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Effect of Gamma Irradiation on Gas and Liquid Chromatographic Profiling and Antioxidant Activity of Propolis from Different Countries

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ABSTRACT

This study evaluated the chemical profiles and antioxidant capacities of five propolis samples sourced from Egypt (EG), Yemen (YE), South Korea (SK), China (CH), and Ireland (IR) - in both their native and gamma-irradiated (10 kGy) states. Chemical characterization was performed using Headspace Solid-Phase Microextraction (HS-SPME) coupled with Gas Chromatography / Mass Spectrometry (GC/MS) and High Performance Liquid Chromatography (HPLC). Antioxidant potential was assessed via 2, 2-Diphenyl-1-picrylhydrazyl (DPPH and 2,2´-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) activity. The irradiated YE sample demonstrated the most potent antioxidant activity, yielding IC_{50} values of 53.86 µg/mL (DPPH) and 50.55 µg/mL (ABTS). Furthermore, the YE and CH extracts contained the highest concentrations of gallic acid, recorded at 590.22 µg/g and 666.41 µg/g, respectively.

Keywords: Propolis, Gamma irradiation, Headspace Solid-Phase Microextraction, Antioxidants, DPPH, ABTS

1. Introduction

Propolis is a resin-based substance harvested by honeybees (*Apis mellifera* L.) from various trees, particularly *Populus* species in temperate zones. After collection from buds and bark, the bees combine the resin with enzymes and salivary secretions. This modified material serves several structural and protective functions: it seals hive openings to prevent drafts and simplifies hive defense. Furthermore, propolis acts as a natural antimicrobial agent, lowering the presence of bacteria and fungi within the colony (Gómez-Caravaca *et al.,* 2006 ; Salatino *et al.,* 2011).

Chemically, propolis is a complex phytochemical matrix consisting of approximately 50% resin rich in flavonoids and phenolic acids alongside 30% wax, 10% essential oils, and 5% pollen, with the remainder being various organic constituents (Gómez-Caravaca *et al.,* 2006). This diverse chemical profile underpins a broad spectrum of pharmacological activities, including potent antimicrobial (antibacterial, antifungal, and antiviral), antioxidant, and immunomodulatory effects (Sforcin and Bankova, 2011). Consequently, propolis is frequently integrated into dermatological formulations, treatments for upper respiratory tract infections, and therapeutic agents for oral inflammatory conditions (Xu *et al.,* 2009).

The therapeutic utilization of propolis by human civilizations dates back to antiquity, driven by its diverse pharmacological profile, which includes potent antibacterial, antineoplastic, immunomodulatory, and anti-inflammatory activities (Maraschin *et al.,* 2016). In the contemporary global market, Brazilian green propolis has emerged as a primary commercial variety. Its high production volume is sustained by significant demand across the nutraceutical, cosmetic, and pharmaceutical sectors (Paviani *et al*., 2010).

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Propolis is categorized into three primary types temperate, tropical, and Pacific based on its morphological characteristics, entomological behavior, and geographic distribution. The temperate variety is distinguished by the presence of flavonoids lacking B-ring substituents, specifically chrysin, galangin, pinocembrin, and pinobanksin. Furthermore, caffeic acid phenethyl ester (CAPE) has been identified as a major bioactive constituent of temperate propolis, independent of its specific provenance (Alvear *et al.,* 2021; Dezmirean *et al.,* 2021). Conversely, tropical propolis is characterized by high concentrations of diterpenes and prenylated phenylpropanoids, while the Pacific (and African) varieties are defined by the presence of geranyl flavanones (Huang *et al.,* 2014).

The qualitative and quantitative profiles of primary polyphenols, specifically flavonoids, in propolis are subject to significant fluctuations governed by the ecological and environmental conditions of the botanical source (Dantas Silva *et al.,* 2017). To address the challenges of analyzing these complex mixtures, headspace solid-phase microextraction (HS-SPME) has emerged as a robust methodology for the evaluation of volatile organic compounds (Mohtar *et al*., 2017). This technique offers a superior alternative to traditional methods by eliminating the need for organic solvents, reducing operational costs, and significantly accelerating extraction timelines.

Solid-phase microextraction (SPME) has emerged as a predominant analytical technique for characterizing the volatile organic compounds (VOCs) within propolis. This method facilitates the isolation of headspace aromatic constituents from the sample matrix via selective adsorption onto a specialized polymeric fiber. Since its introduction into the food science sector, SPME has gained widespread adoption for the precise and efficient analysis of volatile profiles across various matrices (Zhang *et al*., 2007).

Investigations into the effects of gamma irradiation on propolis indicate that the material maintains its thermal stability when exposed to doses up to 10 kGy. Using a gamma cell for treatment, researchers observed that the irradiation process does not significantly alter the thermal properties of the substance within this specific dosage range (Matsuda *et al*., 2002).

The primary objective of the current study was to evaluate the impact of gamma irradiation on the phenolic and flavonoid concentrations within propolis. Furthermore, this research employs headspace solid-phase microextraction (HS-SPME) to characterize the volatile profile of the irradiated propolis matrix. To assess functional stability, the antioxidant capacity was quantified both before and after gamma treatment utilizing DPPH and ABTS radical scavenging assays.

2. Materials and Methods

2.1. Propolis samples

Propolis specimens (*n = 5*) were obtained from diverse geographical regions, including Egypt (EG), Yemen (YE), South Korea (SK), China (CH), and Ireland (IR). Upon collection, the samples exhibited characteristic aromatic profiles and varied in pigmentation from light to dark brown. To maintain chemical stability and prevent degradation, the raw propolis was stored under refrigerated conditions (4 °C) in a light-protected environment prior to analysis.

2.2. Chemicals and Reagents

All analytical-grade reagents and solvents, including HPLC-grade methanol and ethanol, were purchased from Sigma-Aldrich (Steinheim, Germany). The specific antioxidant standards and assay reagents - 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, 98% purity), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and synthetic tert-butylhydroquinone (TBHQ, 99.9% purity) - were likewise obtained from Sigma Aldrich for use in compound isolation and antioxidant activity determination.

2.3. Irradiation treatment

Propolis samples, processed into a fine powder, were subjected to gamma irradiation at doses of 0 and 10 kGy. The treatment was performed using a ^{60}Co Russian Gamma chamber at a dose rate of 665.6 Gy/h, situated at the Cyclotron Project of the Nuclear Research Center (Egyptian Atomic Energy Authority, Egypt). Following the irradiation procedure, all specimens were preserved at 4°C to ensure stability for subsequent experimental analyses.

2.4. Extraction of volatile organic compounds by headspace solid-phase microextraction (HS-SPME)

The extraction of volatile compounds was executed using a manual Solid-Phase Microextraction (SPME) holder equipped with a 50/30 µm StableFlex divinylbenzene-carboxen-polydimethylsiloxane

(DVB-CAR-PDMS) fiber (Supelco, Bellefonte, PA, USA). Approximately 1 g of finely powdered raw propolis was transferred into a 5 mL flat-bottom headspace vial, which was hermetically sealed with a magnetic crimp cap and PTFE/silicone septa. Prior to each analysis, the fiber was reconditioned for 3 min at 250°C within the gas chromatograph (GC) injector port. Extraction was performed by exposing the fiber to the sample headspace for an equilibration period of 30 min at 60 °C. Following extraction, the fiber was immediately inserted into the GC inlet for thermal desorption (1 min at 250°C) in splitless mode, utilizing a modified protocol based on the work of Pellati *et al.* (2013).

2.5. Gas chromatography–mass spectrometry (GC/MS) analysis

Chromatographic analysis was conducted at the Central Laboratory of Chromatography (Radioisotopes Application Division, Nuclear Research Center, Egyptian Atomic Energy Authority) using an Agilent Technologies 8890GC system. The unit was equipped with an automated liquid sampler and coupled to an Agilent 5977B series Mass Selective Detector (MSD). Separation was achieved on a DB-5ms capillary column (60 m x 250 µm x 0.25 µm). The oven temperature program commenced at 50°C (2 min), 5°C/min / 110°C (2 min), and further ramped by 5°C/min to a final temperature of 240°C (5 min). Helium served as the carrier gas at a constant flow rate of 1 mL/min. The temperature of injector was set at 250 °C, transfer line temperature was set as 250 °C and ion source at 250 °C. Injection mode was split ratio 50:1 and solvent delay was 4 min. Mass spectra were acquired in electron impact (EI) mode at 70 eV, covering a scan range of 50–550 amu. Data processing was performed using MSD Mass-Hunter software, with compound identification accomplished by matching mass spectra against the National Institute of Standards and Technology (NIST20) library.

2.6. High Performance Liquid Chromatography (HPLC) analysis

The phytochemical profile was determined via HPLC according to the methodology described by Kim *et al.* (2006), utilizing an Agilent Technologies 1260 Infinity II system equipped with an autosampler and a diode-array detector (DAD). Separation was achieved on an Eclipse XDB-C18 analytical column (150 x 4.6 µm; 5 µm) protected by a C18 guard column (Phenomenex, Torrance, CA). The binary mobile phase consisted of acetonitrile (Solvent A) and 2% aqueous acetic acid (v/v) (Solvent B), delivered at a flow rate of 0.8 mL/min over a 60 min duration. The elution gradient transitioned from 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. Prior to a 50 µL injection, all samples were clarified using 0.45 µm Acrodisc syringe filters. Analytes were monitored at 280 nm (benzoic acid derivatives), 320 nm (cinnamic acid derivatives), and 360 nm (flavonoids), with identification based on UV spectral data and retention time congruence with authentic standards.

2.7. Antioxidant activity analysis

Determinations of antioxidant activities of propolis samples were accomplished using DPPH and ABTS methods as follows:

2.7.1. The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant potential of samples were determined across a concentration gradient (50, 100, 150, and 200 µg/mL) using the DPPH radical-scavenging method (Viuda-Martos *et al*., 2010). Briefly, each concentration was added to 4 mL of a 0.004% methanolic DPPH solution. Following a 30-minute incubation period at ambient temperature, the absorbance was measured at 517 nm against a methanol blank. The inhibition percentage (%) was calculated using the following relationship:

$$\boldsymbol{DPPH\ scavenging\ activity}\ (\%) = \frac{\boldsymbol{A_{blank} - A_{sample}}}{\boldsymbol{A_{blank}}} \times \mathbf{100}$$

Where, A_{blank} denotes the absorbance of the control (reagents only), while A_{sample} represents the absorbance of the poropolis extract. All analyses were performed in triplicate to ensure reproducibility. The IC_{50} value - defined as the extract concentration required to achieve 50% inhibition of the DPPH• radical - was determined by plotting the inhibition percentages against the corresponding extract concentrations. Under this analytical framework, lower IC_{50} values (µg / mL or ppm) signify a more potent antioxidant capacity.

2.7.2. The 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity

The ABTS radical scavenging capacity of the propolis extracts was determined following the protocols of Mohafrash and Mossa (2020); El Kar *et al*., (2011). To generate the ABTS• cation, a 7 mM ABTS solution (0.0384 g in 10 mL distilled water) was reacted with 2.45 mM potassium persulfate (0.0066 g in 10 mL distilled water) in equal proportions. The mixture was incubated in the dark at ambient temperature for 12 – 16 hours to ensure radical stability. Before use, the stock was diluted with distilled water to achieve a working absorbance of 0.700 ± 0.02 at 734 nm. Various sample concentrations (50 – 200 µg/mL) were then mixed with 4 mL of the ABTS working solution. After a 30-minute dark incubation, the absorbance was recorded at 734 nm against a sample-free control.

$$\boldsymbol{ABTS\ scavenging\ activity}\ (\%) = \frac{\boldsymbol{A_{blank} - A_{sample}}}{\boldsymbol{A_{blank}}} \times \mathbf{100}$$

In this calculation, A_{blank} represents the control absorbance, while A_{sample} denotes the absorbance of the propolis extract. To ensure statistical reliability, all assays were performed in triplicate. The resulting antioxidant values are expressed as the mean ± standard deviation (SD) in µg/mL. These data were subsequently compared against the performance of tertiary butylhydroquinone (TBHQ), which served as a synthetic antioxidant reference standard.

2.8. Statistical analysis

All experimental procedures were conducted in triplicate, with results expressed as the mean ± standard error (SE). Data were subjected to a one-way analysis of variance (ANOVA) to determine statistical significance. To identify specific differences between groups, Duncan's Multiple Range Test was employed using SAS software (version 9.1.3; Cary, NC). Statistical significance was pre-defined at a threshold of $P < 0.05$ (Institute 2004).

3. Results and Discussion

Propolis is a resinous substance composed of plant resins, balsams, beeswax, essential oils, and pollen. It boasts a complex chemical profile featuring numerous identified compounds, with polyphenols—specifically flavonoids and phenolic acids—being the most prevalent. As illustrated in Fig. (1), the precise chemical composition of propolis is subject to significant variation based on geographic origin.

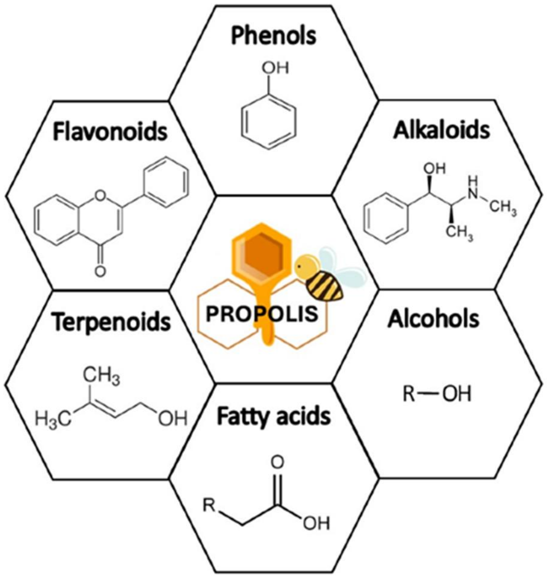

Fig. 1. Major chemical components of propolis

3.1. Analysis of volatile compounds in raw propolis by HS-SPME GC-MS

The selection of an appropriate fiber coating is critical in HS-SPME for analyzing volatile compounds in aromatic and medicinal plants, a point emphasized by Belliardo *et al.* (2006). While the DVB-CAR-PDMS (50/30 µm) fiber is frequently utilized for natural products particularly due to its high affinity for carboxylic acids (Lattuati-Derieux *et al.,* 2008) this study employed a PDMS fiber. The PDMS coating provided a superior overall profile across all chemical classes of propolis volatiles. As noted by Wojtyczka *et al.* (2013), these volatile profiles are primarily influenced by local flora and geographic origin, as evidenced by previous HS-SPME GC-MS analyses identifying 99 compounds in Italian propolis (Salatino *et al*., 2011).

Across the diverse geographical propolis samples, 228 distinct compounds were identified. Detailed in Table 1, the chemical profiles shifted between non-irradiated and irradiated states: EG (39/40 substances), CH (53/90), SK (72/41), IR (70/33), and YE (45/34). EG exhibited the highest concentration of acidic components, with acetic acid appearing as a universal constituent. These fluctuations in acidic content are likely driven by regional climates and the specific local flora of each country. This finding was in agreement with Yang *et al.* (2010) who found that acetic acid in Egyptian propolis had high concentrations in all studied samples.

Each propolis sample is defined by a specific "major" volatile component that reflects its botanical origin. Egyptian propolis showed extremely high Acetic acid content (44.97%). While, China and Ireland propolis dominated by D-Limonene (21.27% and 17.77%, respectively) and Estragole (14.93% and 10.86%). On the other hand, Yemeni propolis shows a high concentration of Hexanedioic acid, bis(2-ethylhexyl) ester (50.02%). South Korea features a more balanced profile with Phenylethyl Alcohol (6.07%) and α-Curcumene (10.46%) as notable markers.

Irradiation treatment at dose 10 kGy, not just degrade compounds; it often triggers a "volatile shift" where complex molecules break down into simpler, often more aromatic, constituents. In Yemen propolis, α-Curcumene saw a massive surge from 7.09% to 31.39%. A similar, though smaller, increase was noted in the China sample (2.27% to 15.37%). While, Nonanal aldehyde increased dramatically in the South Korea sample, jumping from undetected (ND) to 44.06%, and in the Ireland sample to 23.66%. D-Limonene decreased in the China sample; it nearly doubled in the Ireland sample (17.77% to 33.24%) and increased fourfold in the Egypt sample (5.57% to 22.03%). Related to radiation treatment, acetic acid, the primary component of Egyptian propolis, dropped nearly by half, from 44.97% to 24.61%. as well as, in Chinese propolis, estragole Fell from a major component (14.93%) to a trace amount (0.26%).

3.2. HPLC analysis of various propolis samples

Polyphenols function as secondary plant metabolites, with their biosynthesis driven by specific enzymes in response to species-specific requirements and oxidative stress. Consequently, the botanical source of the resin gathered by bees dictates the phenolic profile of propolis across different regions. In this study, high-performance liquid chromatography (HPLC) was employed to evaluate the chemical composition of various propolis extracts (Table 2). The analysis identified 13 phenolic acids—including gallic, caffeic, ρ-coumaric, ferulic, and cinnamic acids alongside 9 flavonoids, such as quercetin, apigenin, rutin, epicatechin, and chrysin.

As shown in Table 2, the propolis exhibited distinct chemical "fingerprints" based on their origin. Yemeni propolis was characterized by high concentrations of Sinapic acid (1506.42 µg/g), Epicatechin gallate (1495.84 µg/g), and Apigenin (980.76 µg/g). while, Egyptian propolis was uniquely rich in Catechin (2753.29 µg/g), though it contained negligible amounts of many other phenolic acids found in the Yemen or China samples. Chinse propolis showed a profile similar to Yemen but with higher Gallic acid (666.41 µg/g) and significant levels of Epicatechin gallate (1152.08 µg/g) and Sinapic acid (1157.97 µg/g). on the other hand, in South Korea & Ireland regions generally displayed lower diversity and lower concentrations of the identified compounds, with South Korea lacking many of the common markers entirely (ND).

The application of 10 kGy radiation caused dramatic shifts in the concentration of bioactive compounds, likely due to radiolytic degradation or the transformation of larger molecules. Conversely, some compounds increased after irradiation, suggesting that the energy might break down complex plant-derived esters or glycosides into simpler phenolic forms. The flavonoid compound (Chrysin), saw a massive surge in the China sample, jumping from 2.83 µg/g to 5894.60 µg/g, and in the Yemen

Table 1: HS-GC/MS analysis of irradiated propolis collected from different regions

		Relative contents (%)									
Time (min)	**Compounds**	**China 0kGy**	**China 10kGy**	**Egypt 0kGy**	**Egypt 10kGy**	**South Korea 0kGy**	**South Korea 10kGy**	**Ireland 0kGy**	**Ireland 10kGy**	**Yemen 0kGy**	**Yemen 10kGy**
5.386	**Acetic acid**	0.38	0.4	44.97	24.61	0.67	1.67	0.26	0.63	0.98	0.73
5.689	**Ethyl acetate**	0.59	--	0.51	--	--	--	0.16	0.43	--	--
6.925	**Propanoic acid**	--	--	--	--	0.17	--	--	--	0.16	--
8.091	**Pyrazine**	0.3	--	--	--	--	--	0.12	--	--	--
8.283	**Isobutyric acid**	--	--	0.92	0.8	--	0.23	--	--	0.43	0.27
8.918	**n-Amyl alcohol**	--	--	--	--	--	0.47	--	--	--	--
9.018	**Butanoic acid**	--	--	0.26	--	0.72	--	--	--	0.14	--
9.676	**octyl alcohol**	--	--	0.42	--	--	--	--	--	--	--
9.956	**n-Octene**	--	--	3.6	--	--	--	--	--	--	--
9.939	**Ethyl butyrate**	0.71	--	--	--	--	--	0.76	0.46	--	--
9.968	**Hexanal**	--	--	--	2.66	--	9.17	--	0.57	--	--
10.37	**Butyl acetate**	0.27	--	0.27	--	--	--	0.36	--	--	--
10.82	**2-Methylpyrazine**	0.49	--	--	--	--	0.34	0.15	--	--	--
11.09	**Furfural**	2.94	--	0.33	--	0.25	1.68	1.33	1.22	0.11	--

11.238	**Isopentanoic acid**	--	--	--	--	0.22	--	--	--	--	--
11.419	**Isobutyl alcohol**	--	--	--	0.59	--	--	--	--	--	--
11.652	**α-Methylbutyric acid**	--	0.27	0.48	--	--	--	--	--	0.35	--
11.856	**Isoprprnyl acetate**	--	--	--	0.79	--	--	--	--	--	--
12.31	**1-Hexanol**	--	--	0.28	--	--	0.28	0.14	--	--	--
12.61	**Isoamyl acetate**	2.2	--	0.23	2.77	0.44	1.66	1.85	2.01	0.22	--
12.68	**2-Methyl-1-butyl acetate**	0.23	--	--	0.3	--	--	0.2	--	--	--
12.847	**3-Methyl-2-butenoic acid**	--	--	--	--	--	--	--	--	0.15	--
13.208	**1-nonene**	--	--	0.38	--	--	--	--	--	--	--
13.348	**Styrene**	--	--	0.3	--	--	--	--	--	--	--
13.535	**Nonane**	--	--	5.98	1.1	--	--	--	--	--	--
13.611	**Heptanal**	--	--	--	0.78	--	0.8	--	--	--	--
13.93	**2-Furyl methyl ketone**	0.22	--	--	--	--	--	--	--	--	--
14.252	**E-3-Methyl-2-hexenoic acid**	--	--	--	--	--	--	--	--	0.15	--
14.567	**2-Methyl-2-butenoic acid**	--	--	--	--	--	--	--	--	0.15	--
15.027	**α-Pinene**	--	--	3.4	0.56	--	--	--	0.51	--	--
15.54	**γ-Pentalactone**	--	--	0.27	--	--	--	--	--	--	--
15.802	**2-Heptanal**	--	--	--	--	--	0.33	--	--	--	--

15.959	**2-Formyl-5-methylfuran**	--	--	--	--	--	1.17	--	--	--	--
15.98	**5-MethyIfurfural**	2.27	--	--	--	--	--	0.72	0.75	--	--
16.164	**2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone**	4.05	--	--	--	--	--	--	--	--	--
16.29	**Hexanoic acid (Caproic acid)**	--	--	--	0.98	1.61	0.48	--	0.42	--	--
16.298	**Benzaldehyde**	0.23	0.45	0.53	1.18	1.12	3.19	3.31	2.47	0.42	0.44
16.572	**Nonanoic acid**	--	0.48	--	--	--	--	--	--	--	--
16.585	**(+)-Sabinene**	--	--	--	--	--	--	0.13	--	--	--
16.659	**1-Octen-3-ol**	--	--	--	--	--	0.69	--	--	--	--
16.77	**2,7-Dimethylocta-2,6-dienol**	--	0.22	--	--	--	--	--	--	--	--
16.84	**(-)-β-Pinene**	0.23	--	--	--	--	0.24	0.42	--	--	--
16.9	**Isovaleric acid**	--	--	--	2.44	--	--	--	--	--	--
17.055	**Nerolidol**	--	--	--	0.3	--	--	--	--	--	--
17.073	**β-Myrcene**	0.69	--	--	--	--	--	0.63	0.32	--	--
17.061	**trans-3-Hexanoic acid**	--	--	--	--	0.17	--	--	--	--	--
17.119	**Furan, 2-pentyl-**	--	--	--	--	1.07	--	--	--	--	--
17.359	**Ethyl hexanoate**	3.78	--	--	0.66	0.56	1.58	3.00	--	0.27	--
17.469	**Decane**	--	--	0.31	0.45	--	--	0.15	--	--	--

17.615	**Octanal**	--	--	0.34	1.98	--	0.93	--	0.35	--	--
17.621	**(3E)-Hexenyl acetate**	0.45	--	--	--	--	--	0.31	--	--	--
17.871	**2,7-Dimethylocta-2,6-dienol**	--	--	0.4	--	--	--	--	--	--	--
17.889	**Hexyl acetate**	3.99	--	--	1.74	0.64	1.38	2.85	1.25	--	--
18.32	**Terpinene 4-acetate**	--	--	--	--	--	--	0.14	--	--	--
18.629	**p-Cymene**	3.43	--	1.57	3.38	0.45	5.02	3.01	2.39	0.2	--
18.804	**D-Limonene**	21.27	0.23	5.57	22.03	3.88	2.84	17.77	33.24	1.63	0.4
18.903	**1-phenyl-1,2-butanediol**	--	--	--	0.33	--	--	--	--	--	--
18.915	**Benzyl alcohol**	--	0.6	0.46	--	1.31	--	--	--	0.5	0.47
18.96	**Pseudolimonen**	--	--	--	--	--	--	0.13	--	--	--
18.979	**Eucalyptol**	0.67	0.21	0.66	--	--	0.99	0.53	0.87	--	--
19.031	**Lanender lactone**	--	--	--	0.53	--	--	--	--	--	--
19.33	**β-cis-Ocimene**	--	--	--	--	--	--	0.14	--	--	--
19.649	**Phenyl acetoacetate**	--	--	--	0.26	--	--	--	--	--	--
19.655	**iso-Amyl iso-butyrate**	--	--	--	--	--	0.36	0.72	--	--	--
19.737	**2-Methylbutyl butyrate**	0.3	--	--	--	--	--	0.23	--	--	--
19.818	**Allyl pentyl ether**	--	--	--	0.27	--	--	--	--	--	--
19.824	**trans-2-Octenal**	--	--	--	--	--	0.54	--	0.27	--	--

19.923	**4-Carene**	--	--	--	0.45	--	--	--	--	--	--
19.935	**γ-Terpinene**	3.43	--	2.3	--	0.35	--	2.94	0.74	0.18	--
20.226	**Isopinocampheol**	--	--	--	--	--	0.79	--	--	--	--
20.33	**Dihydromyrcenol**	--	--	--	--	--	--	0.13	--	--	--
20.716	**Hexanal propylene glycol acetal**	0.48	--	--	--	--	--	0.36	--	--	--
20.249	**Acetophenone**	--	0.21	--	--	0.42	--	--	--	0.35	0.32
20.354	**1,6-Dihydrocarveol**	--	0.26	--	--	--	--	--	--	--	--
20.961	**Guaiacol**	--	0.92	0.71	--	2.02	--	--	--	0.62	0.4
21.007	**o-Methylbenzaldehyde**	0.65	--	--	--	--	--	0.49	--	--	--
21.019	**Isopulegol acetate**	--	--	0.34	--	--	--	--	--	--	--
21.077	**Methyl isoamyl ketone**	--	--	--	0.44	--	--	--	--	--	--
21.14	**Ethoxycitronellal**	--	--	--	--	--	--	0.13	--	--	--
21.409	**Undecane**	--	--	1.34	1.72	--	0.57	--	--	--	--
21.444	**Linalool**	2.37	--	--	--	0.25	--	1.6	0.41	--	--
21.608	**Nonanal**	--	--	1.19	2.96	--	44.06	--	23.66	--	--
21.625	**Isoamyl isovalerate**	0.77	--	--	--	--	--	0.58	--	--	--
21.71	**2-Methylbutyl isovalerate**	--	--	--	--	--	--	0.1	--	--	--
21.917	**cis-p-mentha-1(7),8-dien-2-ol**	0.5	--	--	--	--	--	--	--	--	--

21.92	**Thujone**	--	--	--	--	--	--	0.23	--	--	--
22.103	**Phenylethyl Alcohol**	--	3.17	--	--	6.07	0.27	0.09	--	2.56	3.24
22.231	**1-Terpinenol**	--	0.26	--	--	--	--	--	--	--	--
22.791	**Limona ketone**	--	--	--	--	--	0.26	--	--	--	--
23.047	**Limonene oxide**	0.4	--	--	--	--	0.94	0.42	0.37	--	--
23.589	**L-camphor**	--	--	--	0.3	0.17	0.52	--	0.74	--	--
23.601	**(+)-2-Bornanone**	2.03	--	--	--	--	--	1.46	--	--	--
23.659	**Benzoic acid**	--	--	--	--	0.17	--	--	--	--	--
23.758	**trans-2-Nonenal**	--	--	--	--	--	1.28	--	1.27	--	--
23.881	**Benzyl acetate**	--	--	--	--	1.85	--	--	--	0.84	0.91
23.817	**cis-Menthone**	2.31	--	--	--	--	--	1.62	--	--	--
23.875	**Eucarvone**	--	1.92	--	--	--	--	--	--	--	--
23.884	**Caprylic acid**	--	--	--	--	--	--	--	0.44		--
24.085	**L-(-)-Menthol**	--	0.31	--	--	--	--	0.1	--	--	--
24.178	**D-Isomenthone**	0.61	--	0.25	0.47	--	0.52	--	0.38	--	--
24.248	**Ethyl benzoate**	--	0.43	--	--	0.53	--	--	--	0.33	0.31
24.399	**Hydrocinnamic acid**	--	--	--	--	0.16	--	--	--	--	--
24.837	**(+)-3-Thujanol**	--	1.42	--	--	--	--	--	--	--	--

24.889	**2-Ethylcyclohexyl-3-methylbenzoate**	--	--	--	--	0.21	--	--	--	--	--
25.187	**Methyl salicylate**	--	--	--	1.5	--	--	--	--	--	--
25.198	**2-Propyloyridine**	0.33	--	--	--	--	--	--	--	--	--
25.2	**Dodecane**	--	--	--	--	--	--	0.21	--	--	--
25.303	**Estragole**	14.93	0.26	--	3.11	1.7	2.76	10.86	14.65	--	--
25.379	**cis-Dihydrocarvone**	--	--	--	--	--	0.27	--	--	--	--
25.449	**Decanal**	--	--	--	1.14	--	1.4	--	0.93	--	--
25.857	**5-Isopropenyl-2-methylcyclopent-1-enecarboxaldehyde**	--	--	--	--	--	1.07	--	--	--	--
26.562	**3-Hydroxydodecanoic acid, methyl ester**	--	0.27	--	--	--	--	--	--	--	--
26.76	**1,5,5-Trimethyl-6-methylene-cyclohexene**	--	0.34	--	--	--	--	--	--	--	--
26.877	**Linalyl acetate**	0.47	--	--	--	--	--	0.42	--	--	--
26.88	**Pulegone**	--	--	--	--	--	--	0.32	--	--	--
26.923	**Methyl phenethyl ketone**	--	--	--	--	0.19	--	--	--	--	--
26.976	**(-)-Carvone**	0.65	--	--	--	--	--	0.25	--	--	--
27.046	**β-Phenylethyl acetate**	0.48	--	--	--	--	0.63	--	0.31	3.01	--

27.151	**Anisaldehyde**	--	0.25	--	--	--	--	--	--	--	--
27.279	**Benzenebutanal**	--	--	--	--	--	--	0.12	--	--	--
27.285	**2-phenylethyl octanoate**	--	--	0.95	--	--	--	--	--	--	--
27.331	**Phenethyl acetate**	--	5.75	--	--	6.57	--	--	--	--	8.09
27.384	**Benzyl propionate**	--	0.28	--	--	0.32	--	--	--	--	--
28.013	**4-Ethylguaiacol**	--	0.79	--	--	0.64	--	--	--	--	--
28.112	**Cinnamaldehyde**	0.33	0.65	0.76	0.28	0.34	--	1.06	--	--	--
28.221	**Thymol**	--	--	2.04	--	--	--	--	--	--	--
28.284	**Anethole**	1.34	0.63	0.29	--	0.32	--	2.03	0.53	--	--
28.456	**Carvacrol**	--	--	--	4.22	--	--	--	--	--	--
28.521	**Isobronyl acetate**	3.03	--	--	--	--	--	0.29	--	--	--
28.602	**Eugenol**	0.41	--	0.82	0.43	--	--	--	--	--	--
29.214	**Cinnamyl alcohol**	--	1.93	--	--	0.87	--	--	--	--	0.28
29.459	**4-Ethyl-2-methoxyanisole**	--	0.3	--	--	0.19	--	--	--	--	--
29.884	**Triacetin**	--	0.78	--	--	--	--	--	--	--	--
30.368	**Cinnamyl formate**	--	0.32	--	--	--	--	--	--	--	--
30.56	**Ethyl hydrocinnamate**	--	0.35	--	--	0.18	--	--	--	--	--
30.636	**Phenethyl propionate**	--	2.04	--	--	1.38	--	--	--	--	0.79

30.735	**trans-Isoeugenol**	--	0.24	--	--	--	--	--	--	--	--
30.75	**Eugenol acetate**	--	--	--	--	--	--	0.17	--	--	--
30.944	**Copaene**	0.21	--	--	--	--	--	0.35	0.27	--	--
31.015	**4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptane**	--	0.4	--	--	--	--	--	--	--	--
31.254	**Hydrocinnamyl acetate**	--	--	--	--	0.41	--	--	--	--	--
31.26	**γ-Phenylpropyl acetate**	--	0.94	--	--	--	--	--	--	--	--
31.878	**Methyl cinnamate**	--	0.24	--	--	--	--	--	--	--	--
32.099	**Tetradecane**	0.63	0.65	0.49	0.43	--	1.23	0.34	0.69	--	--
32.169	**Caryophyllene**	0.85	--	0.24	0.27	--	--	0.22	--	--	--
32.187	**β-Patchoulene**	--	2.09	--	--	2.27	--	--	--	0.48	3.44
32.865	**Cyclosativene**	--	0.31	--	--	0.21	--	--	--	--	--
33.003	**β-Cyclogermacrane**	--	--	--	--	0.38	--	--	--	--	--
33.253	**(±)-α-Cedrene**	--	0.36	--	--	0.76	--	--	--	--	0.52
33.347	**β-Longipinene**	--	--	--	--	0.24	--	--	--	--	0.55
33.475	**trans-Valerenyl acetate**	--	--	--	--	0.46	--	--	--	--	--
33.486	**Isovalencenol**	--	0.22	--	--	0.32	--	--	--	--	--
33.661	**α-Guaiene**	--	2.77	--	--	3.67	--	--	--	--	5.12

33.778	**Cinnamyl acetate**	--	0.94	--	--	0.62	--	--	--	0.13	0.27
34.419	**α-Himachalene**	0.37	1.98	--	--	2.63	--	--	--	0.17	3.87
34.46	**(±)-Cadinene**	--	--	--	--	2.19	--	--	--	0.54	0.5
34.477	**β-Guaiene**	--	1.44	--	--	0.41	--	--	--	0.24	--
34.5	**γ-Gurjunene**	--	--	--	--	--	--	--	--	1.38	2.15
34.594	**(+)-Cuparene**	--	--	--	--	1.66	--	--	--	0.73	--
34.617	**α-Curcumene**	2.27	15.37	0.45	--	10.46	1.66	0.75	1.14	7.09	31.39
34.734	**(+)-Ledene**	--	--	--	--	0.75	--	--	--	--	--
34.899	**γ-Patchoulene**	--	--	--	--	5.88	--	--	--	4.32	9.78
35.358	**Heptadecane**	--	--	--	--	--	--	--	0.33	--	--
34.926	**Cyperene**	--	4.35	--	--	--	--	--	--	--	--
35.381	**β-Maaliene**	--	2.63	--	--	2.44	--	--	--	0.42	6.14
35.497	**Dihydroagarofuran**	--	0.88	--	--	1.6	--	--	--	0.79	0.83
35.532	**β-Selinene**	--	--	--	--	2.43	--	--	--	2.04	--
35.661	**(4aS,9aR)-3,5,5,9-Tetramethyl-2,4a,5,6,7,9a-hexahydro-1H-benzo[7]annulene**	--	0.65	--	--	--	--	--	--	--	--
35.754	**α-Selinene**	--	2.95	--	--	2.54	--	--	--	1.63	2.75
35.801	**β-Chamigrene**	0.53	--	--	--	--	--	--	--	--	--

35.813	**δ-Guaiene**	--	--	--	--	3.55	--	--	--	--	5.5
35.83	**Aromandendrene**	--	3.19	--	--	0.42	--	--	--	3.02	0.3
35.969	**α-Longipinene**	--	--	--	--	0.36	--	--	--	1.93	--
36.045	**Methyl laurate**	--	--	--	--	--	0.27	--	--	--	--
36.069	**Sesquicineole**	--	0.85	--	--	1.12	--	--	--	0.41	0.44
36.249	**Tau-Cadinol acetate**	--	0.48	--	--	0.77	--	--	--	--	--
36.401	**L-calamenene**	--	0.44	--	--	0.59	--	--	--	0.73	0.5
36.471	**2-Hydroxy-2,6-dimethyl-1-[(1E)-3-methyl-1,3-butadienyl]bicyclo [4.1.0]hept-3-yl acetate**	--	0.4	--	--	--	--	--	--	--	--
36.605	**Ylangene**	--	0.22	--	--	--	--	--	--	--	0.23
36.762	**trans-α-Bisabolene**	--	0.66	--	--	0.67	--	--	--	0.86	0.66
36.855	**epi-Cubebol**	--	--	--	--	--	--	--	--	--	0.41
36.861	**Italicene ether**	--	0.73	--	--	0.89	--	--	--	--	--
37.06	**Lauric acid**	0.23	--	--	--	--	0.34	0.45	--	--	--
37.124	**Selina-3,7(11)-diene**	--	0.46	--	--	0.59	--	--	--	--	--
37.567	**γ-Selinene**	--	0.8	--	--	--	--	--	--	--	--
37.84	**Amyl salicylate**	--	0.59	--	--	--	--	--	--	--	--
38.103	**α-Vetivol**	--	0.27	--	--	--	--	--	--	--	--

38.371	**Hexadecane**	0.31	0.58	--	--	--	--	--	--	--	--
38.656	**Guaiol**	--	0.91	--	--	0.48	--	--	--	0.28	--
38.74	**2-Hexyl-1-decanol**	--	--	--	--	--	--	0.18	--	--	--
38.907	**(±)-trans-Nuciferol**	--	0.23	--	--	--	--	--	--	--	--
39.41	**2,15-Octadecadienoic acid, methyl**	--	--	--	--	--	--	--	--	--	--
39.752	**γ-Eudesmol**	--	0.96	--	--	--	--	--	--	0.22	--
39.991	**Isoaromadendrene epoxide**	--	0.32	--	--	--	--	--	--	--	--
40.03	**Methyl oleate**	--	--	--	--	--	--	0.54	--	--	--
40.125	**(+)-β-Costol**	--	0.21	--	--	0.19	--	--	--	--	--
40.341	**α-Bisabolol oxide B**	--	0.38	--	--	0.16	--	--	--	--	--
40.364	**Hexacosane**	0.62	--	--	--	--	--	--	--	--	--
40.399	**Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)-**	--	0.22	--	--	--	--	--	--	--	--
40.42	**2-Methylhexadecan-1-ol**	--	--	--	--	--	--	0.18	--	--	--
40.487	**α-Eudesmol**	--	1.18	--	--	0.74	--	--	--	0.34	--
40.656	**β-Acorenol**	--	0.72	--	--	--	--	--	--	0.15	0.25

40.854	**4a,10a-Methanophenanthren-9β-ol, 11-syn-bromo-1,2,3,4,4a,9,10,10a-octahydro-**	--	0.37	--	--	--	--	--	--	--	--
40.959	**hexadecyl acrylate**	--	0.52	--	--	--	--	--	--	--	--
41.035	**7-epi-cis-sesquisabinene hydrate**	--	0.5	--	--	--	--	--	--	--	--
41.157	**2,6,10-Trimethyltetradecane**	--	0.82	--	--	--	--	--	--	--	--
41.227	**(8Z)-7-Methyl-8-tetradecenyl acetate**	--	0.22	--	--	--	--	--	--	--	--
41.59	**Capric ether**	--	--	--	--	--	--	0.42	--	--	--
41.73	**Methyl stearate**	--	--	--	--	--	--	0.37	--	--	--
42.06	**Linoleic acid**	--	--	--	--	--	--	8.24	--	--	--
42.445	**Myristic acid**	--	0.39	--	--	--	--	--	--	--	--
42.47	**trans-13-Octadecenoic acid**	--	--	--	--	--	--	13.06	--	--	--
42.486	**2-Propylheptanol**	--	--	--	2.62	--	--	--	--	--	--
42.649	**α-Bisabolol oxide A**	--	0.93	--	--	--	--	--	--	--	--
43.354	**Heptacosane**	0.6	0.28	--	--	--	--	0.23	--	--	--
43.38	**Stearic acid**	--	--	--	--	--	--	3.69	--	--	--
43.55	**Oleic Acid**	--	--	--	--	--	--	0.39	--	--	--
43.792	**Hexanedioic acid, bis(2-ethylhexyl) ester**	4.9	--	--	--	--	--	--	--	50.02	--

43.803	**Isopropyl myristate**	--	0.22	--	--	--	--	0.18	--	--	--
44.526	**Pentadecylic acid**	--	0.26	--	--	--	--	--	--	--	--
44.602	**Hexanedioic acid, mono(2-ethylhexyl)ester**	--	1.19	--	--	--	--	--	--	--	--
44.7	**Ethyl stearate**	--	--	--	--	--	--	0.14	--	--	--
45.989	**Methyl palmitate**	--	1.11	--	--	0.3	--	--	--	--	--
46.298	**Hexadec-9-enoic acid**	--	0.63	--	--	--	--	--	--	--	--
46.91	**Palmitic acid**	--	7.44	0.49	--	--	--	--	--	--	--
47.11	**Hexanedioic acid, (ethylhexyl) ester**	--	--	--	--	--	--	0.8	--	--	--
47.586	**Ethyl palmitate**	--	0.32	--	--	--	--	--	--	--	--
47.79	**3-Deoxyestradiol**	--	0.21	--	--	--	--	0.27	--	--	--
	Identified Compounds	96.7	96.32	85.1	91.8	91.66	94.88	96.5	95.02	92.47	92.25
	Unidentified Compounds	3.3	3.68	14.9	8.2	8.34	5.12	3.5	4.98	7.53	7.75

Table 2: Phenolic profile of irradiated and non-irradiated propolis

Compounds	**Phenolic Compounds Type**	**Time (min)**	**Concentration (µg/g)**									
			Yemen		**Egypt**		**South Korea**		**China**		**Ireland**	
			0 kGy	**10 kGy**	**0 kGy**	**10 kGy**	**0 kGy**	**10 kGy**	**0 kGy**	**10 kGy**	**0 kGy**	**10 kGy**
Gallic acid	**P**	**4.01**	590.22	525.03	6.34	6.64	ND	ND	666.41	70.85	28.22	16.36
Protocatechuic acid	**P**	**7.18**	58.27	340.06	10.75	68.81	89.09	34.15	4.37	28.29	265.56	215.21
Gentisic acid	**P**	**11.01**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
***ρ*-hydroxybenzoic acid**	**P**	**11.41**	27.36	27.65	ND	ND	ND	ND	25.63	28.01	25.11	14.86
Catechin	**F**	**12.28**	190.30	197.31	2753.29	351.24	ND	ND	19.70	136.79	ND	ND
Chlorogenic acid	**P**	**14.19**	5.21	12.91	148.03	122.02	ND	66.94	4.02	8.72	ND	ND
Caffeic	**P**	**14.99**	ND	1.58	6.43	32.63	ND	ND	ND	ND	ND	ND
Syringic acid	**P**	**16.15**	ND	1.70	2.05	15.85	3.64	ND	ND	7.45	10.86	ND
Epicatechin	**F**	**16.77**	203.56	249.20	21.05	ND	ND	ND	211.38	212.54	32.64	42.75
Vanillic	**P**	**17.49**	3.61	3.61	34.42	220.88	ND	ND	7.13	ND	ND	ND
Ferulic acid	**P**	**22.41**	16.47	15.82	3.12	25.73	ND	ND	3.63	13.32	10.61	ND
Sinapic	**P**	**13.18**	1506.42	1374.35	4.95	1.93	ND	ND	1157.97	1145.75	16.31	3.58
Epicatechin gallate	**F**	**23.65**	1495.84	10.51	3.45	2.75	ND	ND	1152.08	ND	20.24	ND
Rutin	**F**	**25.41**	19.15	28.25	ND	14.82	ND	ND	19.28	21.01	ND	ND
***ρ*-coumaric**	**P**	**26.99**	15.68	ND	ND	ND	ND	ND	27.08	ND	ND	ND
Rosmarinic	**P**	**29.54**	ND	61.65	ND	3.10	ND	ND	ND	70.82	ND	ND
Apigenin-7-glucoside	**F**	**30.20**	51.46	ND	ND	ND	ND	ND	30.05	ND	4.51	ND
Cinnamic	**P**	**35.70**	679.60	160.29	3.52	3.70	142.09	ND	12.02	114.55	0.69	ND
Qurecetin	**F**	**36.82**	92.04	22.18	2.96	2.90	7.37	14.73	6.24	76.11	ND	ND
Apigenin	**F**	**41.04**	980.76	6.97	6.92	7.37	ND	ND	ND	38.45	ND	ND
Kaempferol	**F**	**41.83**	428.81	277.11	2.67	ND	ND	ND	ND	336.39	ND	ND
Chrysin	**F**	**53.54**	61.68	2689.56	62.49	39.80	3.34	9.93	2.83	5894.60	4.67	16.32

ND = Not detected, P = phenolic acids, F = Flavonoids sample, rising from 61.68 µg/g to 2689.56 µg/g. as well as, Protocatechuic acid levels increased across several regions post-irradiation, most notably in Yemen (from 58.27 to 340.06 µg/g) and China (from 4.37 to 28.29 µg/g). while, Rosmarinic acid not detected (ND) at 0 kGy in Yemen or China, it appeared at significant levels (61.65 µg/g and 70.82 µg/g, respectively) after 10 kGy treatment. The results indicate that irradiation does not merely "destroy" antioxidants but rather restructures the phenolic profile. While sensitive compounds like Epicatechin gallate and Apigenin are prone to degradation, others like Chrysin and Protocatechuic acid may become more concentrated, which correlates with the changes in antioxidant activity (IC_{50} values) observed in your previous data.

The therapeutic potential of propolis specifically its antioxidant, antifungal, and antibacterial properties—is intrinsically linked to a chemical composition that fluctuates based on seasonal variations and botanical sources. High concentrations of flavonoids, phenols, and organic acids exert antimicrobial effects by compromising the structural integrity of microbial cells, leading to the degradation of the cell wall and cytoplasm. Furthermore, these compounds interfere with critical metabolic pathways and inhibit cell division, reflecting mechanisms of action common to several conventional antibiotics (Kalogeropoulos *et al.,* 2009).

3.4. Antioxidant activity of propolis extract

3.4.1. DPPH Radical Scavenging Activity

The antioxidant capacity of various propolis samples was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a well-established method based on the hydrogen-donating ability of antioxidants (Nagai *et al.,* 2003). This study measured the reduction of the stable DPPH radical to evaluate the scavenging efficiency of both irradiated and non-irradiated propolis. Results were compared against tert-butylhydroquinone (TBHQ) as a reference standard, as detailed in Table 3.

In this study, the highest DPPH radical scavenging activity with minimum IC_{50} was observed for China (CH) propolis (IC_{50} = 60.48 µg/ml), followed by Yemen (YE) propolis (IC_{50} = 70.72 µg/ml), Egypt (EG) propolis (IC_{50} = 235.74 µg/ml), Ireland (IR) propolis (IC_{50} = 1362.95 µg/ml), South Korea (SK) propolis (IC_{50} = 1940.31 µg/ml). The results of DPPH radical scavenging activity of all samples are represented in Table 3. Regarding the most potent activity, (CH) and (YE) propolis exhibited the highest activity, with IC_{50} values for DPPH at 60.48 µg/ml and 70.72 µg/ml, respectively. While, (EG) and (IR) propolis showing significantly lower activity with IC_{50} value for (IR) (1362.95 µg/ml) than (EG) (235.74 µg/ml), that gave moderate potency. On the other hand, in the least potent activity, (SK) showed the weakest radical scavenging capacity, requiring 1940.31 µg/ml to reach 50% inhibition. These variations are likely due to the different floral sources and botanical environments available to the bees in each country, which dictate the phenolic and flavonoid composition of the propolis as well as, its aromatic compounds (Ahn *et al.,* 2007; Kumazawa *et al.,* 2004; Kurek-Górecka *et al.,* 2022).

The antioxidant potency of dihydroxy benzoic acid derivatives is maximized when hydroxyl groups are positioned at the third and fifth carbons. Among these, gallic acid exhibits exceptional radical-scavenging activity due to the presence of three hydroxyl groups at the 3, 4, and 5 positions (Kurek-Górecka *et al.,* 2013). Furthermore, recent studies highlight the dual antioxidant and anticancer properties of apigenin (Kowalczyk *et al.,* 2017). In this study, propolis from Yemen and China yielded the highest concentrations of cinnamic acid, epicatechin gallate, sinapic acid, chrysin, and gallic acid. The abundance of these compounds, alongside ferulic acid and apigenin, underpins the robust antioxidant capacity of these specific extracts (Kurek-Górecka, *et al*., 2022).

Furthermore, the application of 10 kGy irradiation gave promising effects (Matsuda *et al*., 2002; Heidarieh, *et al*., 2021) across the samples especially from selected countries. However, an improvement activity in (YE) propolis, The IC_{50} dropped from 70.72 to 53.86 µg/ml (DPPH), suggesting that irradiation may have liberated more bioactive compounds or caused the breakdown of larger molecules into more active antioxidants. On the contrary, reduction activity in China's propolis saw a significant *increase* in IC_{50} (from 60.48 to 153.91 µg/ml), indicating a loss of antioxidant power following irradiation. While, Egyptian propolis showed remarkable stability, with almost no change in activity (235.74 vs 234.92 µg/ml). A study by (Heidarieh *et al.,* 2021) demonstrated that escalating irradiation doses from 30 to 50 kGy resulted in a reduction of the antioxidant capacity in ethanolic propolis extracts.

These findings align with observations by Miguel *et al*. (2014) regarding thirteen propolis varieties from southern Portugal, where antioxidant activity varied significantly between 35.76% and 83.27%.

Such fluctuations in bioactive potential are primarily attributed to variations in total phenolic and flavonoid content across different geographical regions.

3.4.2. ABTS Radical Scavenging Assay

An additional *in vitro* approach used to quantify the antioxidant capacity of the propolis extracts was the ABTS assay. As shown in Table 3, the samples demonstrated significant scavenging activity against the ABTS radical cation. This spectrophotometric technique is a well-established method for evaluating the antioxidant potential of diverse compounds (Erel, 2004). The process involves generating the ABTS$^+$ radical through the oxidation of ABTS with potassium persulfate; the radical is subsequently reduced in the presence of hydrogen-donating antioxidants, resulting in a measurable decolorization.

As shown in Table 3, (CH) and (YE) showed the highest natural antioxidant activity, with IC$_{50}$ values of 53.97 µg/ml and 65.36 µg/ml, respectively. (EG) sat in the middle range (219.35 µg/ml), while (IR) and (SK) were significantly less effective, with SK requiring a very high concentration of 1818.26 µg/ml. All propolis samples collected from different country origin are significantly different (≤ 0.05), likely reflecting the diverse botanical sources available to bees in these specific climates.

The response to irradiation was not uniform across all propolis origins, suggesting that the chemical stability of propolis is region-dependent. In the cases of (YE) and (SK), irradiation actually improved antioxidant capacity. Most notably, SK's IC$_{50}$ dropped sharply from 1818.26 to 1065.62 µg/ml. This could be due to the radiolytic breakdown of complex polyphenols into smaller, more active phenolic monomers. Conversely, the propolis from China (CH) and Ireland (IR) saw a decrease in potency. China's IC$_{50}$ nearly tripled to 140.15 µg/ml, suggesting that its specific antioxidant compounds may be sensitive to gamma radiation. While, Egypt (EG) remained remarkably stable, with only a marginal change from 219.35 to 217.02 µg/ml. The synthetic antioxidant TBHQ serves as a benchmark with an IC$_{50}$ of 73.22 µg/ml. It is impressive to note that Yemen propolis (at both 0 and 10 kGy) and China propolis (at 0 kGy) outperformed the synthetic TBHQ. Even after irradiation, Yemen propolis (50.55 µg/ml) remained significantly more potent than the commercial standard.

In both the DPPH and ABTS assays, propolis samples from various global regions exhibited differing levels of radical-scavenging efficiency, with certain samples showing significantly lower activity than others. these variations suggest that antioxidant properties are influenced not only by geographical origin but also by the specific chemical profiles derived from diverse botanical sources. It is evident that phenolic compounds serve as the primary determinants of the antioxidant potential in propolis, a finding consistent with the research of Olczyk *et al.* (2017). Moreover, the trends between the two assays are highly consistent. Generally, the IC$_{50}$ values for ABTS are slightly lower than those for DPPH across all samples. This suggests the propolis extracts may have a slightly higher affinity for the ABTS radical cation or that the ABTS assay is more sensitive to the specific hydrophilic/lipophilic balance of the propolis compounds.

TBHQ, a standard synthetic antioxidant, has IC$_{50}$ values of 91.32 µg/ml (DPPH) and 73.22 µg/ml (ABTS). Notably, Yemen propolis (at 10 kGy) and China propolis (at 0 kGy) outperformed the synthetic standard, highlighting their potential as powerful natural alternatives to commercial antioxidants.

Both the ABTS and DPPH assays corroborated that propolis samples with the highest phenolic concentrations possess the most robust antioxidant capacity. Specifically, the ABTS radical cation inhibition test highlighted the superior activity of propolis from Yemen and China. This performance is likely attributed to their elevated levels of phenolic acids, such as gallic and ρ-coumaric acid. Additionally, the Yemen sample was characterized by a particularly high concentration of apigenin, further contributing to its bioactive potential (Kurek-Górecka *et al.,* 2022).

Table 3: Radical scavenging activity of propolis at different irradiation doses

Irradiation treatment	**Propolis Origin**	**IC50 (µg / ml)**	
		DPPH	**ABTS**
0 kGy	YE	70.72 ± 0.577^{h}	65.36 ± 1.00^{I}
	EG	235.74 ± 0.565^{e}	219.35 ± 0.577^{E}
	SK	1940.31 ± 0.588^{a}	1818.26 ± 0.577^{A}
	CH	60.48 ± 0.589^{i}	53.97 ± 0.566^{J}
	IR	1362.95 ± 0.565^{d}	1255.78 ± 0.571^{C}
10 kGy	YE	53.86 ± 0.583^{j}	50.55 ± 0.606^{K}
	EG	234.92 ± 0.589^{e}	217.02 ± 0.577^{F}
	SK	1751.04 ± 0.577^{c}	1065.62 ± 0.583^{D}
	CH	153.91 ± 0.571^{f}	140.15 ± 0.606^{G}
	IR	1822.2 ± 0.462^{b}	1626.82 ± 0.568^{B}
TBHQ		91.32 ± 0.574^{g}	73.22 ± 0.580^{H}

YE= Yemen, EG= Egypt, SK= South Korea, CH=China, IR= Ireland, DPPH= 2, 2-Diphenyl-1-picrylhydrazyl, ABTS= 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic, TBHQ= Tertiary butylhydroquinone, Calculated mean is for triplicate evaluations ± SD; means with different superscripts in the same column are regarded statistically different ($p \leq 0.05$).

4. Conclusion

This study evaluated both unirradiated and gamma-irradiated (10 kGy) propolis samples, all of which were found to possess significant concentrations of phenolic and flavonoid compounds with measurable antioxidant activity. Notably, Yemen and Chinese samples irradiated at 10 kGy exhibited a high abundance of phenolic constituents, particularly gallic acid. Consequently, gamma irradiation at a dose of 10 kGy emerged as a promising treatment for most of the international propolis samples investigated, enhancing or maintaining their bioactive potential.

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