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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF GAMMA-IRRADIATED MISWAK (*SALVADOR PERSICA* L.) STICKS

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Abstract

Salvadora persica extract displays valuable biotherapeutic capacities such as antimicrobial and antioxidant traits. The current study was designed to evaluate the antioxidant activity (FRAP and DPPH· assay), antioxidative stability (Rancimat test), and chemical composition (GC/MS analysis) of *S. persica* extract as affected by different irradiation doses. Furthermore, the study evaluated in vitro the microbicidal action of the ethanol (70%) extract of *S. persica* sticks in terms of radial growth rate and inhibition zone versus pathogenic microbial strains, including pathogenic bacteria, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhimurium*, and pathogenic fungi, *Aspergillus brasiliensis* and *Candida albicans*. In DPPH· and FRAP assays, oxidative stability displayed that the antioxidant capacities in extracts reached maximum values at an irradiation dose of 2 kGy. Both irradiated and non-irradiated extracts exhibited prohibiting influences (strong and moderate) on the growth of the tested bacterial strains. At the same time, the fungal strains, *A. brasiliensis* and *C. albicans* showed sensitive and resistant potentials against plant extract.

Keywords*:* Oxidative stability, benzyl isothiocyanate, microbicidal, pathogenic fungi, pathogenic bacteria, phytochemicals, GC/MS

Introduction

Salvadora persica, frequently known as Miswak or Siwak, is a member of the plant family Salvadoracea (Khatak *et al*., 2010). It is mainly distributed in dry and subtropical regions of Africa, the Middle East, and the Indian subcontinent (Mansour *et al*., 2020). Miswak is known long back in history for use as a natural toothbrush for dental and oral hygiene. Moreover, the aqueous extract of *Salvadora persica* roots was reported to have antimicrobial, anti-inflammatory, and antioxidant traits.

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These activities have been ascribed to several detectable substances in its natural extracts, such as potassium and sodium chloride, as well as salvadorine, vitamin C, salvadourea, silica, saponins and different minerals (Almas, 1999; Al Bratty *et al*., 2020; Mohamed and Khan, 2013).

Salvadora persica has a number of phytochemicals, including antiseptic and antibacterial compounds that are responsible for effective biological properties. The antimicrobial activity against bacterial and fungal strains was reported in different parts of Miswak. In Miswak, these active components are very stable and are reported to have activity in water, alcohol, and acetone-based extracts (Al Bratty *et al*., 2020; Khan *et al*., 2020). Phytochemical screening of the aqueous extract of *Salvadora persica* leaves revealed the presence of sterols, terpenes, flavonoids, flavone aglycone, saponins, and tannins (Aljarbou *et al*., 2022). Furthermore, a study screened the extracts (n-hexane, chloroform, ethanol, and water extracts) of *Salvadora persica* twigs and stem for the presence of phytochemicals, including alkaloids, glycosides, tannins, saponins, and flavonoids. All tested phytochemicals were absent in the n-hexane extract, and only alkaloids were present in the chloroform extract. Ethanol extract contained all tested phytochemicals except for alkaloids and tannins. The aqueous extract contained all tested phytochemicals except alkaloids. These variations could be explained by the differences in solvent polarity to extract the phytochemical compounds (Blažević *et al*., 2017). Moreover, this could be clarified strongly by using aqueous extracts that are less active in the alcoholic and non-polar extracts.

Isothiocyanate (benzyl mustard oil) is the principal consistent and showed the strongest activity against most foodborne pathogens (Dufour *et al*., 2015; Saladino *et al*., 2017). Isothiocyanates are produced via enzymatic hydrolysis of glucosinolates, a class of sulfur-containing secondary metabolites occurring exclusively in the botanical order Brassicales, by myrosinase (Ishida *et al*., 2014; Blažević *et al*., 2017).

Irradiation can influence the level of antioxidants/phytochemicals and the capacity of a specific plant to produce them at different levels. Under certain favorable conditions, the concentration of phytochemicals might be enhanced. These conditions include exposure to radiation sources, wounding, storage at low temperatures, and/or exposure to extreme temperatures (Zobel, 1997). Although some studies reported that gamma irradiation maintains or enhances the antioxidant properties, there are few examples wherein the antioxidant potential of the plant material was decreased (Alothman *et al*., 2009). It was reported that the active components of Miswak's main root are stable to gamma irradiation. The antioxidant activity was increased when the soft gamma-irradiated samples were used. It was mentioned that root Miswak treated by gamma irradiation at 20 kGy can be used in oral hygiene products as a source of natural antioxidants with antimicrobial activities (Huang *et al*., 2021; Baky *et al*., 2024).

To the best of our knowledge, no studies have been published on ionizing radiation's effects on the phytochemical content, antioxidant, and antimicrobial activities of Miswak (*Salvadora persica*). The current study aimed to assess the antibacterial and

antioxidant activity of the ethanol (70%) extract and determine the chemical profile of irradiated and non-irradiated Miswak.

Materials and methods

Materials

2,2-diphenyl-1-picrylhydrazyl (DPPH·), ferric chloride hexahydrate ($FeCl_3$ 6H2O), ferrous sulfate heptahydrate ($FeSO_4$ $7H_2O$), and butylated hydroxyl toluene (BHT) were purchased from Sigma-Aldrich (Germany). Refined sunflower seed oil (free from natural antioxidants) was obtained from Arma Oils Company, 10th of Ramadan City, Egypt. The Miswak sticks *Salvadora persica* L. sticks were obtained from the local market in Zagazig City (Egypt). The stem bake of *S. persica* was dried in a 55^0C oven for 3 days and then sliced into discs or ground into a fine powder using a coffee grinder.

Irradiation treatment

Fine powder of the Miswak was irradiated with gamma irradiation at doses of 0, 0.25, 0.5, 1, 2, 4, and 6 kGy using an experimental 60Co Russian Gamma chamber (dose rate 665.6 Gy/h), Cyclotron Project, Nuclear Research Center, Egyptian Atomic Energy Authority, Egypt.

Preparation of S. persica extract

Ethanol extract was made by soaking 25 g *S. persica* powder in 500 mL ethanol 70% (Sigma-Aldrich, St. Louis, USA) in a closed container and soaking at room temperature for 48 h. Following the soaking period, the miswak extract was filtered with Whatman No. 1 filter paper, and the filtrate was evaporated in a vacuum evaporator at 50°C. The miswak extract was kept on ice at -18°C in a dark vial for further research.

DPPH· Radical-scavenging Activity

The DPPH·test was used to measure the antioxidant activity of the *S. persica* extract.The DPPH·radical-scavenging activity (DPPH·-RSA) was assessed according to Gülçin et al. (2004). One mL of DPPH· solution in ethanol (0.1 mM) was shaken with 3 mL of the *S. persica* extract and left for 30 min at room temperature. After that, the absorbance was spectrophotometrically measured at 517 nm (equation 1).

$$\text{Antioxidant activity (inhibition) \%} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (1)$$

where:

$A_{control}$ is the absorbance of the control reaction (absorbance of DPPH solution), and A_{sample} is the absorbance of *S. persica* extract with DPPH· solution. Samples were analyzed in triplicate. The synthetic antioxidant of BHT was applied as the positive control.

Ferric Reducing Antioxidant Power (FRAP) assay

The methods of Benzie and Strain (1996) was modified to be carried out in microplates. A freshly prepared TPTZ reagent was prepared [300 mM acetate buffer

(pH=3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM$FeCl_3$, in a ratio of 10:1:1 (v/v/v)]. A volume of 190 uL TPTZ reagent was mixed with 10 uL of the sample in a 96-well plate (n=3). The reaction was incubated at room temperature for 30 min in the dark. At the end of the incubation period, the resulting blue color was measured at 593 nm. The ferric-reducing ability of the samples was reported as µM TE/mg.

Determination of antimicrobial activity

A diffusion assay was used to screen the inhibitory effect of *S. persica* extract. *In vitro* antibacterial and antifungal activities of the extract against human pathogenic bacteria, two Gram-positive *Staphylococcus aureus* (ATCC 20231) and *Bacillus subtilis* (ATCC 9372) and two negative strains (*Escherichia coli* (ATCC 35218) and *Salmonella typhimurium* (ATCC 98031) as well as pathogenic fungi *Aspergillus brasiliensis* (ATCC 16404) and *Candida albicans* (ATCC 10231) were investigated by the agar well diffusion method (Ben Hsouna *et al*., 2017; Edogbanya *et al*., 2019) with some modifications. Accordingly, 250 mL culture media (Mueller-Hinton sterile agar for antibacterial test and sterilized potato dextrose agar for antifungal test) were suspended by 2.5 mL of the test microbial suspension (105 CFU/mL for bacteria and yeast and 104 spore/mL for fungi). The inoculated media were poured on Petri dishes and allowed to solidify at room temperature. A cork borer (8 mm diameter) was flamed and used to bore one central well in each plate, which contained 50 µL (for the antibacterial test) and 100 µL for (for the antifungal test) of *S. persica* extract, each separately. The plates were kept at 7°C for 3 h to allow the extract diffusion and then incubated at 37°C for 24 h (for bacterial growth) and 28°C for 72 h (for fungal growth) (Farouk *et al*., 2022). The zones of growth inhibition around the well were measured in millimeters. The experiments were performed in triplicate.

Sunflower oil preparation for oxidative stability test

Sunflower oil was modified with irradiated Miswak extract at doses of 0, 0.25, 0.5, 1, 2, 4 and 6 kGy at a concentration of 0.02% (200 ppm) and mixed well using a magnetic stirrer to complete dispersion in the sunflower oil. All samples were kept without any addition as a control, and BHT (200 ppm) was used as the reference for antioxidants. All samples were kept in brown glass bottles for further determination of the oxidative stability index.

Determination of Oxidative stability

The oxidative stability of sunflower oil samples was determined using the Rancimat equipment (Metrohm 679), as described by Gutiérrez (1989). The induction period was determined using a 5 g oil sample, and the air supply was 20 L/min. Deionized water was used as absorption solution for conductivity measurement. Determinations were conducted at 100°C, and the conductivity curves were recorded.

Gas Chromatography/Mass Spectroscopy of extract

Gas chromatography (Agilent 8890) with a mass spectrometer detector (Agilent 5977B) series with capillary column DB-5MS, 60 m×250 µm id×0.25 µm film thicknesses. Helium gas was the carrier gas at a constant pressure of 65 kPa. The solvent extract was injected with a volume of 1 µL in a split ratio of 1:50 and a

solvent delay of 4 min. The oven temperature increase was programmed from 50-240°C with a step of 5°C per min until reaching 240°C.

Statistical analysis

All independent analyses were carried out in triplicate (n= 3), for which the results were expressed as mean ± standard error. Data were analyzed using SPSS 2009 analytical software version 18.0 (SPSS Inc., Illinois, USA). Data were subjected to a one-way analysis of variance (ANOVA) followed by the Duncan test for comparison of means as a post-hoc test. Significant levels were based on the confidence level of 95% ($p < 0.05$).

Results and discussion

Antioxidant activity

The antioxidant activity of *S. persica* extract was examined by assessing the inhibition of the DPPH· and FRAP free radicals. The results of the DPPH-RSA exerted by the *S. persica* extracts treated with different doses of gamma irradiation (0, 0.25, 0.5, 1, 2, 4 and 6 kGy) are presented in Table 1. The DPPH-RSA of the control sample was 68.45% and the irradiation treatment resulted in the significant increase up to a maximum of 85.23% for the sample treated with 2 kGy (Table 1). Further increase in the irradiation dose up to 6 kGy caused a slight decrease of the DPPH-RSA to 76.71%, which is higher compared to the DPPH· inhibition value of 50.91% for BHT. Meanwhile, the FRAP value of the control sample was 46.83, and there was a significant increase in FRAP after being subjected to gamma irradiation, with the samples treated with 2 kGy having the highest FRAP among all treatments.

Table 1. Influence of irradiation dose on the antioxidant activity of *Salvadora persica* extracts.

	DPPH·-RSA (%)	**FRAP (**µMTE/mg)
0.00 kGy	68.45^{e} ± 0.07	46.83^{f} ±0.03
0.25 kGy	67.01^{g} ± 0.12	47.73^{e} ±0.01
0.50 kGy	67.67^{f} ±0.02	48.22^{b} ±0.07
1.00 kGy	77.52^{b} ±0.04	47.90^{de} ±0.03
2.00 kGy	85.23^{a} ±0.05	48.65^{a} ±0.14
4.00 kGy	76.89^{c} ±0.03	48.00^{cd} ±0.012
6.00 kGy	76.71^{d} ±0.05	48.12^{bc} ±0.204
BHT (200 ppm)	50.91^{h} ±0.001	47.02^{f} ±0.004

Results are presented as mean ± S.D. The mean values in the same row with different superscript alphabets are significantly different (P<0.05) according to Duncan test.

The antioxidant activities of Miswak methanol extract with increasing gamma radiation doses on individual antioxidant assays were reported. Different gamma irradiation doses enhanced the antioxidant activities of obtained methanol extracts

in most assessments compared to the control. However, a discrepancy in the bioactivities was observed in the dosage effect. According to the performed individual evaluation, the highest dose exhibited the strongest antioxidant activities, with significant differences compared to the control (Chaudhari *et al*., 2023; Mohammed *et al*., 2023).

Oxidative stability

The Rancimat test is a technique based on the conductometric determination of volatile degradation products, which is currently used to evaluate the oxidative stability of oils and fats and to study the antioxidant potentiality of new molecules. A longer induction period indicates higher resistance to oxidation or good efficiency of the added antioxidants. The effects of gamma irradiation with different doses (0.25, 0.5, 1, 2, 4, and 6 kGy) on the oxidative stability of refined sunflower oil compared with control (non-irradiated samples) and oil enriched with BHT are presented in Table 2. There was a significant increment in the induction period at 110ºC/h after being subjected to gamma irradiation and BHT as compared with control samples, which were 3.36, 4.13, 4.64, 4.67, 4.72, 4.69, 4.52, and 4.13 h, for control, 0.25, 0.5, 1, 2, 4, 6 and BHT, respectively. The same trend was observed with the results of shelf life at 25ºC (months) when gamma irradiation was used at different doses, and BHT prolonged the shelf life of sunflower oil. The values were 6.72, 8.16, 9.19, 9.34, 9.44, 9.20, 9.03 and 8.25 for control, 0.25, 0.5, 1, 2, 4, 6 and BHT, respectively. Therefore, gamma-irradiated *S. persica* extracts could be used as an antioxidant agent for extending the shelf life of edible oils. Regarding the antioxidant activity, samples treated with gamma irradiation with different doses showed good oxidative stability compared to control and BHT samples. Meanwhile, the sample treated with 2 kGy exhibited better oxidative stability than the samples containing BHT, being characterized by shelf life at 25ºC of 9.44 months, antioxidant activity of 1.37%, and increasing index of 182.3. The results are in agreement with Lante *et al*. (2011).

Table 2. Oxidative stability of sunflower oil treated with different doses of ethanol extract of irradiated Miswak sticks (0.02%) and BHT (0.02%).

Treatment (kGy)	Oxidative stability			
	Induction period at 110ºC/h	Shelf life at 25ºC (months)	Antioxidant activity (%)	Increasing Index
0.00	$3.36^{d} \pm 0.033$	$6.72^{e} \pm 0.067$	---	---
0.25	$4.13^{c} \pm 0.053$	$8.16^{d} \pm 0.123$	$1.23^{a \pm} 0.028$	$129.37^{c} \pm 20.869$
0.50	$4.64^{ab} \pm 0.049$	$9.19^{bc} \pm 0.167$	$1.39^{a} \pm 0.078$	$165.53^{ab} \pm 11.153$
1.00	$4.67^{ab} \pm 0.073$	$9.34^{ab} \pm 0.053$	$1.38^{a} \pm 0.042$	$181.77^{a} \pm 3.073$
2.00	$4.72^{a} \pm 0.078$	$9.44^{a} \pm 0.054$	$1.37^{a} \pm 0.041$	$182.39^{a} \pm 5.778$
4.00	$4.69^{a} \pm 0.078$	$9.20^{bc} \pm 0.077$	$1.37^{a} \pm 0.012$	$178.21^{ab} \pm 3.012$
6.00	$4.52^{b} \pm 0.066$	$9.03^{c} \pm 0.098$	$1.35^{a} \pm 0.037$	$169.95^{b} \pm 2.450$
BHT (200 ppm)	$4.13^{c} \pm 0.098$	$8.25^{d} \pm 0.106$	$1.22^{a} \pm 0.064$	$145.39^{c} \pm 1.604$

Results are presented mean ± S.D. The mean values in the same column with different superscript alphabets are significantly different ($P<0.05$) according to Duncan test.

***Salvadora persica* bioactive compounds**

Miswak is a widely used chewing stick in Middle Eastern and African cultures that is prepared from twigs and roots of the plant *Salvadora persica*. Benzyl isothiocyanate (benzyl mustard oil) is the dominant component in *Salvadora persica* L., a naturally occurring constituent in many cruciferous vegetables. Benzyl isothiocyanate inhibits chemically induced cancer in animal models (Srivastava and Singh, 2004; Aljarbou *et al*., 2022). Besides, benzyl isothiocyanate is the major antimicrobial component in Siwak (Sofrata *et al*., 2011).

As shown in Figure 1, the effect of irradiation doses on the major compound of Salvadora persica, benzyl isothiocyanate, was clearly illustrated, where the percentage of benzyl isothiocyanate was 11.69, 15.15, 21.1, 26.61, 15.18 and 11.45% at doses 0.25, 0.5, 1, 2, 4 and 6 kGy in comparison with the control sample (9.01%). The results revealed that the irradiation dose positively affected the percentage of samples, which increased after the treatments. The most effective irradiation dose was 2 kGy. The effect of the irradiation process could result from a chemical modification of terpenes, including the oxidative effect; this effect of the irradiation process was degreed according to the content of terpenes and other hydrocarbons in the plant terpenoids.

Organic sulfur compounds and elemental sulfur were also present as small amounts of fluoride, calcium, phosphorus, silica, and ascorbic acid (Farag et al., 2021). This compound was reported as the most robust antibacterial component with high bactericidal activity against Gram-negative periodontal pathogens.

Antimicrobial activity

The inhibition zone ≥ 20 mm was associated with the strong activity, the moderate activity was assigned to the inhibition zone of 12 to 20 mm and no inhibition activity was considered the zone was ≤ 12 mm (Ait-Ouazzou *et al*., 2011). The growth inhibitory effects of both irradiated and non-irradiated ethanol extracts of *Salvadora persica* sticks against six pathogenic strains, four bacteria and two fungi, in terms of inhibition zone are listed in Table 3. It can be noticed that the ethanol extract exhibited a stronger antibacterial activity against *S. aureus*, and the highest antibacterial activity (24 mm) was at 0.5 kGy. On the other hand, the inhibitory effect was at moderate activity (14.33-19.00 mm) in the case of *B. subtilis* in all irradiation treatments. In the case of Gram-positive bacteria, irradiation doses showed stronger antibacterial activity (21-29 mm) compared to non-irradiated samples (19 mm) for *E. coli*. Like *E. coli*, stronger antibacterial activity at doses 0.25, 0.5, 1, and 2 kGy was shown against *S. typhimurium*, while 0, 4 and 6 kGy showed moderate antibacterial activity.

Baur *et al.* (1996) reported that the microorganism is resistant if the inhibition zone is below 8.00 mm and sensitive if it is above 11.00 mm. According to this report, our data may suggest that the plant extract effectively has an inhibitory bactericidal influence against the tested bacterial strains. Also, it has been found that *S. persica* has a potent antimicrobial influence versus *Streptococcus* sp, *Staphylococcus aureus*, and *Enterococcus faecalis* (Al Lafi and Ababneh, 1995; Almas *et al*., 1997).

The data showed that the plant extract suppresses the growth of the tested fungal strain, as confirmed by the highest inhibition zone at doses 1 and 2 kGy. All treatments and the control one showed stronger antibacterial activity against *Aspergillus brasiliensis*. On the contrary, *Candida albicans* was the most resistant fungus to the plant extract, as it recorded the lowest inhibition zones in all treatments and the control. The present fungicidal property of the plant extract is compatible with other studies. An *in vitro* research revealed that the aqueous extract of miswak had to prohibit efficacy on the growing rate of *Candida albicans* that may be ascribed to its high sulfate concentration (Al-Bagieh *et al*., 1994; Al-Bayati and Sulaiman, 2008). The effect of the irradiation process could be related to the chemical modification of terpenes, including the oxidative effect. This effect of the irradiation process was ranked according to the content of terpenes and other hydrocarbons.

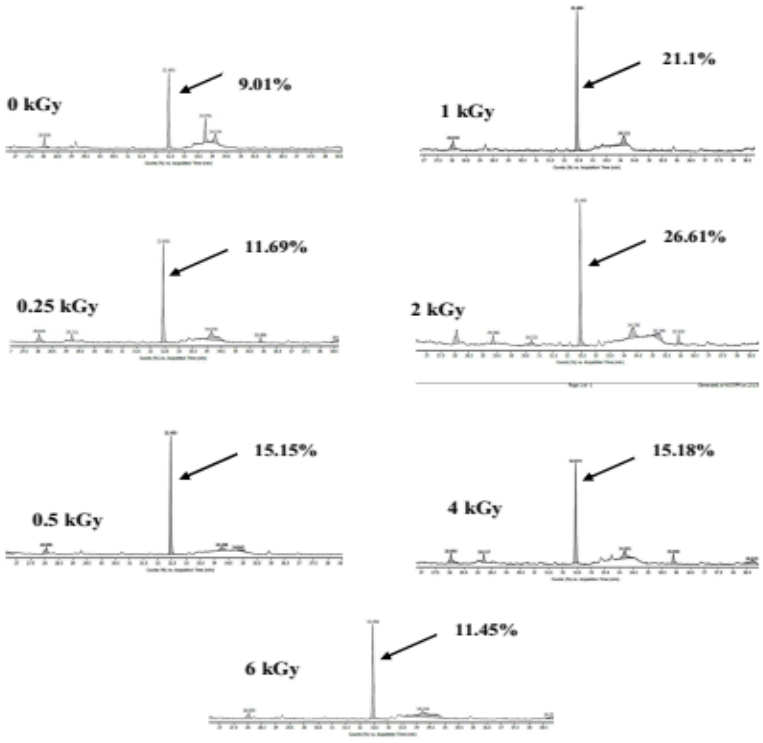

Figure 1. Effect of irradiation doses on the levels of benzyl isothiocyanate in *Salvadora persica* root.

The Miswak antimicrobial action has been attributed to the presence of relatively high levels of volatile oils. These include the germicides and bactericides benzoic acid, benzaldehyde, trimethylamine, the three siloxides, barbituric acid, vitamin C, tannic acid, and other acids, which have a beneficial effect on oral hygiene and dental health. Miswak was effective for the treatment of common oral pathogens, including

Actinobacillus actinomycetemcomitans, *Actinomyces naeslundii*, *Porphyromonas gingivalis*, and *Prevotella intermedia*, as well as the periodontopathic *Streptococcus* and *Candida* species (Al Bratty *et al*., 2020; Nordin *et al*., 2020; Naiel *et al*., 2021; El-Sherbiny *et al*., 2023).

Table 3. Antimicrobial activity (zone of inhibition in mm) of ethanol stick extract of *Salvadora persica* against pathogenic bacteria and fungi.

Irradiation treatment (kGy)	Bacterial strain				Fungal strain	
	Gram-negative		Gram-positive			
	Staphylococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Salmonella typhimurium***	***Aspergillus brasiliensis***	***Candida albicans***
0.00	$20.01^{cd} \pm 0.4$	$15^{c} \pm 0.4$	$19^{a} \pm 0.2$	$17.33^{e} \pm 0.57$	$21^{c} \pm 0.52$	$4^{d} \pm 0.5$
0.25	$20^{d} \pm 0.42$	$19^{a} \pm 0.42$	$22d^{c} \pm 0.3$	$22.5^{b} \pm 0.5$	$20^{c} \pm 0.43$	$4^{d} \pm 0.5$
0.50	$24^{a} \pm 0.44$	$19^{a} \pm 0.44$	$29^{a} \pm 0.42$	$25.5^{a} \pm 0.5$	$23^{b} \pm 0.34$	$5.17^{c} \pm 0.21$
1.00	$20^{d} \pm 0.5$	$15^{c} \pm 0.5$	$25^{b} \pm 0.5$	$20^{c} \pm 0.5$	$25.3^{a} \pm 0.55$	$6^{b} \pm 0.53$
2.00	$22^{b} \pm 0.11$	$16.93^{b} \pm 0.11$	$23.8^{c} \pm 0.76$	$21.87^{b} \pm 0.23$	$26^{a} \pm 0.23$	$7.7^{a} \pm 0.57$
4.00	$21.33^{bcd} \pm 0.21$	$15^{c} \pm 0.21$	$21^{f} \pm 0.33$	$16.5^{f} \pm 0.5$	$20.33^{c} \pm 0.57$	$6^{b} \pm 0.52$
6.00	$21.67^{bc} \pm 0.57$	$14.33^{c} \pm 0.57$	$19^{e} \pm 0.32$	$19.5^{d} \pm 0.57$	$20.67^{c} \pm 0.55$	$5.7^{bc} \pm 0.57$

Results are presented as mean ± S.D. The mean values with different superscript alphabets indicate significant differences ($P<0.05$) using Duncan test.

The extract of Miswak is effective against oral bacteria, including *Staphylococcus aureus*, *Porphyromonas gingivalis*, *Streptococcus mutans*, *Streptococcus salivarius*, and *Staphylococcus albus*. It is widely believed that the substance released by the bristle during chewing has an antimicrobial action because it contains fluoride. It is documented that salvadorine, an ingredient in *Salvadora persica*, exhibits antibacterial, antifungal, antiviral, and pasteurization effects against dental pathogens (Nordin *et al*., 2020; Naiel *et al*., 2021; El-Sherbiny *et al*., 2023).

The increase in the antimicrobial action of gamma-irradiated Miswak was attributed to the higher concentration of silica and free radicals formed from gamma radiation. Previous findings support our study by demonstrating the potential use of gamma-irradiated Miswak as a natural antioxidant and antimicrobial source (Asdaq *et al*., 2021; Mosaddad *et al*., 2023; Baky *et al*., 2024; Yu *et al*., 2024).

Conclusions

This study showed that irradiated and non-irradiated *S. persica* sticks (Miswak) have broad antimicrobial activity against gram-positive and gram-negative bacteria. Evaluation of DPPH, FRAP, and oxidative stability of Miswak stick extracts indicate that the most effective irradiation dose was 2 kGy. The GC-MS analysis showed a significant increase of the benzyl isothiocyanate in the irradiated samples compared to the control.

In conclusion, the results suggest that gamma radiation is a safe and effective method to enhance the antimicrobial and antioxidant traits of Miswak for food preservation applications. This is important as consumers are more interested in using natural antimicrobial compounds to prevent spoilage and maintain the quality of food. Gamma irradiation is a cost-effective natural method to target foodborne pathogens, as it increases antioxidant and antibacterial impact. In the absence of antimicrobials, irradiated Miswak sticks could be introduced to the packaging materials to reduce contamination by prepared food or moisture-susceptible foods stored in hermetically sealed packages.

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