 0.060$	$0.394^{a} \pm 0.0280$
10.0	$15.73^{b} \pm 1.09$	$2.666^{a} \pm 0.158$	$0.375^{ab} \pm 0.0390$
LSD	2.40	0.3666	0.06567

The findings are donated as the means of triplicate determinations along with the corresponding standard errors (±). Different letters (a, b, c, d) denote substantial variance among means at a threshold of $p < 0.05$.

Γ-irradiation appears to have a negative impact on the protein-related components of the truffle, including crude protein and soluble protein. The decrease in crude protein and soluble protein content with increasing irradiation doses suggests a potential degradation or alteration of protein structures under irradiation. Similar observations were made in both shiitake and white button mushrooms treated with doses of 1.2 and 1.6, showing initial minor decreases in soluble protein content, indicating a negative effect of higher doses (Jiang et al., 2010). Total free amino acids show some variation, with the highest content at 7.5 kGy, possibly indicating changes in amino acid composition in response to irradiation stress. It was observed that the truffles contain a quantity of amino acids (El Enshasy et al., 2013), which increased with the irradiation dose at 7.5 and 10 kGy. The significant LSD values for all three parameters suggest that the observed differences in protein-related components among the irradiation dose levels are not likely due to random variability but are statistically significant.

3.1.3 Amino acids composition

Table 3 illustrates the effect of γ-irradiation on the amino acid composition of truffles at different irradiation dose levels (0.0, 2.5, 5.0, 7.5, 10 kGy). The content of arginine remains relatively stable across the irradiation dose levels, with a slight decrease at 7.5 kGy. Histidine content shows a slight decrease with increasing irradiation dose levels, reaching its lowest at 10 kGy. Isoleucine c ontent decreases slightly with increasing irradiation dose levels, indicating a minor impact on this amino acid. Leucine content shows a decreasing trend with higher irradiation doses, suggesting some sensitivity to gamma irradiation. Lysine content remains relatively stable across the γ-irradiation dosage levels, with a minor decrease at 10 kGy. Methionine content declines with intensifying γ-irradiation dosage levels, with the lowest value at 10 kGy. Phenylalanine content shows a slight decrease, particularly at 10 kGy. Threonine content exhibits a decreasing trend with higher irradiation doses. Valine content decreases with increasing irradiation doses, with a noticeable decrease at 10 kGy. Alanine content shows a decreasing trend with higher irradiation doses, with the lowest value at 10 kGy. Aspartic acid content decreases with increasing irradiation dose levels, indicating some sensitivity to γ-irradiation. Cysteine content varies, showing an increase at 7.5 kGy and then a decrease at 10 kGy. Glutamic acid content decreases with increasing irradiation dose levels, indicating sensitivity to gamma irradiation. Glycine content fluctuates across irradiation dose levels, with an increase at 7.5 kGy. Proline content decreases with higher irradiation doses, with the lowest value at 10 kGy. Serine content shows variability, with a decrease at 10 kGy. Tyrosine content decreases with accumulative irradiation dosage levels, with the lowest value at 10 kGy.

The influence of γ-irradiation on amino acid composition varies among different amino acids. Some amino acids show a general decreasing trend with higher irradiation doses, suggesting sensitivity to irradiation. Certain amino acids, such as lysine, arginine, and glycine, appear relatively stable across irradiation dose levels. According to Jiang et al. (2010), phenylalanine and tyrosine were minimally affected by the irradiation of *Agaricus bisporus* mushroom. The fluctuations in amino acid content may have implications for the nutritional value and sensory attributes of the truffles. Further analysis and interpretation may be needed to understand the specific impacts of γ-irradiation on the truffle's amino acid constituents in the context of nutritional quality and potential changes in flavor.

3.1.4 Radical scavenging effects

Figure 2 depicts the DPPH free radical scavenging effects of truffles exposed to γ-irradiation at variant dosage levels. The radical scavenging effects are presented as a percentage, indicating

Table 3. The effect of γ-irradiation on amino acids constitutes of truffle exposed to γ-irradiation.

Amino Acid	**Irradiation dose level (kGy)**				
g/100 g DW	**0.0**	**2.5**	**5.0**	**7.5**	**10**
Arginine	0.95	0.98	0.96	0.88	0.92
Histidine	0.47	0.45	0.46	0.44	0.43
Isoleucine	0.95	0.91	0.91	0.88	0.86
Leucine	1.20	1.18	1.18	1.09	1.07
Lysine	0.68	0.64	0.68	0.65	0.64
Methionine	0.35	0.26	0.25	0.26	0.24
Phenylalanine	0.84	0.79	0.85	0.79	0.72
Therionine	1.42	1.42	1.37	1.37	1.29
Valine	1.07	0.96	0.99	0.94	0.90
Alanine	1.41	1.38	1.20	1.10	1.18
Aspartic	1.95	1.90	1.90	1.83	1.79
Cysteine	0.48	0.46	0.46	0.52	0.53
Glutamic	2.16	2.10	2.10	1.98	1.99
Glycine	0.48	0.97	0.97	0.92	0.91
Proline	1.06	1.02	1.02	0.98	0.97
Serine	0.96	0.96	0.96	0.97	0.88
Tyrosine	0.89	0.89	0.89	0.84	0.82

the ability of the truffle to neutralize DPPH free radicals. For the control sample (0.0 kGy), the DPPH radical scavenging activity is measured at 65.3%, representing the baseline activity of the non-irradiated truffle. At this γ-irradiation dosage level of 2.5 kGy, the DPPH radical scavenging effects increases to 70.1%, indicating a positive impact on the antioxidant activity. The scavenging activity further improves to 72.5%, suggesting that γ-irradiation at this level (5.0 kGy) enhances the truffle's capability to neutralize free radicals. The DPPH radical scavenging effects continue to rise at level 7.5 kGy, reaching 75.7%. This suggests a dose-dependent relationship, where higher irradiation levels correspond to increased antioxidant activity. The highest level of irradiation (10 kGy) in the study shows the most significant improvement in DPPH radical scavenging activity, reaching 80.2%. This suggests that gamma irradiation, even at higher doses, positively influences the truffle's antioxidant capacity.

The results from Figure 2 suggest that γ-irradiation has a positive impact on the DPPH free radical scavenging effects of the truffle. The raise in scavenging activity with superior irradiation doses indicates a potential enhancement of antioxidant properties, which can be attributed to changes in the truffle's chemical composition. It is noteworthy that while γ-irradiation may influence antioxidant activity, the overall quality and nutritional value of the truffle may be subject to a balance between the positive and potentially negative effects of irradiation. Further studies and analyses would be beneficial to understand the specific compounds responsible for the observed antioxidant effects and to estimate the overall influence of γ-irradiation on the truffle's health-promoting properties. The results obtained by El-Beltagi et al. (2019) showed that γ irradiation at a dosage of 5 kGy amended the natural polyphenolic components, TPC, TF, and antioxidant scavenging effects (DPPH%).

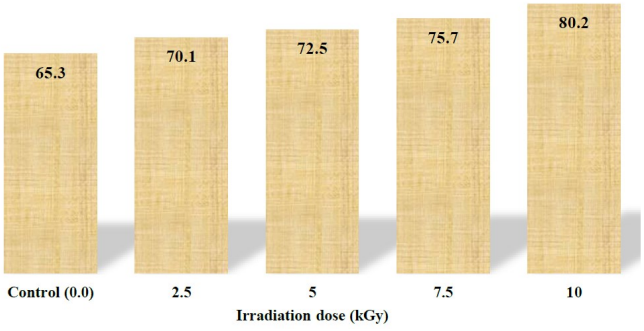

Figure 2. DPPH free radical scavenging activity of truffle exposed to γ-irradiation.

3.2 Impact of γ-irradiation on the Microbial Attributes of Truffles

Table 4 presents the susceptibility of tested microbial strains to irradiated truffle extracts, measured in the zone of inhibition (in mm), and compares these results to standard antibiotics. The zone of reticence for both *S. aureus* and *B. cereus* varies across irradiation dose levels. For *S. aureus*, the zone of inhibition increases from 15 mm at the control to 16 mm at 2.5 kGy and then fluctuates with higher doses. For *B. cereus*, there is a general increase in the zone of inhibition up to 7.5 kGy, but a notable decrease at 10 kGy. The control shows an initial zone of inhibition for *B. cereus*, indicating some inhibitory effect. The zone of inhibition for both *E. coli* and *P. aeruginosa* varies across irradiation dose levels. For *E. coli*, there is a general decrease in the zone of inhibition with higher doses. For *P. aeruginosa*, the zone of inhibition decreases with increasing irradiation doses, with a significant decrease at 10 kGy. The zone of inhibition for these fungi varies across irradiation dose levels. For *Aspergillus flavus* and *Aspergillus niger*, there is a general increase in the zone of inhibition with higher irradiation doses. *Aspergillus terreus* shows fluctuations in the zone of inhibition, reaching the highest value at 22 mm for 5.0 kGy. *C. albicans* shows a general increase in the zone of inhibition with higher irradiation doses, reaching the highest value at 23 mm for 10 kGy. Instances marked as "R" indicate resistance to either irradiated truffle extracts or standard antibiotics. For example, *C. albicans* shows resistance at 10 kGy. The zone of inhibition with Gentamycin is mentioned for

Table 4. Sensitivity of verified strains toward irradiated truffle extracts (zone of inhibition in mm) compared to standard antibiotics.

Microbial strains	Irradiation dose level (Gy)						
	Control (0.0)	2.5	5.0	7.5	10.0	CN	NS
Tested bacteria (Gram positive)							
S. aureus	15	16	11	16	14	15	NT
B. cereus	12	15	133	13	13	12	NT
Tested bacteria (Gram negative)							
Escherichia coli	19	19	16	18	22	10	NT
P.aeruginosa	16	15	16	16	13	11	NT
Tested fungi							
Aspergillus flavous	15	12	11	11	9	NT	R
Aspergillus niger	15	12	14	11	13	NT	10
Aspergillus .	20	22	22	22	23	NT	12
C. albicans	20	20	20	19	22	NT	R

CN: Gentamycin; NS: Nystatin; NT: Not Tested; R: Resistant.

reference and comparison. The zone of inhibition with Nystatin is also provided for reference.

The data suggest that irradiated truffle extracts exhibit varying degrees of antimicrobial effects *versus* both Gram+ and Gram$^-$ bacteria, alongside fungi. The zone of inhibition varies across different irradiation dose levels, indicating a dose-dependent response. Comparisons with standard antibiotics provide a reference for the effectiveness of the irradiated truffle extracts. Instances of resistance highlight variability in microbial responses, emphasizing the need for further investigation into the specific mechanisms involved. The irradiation process is effective in reducing the bacterial load on fresh lettuce leaves (Niemira, 2008). Additionally, irradiation, being a non-thermal process, can be utilized to enhance the microbiological safety of food (Beaulieu et al., 2002).

4 CONCLUSION

The comprehensive analysis of the impact of γ-irradiation on a wild edible desert truffle (Zubaidi) from Saudi Arabia has provided valuable insights into its chemical composition, antimicrobial properties. The research explored various parameters, including phenols, flavonoids, soluble sugars, reducing sugars, protein content, amino acid constitutes, and the susceptibility of microbial strains to irradiated truffle extracts. Γ-irradiation did not significantly alter TPC and TF of the truffle, indicating the robustness of these bioactive compounds under irradiation. The levels of soluble sugars and reducing sugars exhibited minor variations, with a notable increase at the highest irradiation dose in reducing sugars. Amino acid composition showed dose-dependent changes, with some amino acids decreasing with higher irradiation doses, highlighting the need for a balanced consideration of the nutritional impact of irradiation. The protein content in the truffle decreased with higher irradiation doses, suggesting potential protein degradation. Gamma-irradiated truffle extracts demonstrated antimicrobial effects *versus* both Gram$^+$ and Gram$^-$ bacteria, alongside fungi. The zone of inhibition varied with irradiation doses, indicating a dose-dependent impact on microbial growth. The antimicrobial activity of irradiated truffle extracts was comparable to standard antibiotics in certain cases. The research highlights the potential of γ-irradiation as a non-thermal process to enhance the microbiological safety of fresh produce, as demonstrated with lettuce leaves. The antimicrobial properties of irradiated truffle extracts suggest their potential application in food preservation and safety. Further investigations are warranted to elucidate the specific compounds responsible for the observed antimicrobial effects. Nutritional assessments, including vitamins and minerals, would provide a more comprehensive understanding of the truffle's overall quality after irradiation. Sensory evaluations and consumer studies could shed light on the acceptability of irradiated truffle products. In summary, this research contributes to the understanding of the impacts of γ-irradiation on the chemical and antimicrobial properties of a wild edible desert truffle. The findings offer valuable insights for both food scientists and consumers, informing potential pharmaceutical applications in addition to food safety and preservation.

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