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ORIGINAL PAPER

Biological activities of Egyptian grape and mulberry by-products and their potential use as natural sources of food additives and nutraceuticals foods

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Abstract
Interest in the biological role of bioactive compounds present in plant by-products has increased over the last few years. This study aimed to investigate the nutritive value and biological activities of Egyptian Grape leaves (GL), Grape seeds (GS) and Mulberry leaves (ML), as well as investigate the impact of γ-irradiation for improving the utilization of these plant by-products. The dose level 5.0 kGy showed highest the content of crude protein (24.42, 19.41 and 13.50 mg/100 g), as well as crude fiber (34.26 and 21.18 mg/100 g) for ML, GL and GS, respectively. Mulberry leaves has a highest content of protein and fiber at dose 5.0 kGy compared with GL and GS. The highest total phenolic content was found in GS (9.75 mg/g DW), followed by GL (7.32 mg/g DW) and the lowest in ML (5.97 mg/g DW). While ML had a higher total flavonoids content (5.61 mg/g DW) than GS (4.88 mg/g DW) and GL (2.86 mg/g DW). Total phenolic and flavonoid contents were significantly increased at 5.0 kGy. The highest level (83.25% and 80.24%) of scavenging activity (DPPH %) and inhibition activity of HCT 116 cells was recorded at 5.0 kGy by GS. All extracts irradiated at 5.0 kGy exhibited varying degrees of antibacterial activity against (Gram+ve and Gram–ve), the GS followed by GL then ML showed strong antibacterial activity with a diameter of inhibition zone of 26.2, 24.5 and 19.7 mm, against *L. monocytoganes*, respectively and 24.4, 21.4 and 17.2 against *S. typhimurium*, respectively. This study suggests that γ-irradiation is an effective technique to enhance the recovery of phenolics and flavonoids from GL, GS and ML. Also in current study, antioxidant, antibacterial and anticancer activity has been suggested to appear a clear positive relationship with the total phenolic material. This study has proved that the Egyptian GL, GS and ML are rich sources of valuable phytochemicals and nutrients that can serve as a potential source of nutraceuticals and multifunctional food additives (antimicrobial, antioxidant, and anticancer). Phenolic compounds recovered from GL, GS and ML may have a potential role in fighting the COVID-19.

Keywords Egyptian grape and Mulberry-by-products · Antimicrobial · Antioxidant · Anticancer · Food additives · Nutraceuticals foods

Introduction

Food loss and waste (FLW) along the food value chains in the Near East and North Africa (NENA) is estimated to reach 250 kg per individual and over USD 60 billion annually, based on a study prepared by the Food and Agriculture Organization [1]. For an area that relies heavily on global food imports, has limited capacity to increase food production, and faces shortages of water and arable land, the social, economic and environmental impacts are serious. Although food requirements are increasing, FLW is high in Egypt, especially for perishable products. Fruit and vegetable FLW is expected to 45–55% of production across the country annually. Therefore, attention must be paid to the fruits and vegetables by-products and to make maximum use of them in order to contribute to solve these problems [2]. The residues resulting from agro-industrial processes could lead to a major pollution problem are important sources of natural products with antimicrobial, antioxidant

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and anti-cancer properties [3, 4]. The large quantity of waste generated by agro-industries, in addition to the significant loss of useful resources, also poses serious management problems, both from an economic and environmental perspective [5].

Plant By-products contain oligosaccharides, phenolic compounds, proteins and other substances, which make them a rich source of natural compounds which can potentially be used in the food industry as sources of food additives at no additional cost of production and at reduced industrial costs [6, 7].

Artificial additives change human enzymes and lipids and have a possible carcinogenic effect [8]. Therefore, recent research has been carried out to replace chemical additives with natural additives that can be produced from agricultural wastes [9]. Growing attention to natural antioxidants, anticancer and antimicrobial compounds has led to plant by-product research as a source of these bioactive compounds [10]. Consumers today look forward not only to food items that are safe or nutritious but also to the need for natural, organic, or healthy foods. Increasing the consumer attention on functional foods has contributed to an increase in demand for natural foods [11]. For certain food additives already in use, the reuse of fruit waste or by-products can reflect a sustainable source or even produce new added-value ingredients with functional compounds and properties that will support the entire food system [12, 13].

The bioactive compounds produced by plant by-products are the source of phenolic bioactive compounds, and their pharmacological activities, such as anti-inflammatory, anti-allergic, antimicrobial, antiviral, anti-cancer, cardioprotective and vasodilatory activities, play an important role in human health [14, 15]. Grape and mulberry leaves are a major source of phenolic compounds. Recently, growing interests in phenolic compounds from grapes and mulberry leaves have focused on their biological activities linking to human health benefits, such as antioxidant, cardioprotective, anticancer, antiinflammation, antiaging and antimicrobial properties [16–18].

Due to the phenolic content of grape leaves and berries, it may have a possible effect on the Corona virus. Several studies and clinical trials increasingly proved the role of polyphenols in controlling various human pathogens, like SARS and MERS which are quite similar to COVID-19. As a result, polyphenols may potentially fight the Coronavirus by enhancing the host immune response against viral infections by different biological mechanisms. Thus, polyphenols ought to be considered as a potential source for designing new drugs that could be used effectively in the combat against COVID-19 and other rigorous diseases [19, 20].

Gamma irradiation as a phytosanitary treatment has been proven to be safe and effective in improving the hygienic quality of various foods and herbal materials in order to extend their shelf life [21]. Irradiation of any commodity up to an overall average dose of 10 kGy presents no toxicological hazard and introduces no special nutritional or microbiological problems World Health Organization (WHO) and Food and Agricultural Organization (FAO) [22]. Studies have shown that irradiation treatment can increase the contents of certain phytochemicals and enhance the biological value of some plants [23, 24].

Egypt is one of the largest countries in Africa and the Middle East producing grapes and berries, which results in the production of large quantities of secondary waste. Currently, grapes seeds and leaves and mulberry leaves as plant by-products have attracted scientific interest to confirm their production of nutrient and bioactive compounds and which can they are used in the food and pharmaceutical applications to the development of innovative added-value products [18, 19, 25]. The biological functions of these plants by-products are responsible for multiple benefits and that is through their integration into functional foods, nutraceuticals, nutritional therapy and cosmetics. Therefore, this study was conducted to identify the bioactive compounds of Egyptian grape seeds, grape leaves and mulberry leaves to estimate their nutritive values besides the biological activities (antioxidants, antibacterial and anticancer activities), as well as studying the effect of the role of γ-irradiation in improving these activities, so that they can be potentially used as a multifunctional food additive or as nutraceutical ingredients.

Materials and methods

Plant by-products

Grape leaves (GL), Grape seeds (GS) and Mulberry leaves (ML) samples were collected from the Agricultural Research Center (Cairo, Egypt). The obtained plant by-products were washed with distilled water, dried, and powdered with an electric grinder.

Irradiation treatment

Gamma irradiation of grape leaves, grape seeds and mulberry leaves was performed in the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt by using ^{60}Co (Indian Gamma cell) Ge-4000 A. Samples were placed in clean plastic bags and exposed to various dose levels; 0.0, 3.0 and 5.0 kGy at the time of the experiment the dose rate was 1.49 kGy/h.

Determination of nutritive values (crude protein, fiber and carbohydrates) of plant by-products

According to A.O. A. C, [26] methods were used to calculate crude protein and crude fiber. All of the above measurements were made in triplicate and expressed as g/100 g dry samples. The total carbohydrates were calculated as %. Carbohydrates % = 100 – % (protein, fat, ash and fibers).

Determination of biological activity

Preparation of plant by-products extracts

Unirradiated and irradiated (3.0 and 5.0 kGy) GL, GS and ML extracts were prepared by transferring 20 g from each powder sample to dark bottles and blended with 200 ml of methanol solvent and stored at room temperature. Extracts were filtered with filter paper after 24 h and residues were re-extracted with an equal solvent volume. The method was repeated after 48 h. The combined supernatant was evaporated to dryness using a rotary evaporator, then converts to a powder form and stored at 4 °C.

Determination of total phenolics

Folin Ciocalteu's method was used to assess the total content of phenols as defined by Singleton et al. [27]. An aliquot of methanol extract (300 µl) was mixed with 0.5 ml of Folin-Denis and 1.0 ml of concentrated Na_2CO_3 solution, total volume was adjusted to 10 ml by distilled water. The absorbance was evaluated at 765 nm against the blank after an hour. Mean of three readings has been used to calculate total phenol content; results had been displayed as gallic acid equivalent (GAE) milligram per g of samples dry weight.

Determination of total flavonoids

According to Jelena et al. [28], the total flavonoid content of plant by-product extracts were calculated using aluminium chloride. Methanolic extract sample (600 µl) was combined with 0.3 ml of 5% sodium nitrite. Five minutes later, 0.3 ml of 10% aluminum chloride was added, 2.0 ml of 1.0 M sodium hydroxide was added after 6 min, and the complete volume with distilled water was made up to 5.0 ml. The absorption of the mixture against the reagent blank was recorded at 510 nm. Results were displayed as the samples dry weight of mg quercetin equivalent (QUE)/g.

Phenolic HPLC analysis

Phenoilc compounds were determined according to Goupy et al. [25], In brief, one gram of each ethanol plant extract individually was mixed with 2 ml methanol for 5 min and then centrifuged. The supernatant was filtered using a 0.2 mm Millipore membrane filter and then 1 ml of filtrate was injected into HPLC Win Chrome Chromatography (GBC 1100) equipped. The phenolic acid standard (Sigma) was dissolved in a mobile phase, and then injected into HPLC. The phenolic compounds concentration was calculated from retention time and peak area. The data were analyzed using Win Chrome Chromatography Ver.13 software.

Based on the dose (5.0 kGy) that resulted in an increase in total phenolics and flavonoids content, all following biological tests were performed on irradiated samples at 5.0 kGy.

Antioxidant activity

DPPH radical-scavenging activity

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of sample extracts was determined according to the previous method reported by Park et al. [29], and was conducted at 515 nm. The following formula was used to calculate the radical scavenging activity.

$$\text{DPPH scavenging effect\%} = [(A0 - At/A0] \times 100$$

A0 = The absorbance of control reaction (containing reagents except the test compounds).

A1 = The absorbance in the presence of the tested extracts.

The IC_{50} is defined as the concentration of antioxidants necessary to decrease the initial DPPH concentration by 50%.

Antibacterial activity

Bacterial strains

Following bacterial food-borne pathogen was used in our study comprising both Gram-negative and Gram-positive species: *Listeria monocytoganes, Salmonella typhimurium, Pseudomonas. aeruginosa* and *Escherichia. coli.* They were obtained from Microbiology Resources Centre (MIRCEN), Faculty of Agriculture, Ain- Shams University, Cairo-Egypt. All bacteria strains were employed to test the antimicrobial activities of tested plant by-product extracts.

Disc diffusion method

National Committee for Clinical Laboratory Standards (NCCLS) [30] has proposed this procedure as a consensus standard. Plant by-product extracts were dissolved at a concentration of 10 mg/ml of Dimethyl sulfoxide (DMSO) (10%). Antibacterial tests were carried out by the disc-diffusion method using 100 µl 10^8 CFU/ml bacterial inoculum on Mueller Hinton agar (MHA, Torlak) in sterilized Petri dishes. The discs (6mm in diameter, Hi Media Laboratories Pvt. Limited) were impregnated with 40 µl of the solution extracts and placed on the inoculated agar. As negative and positive controls, two control discs containing DMSO (10%, in sterile water) and Gentamicin (10 µg/disc) were used, respectively. The plates were incubated at 37 °C for 24 h, and the experiments were carried out in duplicate.

Determination of minimal inhibitory concentration (MIC)

Minimal Inhibitory Concentration (MIC) was determined by the micro-broth dilution assays, according to Wiegand et al. [31]. The concentrations of the extracts used for MICs were ranged from 1600 to 6.25 µg/ml. In brief, the 100 µl culture (MHA) containing two-fold dilutions of extracts for each strain were loaded in sterile 96-well plates duplicate wells, 100 µl of tested bacteria inoculum (10^8 CFU/ml) was added to each test well. Positive and negative controls consisted of wells without plant extracts and without microorganisms, respectively. Plates were then incubated at 37 °C, for 24 h. Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration of sample tested at which microorganisms show no visible growth.

Anticancer activity

Cell culture

In a humidified CO_2 incubator with a 5% CO_2 atmosphere at 37 °C, human colorectal HCT 116 cancer cells were preserved in Roswell Park Memorial Institute (RPMI 1640 medium) supplemented with 10% (v/v) Fetal Bovine Serum (FBS), and 1% penicillin-streptomycin.

Cell viability assay

Using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, cell viability was calculated [32]. At a density of 1×10^4 cells/well, HCT 116 cells were seeded into 96 well plates. After an incubation time of 24 h, the cells were treated with different sample concentrations (100, 200 and 400, 800 µg/ml) for 24 h. Half mg/ml of MTT reagent (100 µl) was added to each well after incubation with samples, and the cells were incubated at 37 °C in a humidified incubator to allow the MTT to be metabolized. The sample absorbance was measured using a microplate reader at a wavelength of 540 nm.

$$\text{Inhibition percentage} = 100 - (\text{abs of treated cells}/\text{abs. of untreated cells}) \times 100$$

Statistical analysis

The Randomized Complete Block Design (RCBD) was used for the experiment using three replications per treatment. The data was analyzed using an ANOVA and Duncan's multiple range test Duncan, [33] were applied to compare the results of the experiment ($p \leq 0.05$).

Results and discussion

Crude protein, crude fiber, and carbohydrates

The nutritive values of GL, GS, and ML were provided in Fig. 1A, B and C. The results indicate that ML had higher total crude protein content (22.80 g/100 g DW) than GL and GS (17.41 and 13.50 g/100 g DW), respectively. The highest total crude fiber was recorded in ML and GL, (33.36 and 31.46 mg/100 g DW), respectively, it was the lowest in GS (18.38 mg/100 g DW), while the GL contained the highest total carbohydrates (49.92%) than ML (44.58%) and GS (43.53%). For all the analyzed parameters, there are significant differences among the three varieties. There are limited studies on the nutritional values of the plant's by-products understudy. The protein content found in this study is lower than those found by Yu et al. [34] in China Mulberry leaves of nineteen varieties (27.63–37.36 g/100 g DW), while, the content of fiber in this study was higher than that reported by Yu et al. [34] in China Mulberry leaves of 19 varieties (11.46–16.61 g/100 g DW). In this study, the protein and carbohydrate values of the grape seeds were greater than those observed by Hanaa et al. [35], they found that the protein and carbohydrate values in the grape seeds are 10.7 and 22.37 g/100 g, respectively. The differences could be due to the different cultivars, ecology, etc. The plant by-products in this study revealed that their content of crude protein, fiber and carbohydrates was equal to or greater than that of common leafy vegetables recorded in the Egypt Food Composition Tables, like cabbage, pakchoi and spinach.

This study proved that GL, GS, and ML are good resources of protein, fibers and carbohydrates. Fiber-rich by-products may be incorporated into food products as inexpensive, non-caloric bulking agents to replace flour, fat or sugar, and enhances water and oil retention to improve emulsion or oxidative stabilities [36], besides the health benefits, dietary fibers have several functional properties, like water-holding capacity, swelling capacity, raising viscosity, and gel formation, all of which are important in the formulation of specific foods [37].

Serious protein deficiencies and the high costs of animal protein sources have stimulated research on developing new sources of protein from unexploited sources of wastes and by-products [38]. The plant by-products which have high protein content can be contributed to protein's recommended

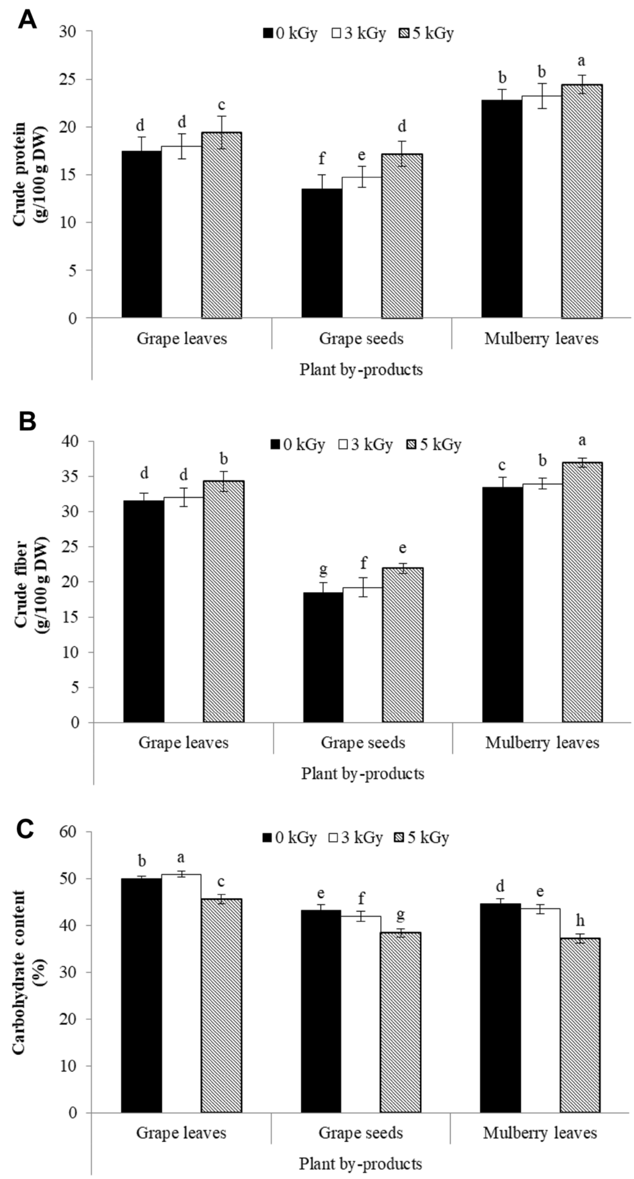

Fig. 1 **A** Effect of γ- irradiation on crude protein values of Egyptian grape leaves, grape seeds and mulberry leaves. **B** Effect of γ- irradiation on crude fiber values of Egyptian grape leaves, grape seeds and mulberry leaves. **C** Effect of γ- irradiation on carbohydrate % values of Egyptian grape leaves, grape seeds and mulberry leaves. Bars ± SD (n = 3). Different letters indicate statistically significant differences at $p \leq 0.05$

dietary allowance (RDA), which is 0.8 g/kg of body weight [39]. The current study indicated that, the utilization of GL, GS, and ML in the production of value-added protein adjuncts can help to reduce waste and hence contribute to environmental sustainability. Although plants are high in protein, carbohydrate fibers and other bioactive nutrients, due to the presence of high proportions of different antinutrients, their bioavailability and utilization are relatively low for humans or animals, a potential strategy for inactivating these compounds may be through the irradiation process. This study aims at the possibility of using gamma irradiation for enhancing nutrient availability of the tested plant by-products for their potential use in the food industry as food additives sources.

Figure 1A, B and C also, presents the results obtained by investigating the effect of gamma irradiation dose levels (3.0 and 5.0 kGy) on the nutritive values of GL, GS and ML. No significant changes in the protein and fiber content of the tested samples were detected by gamma irradiation at a dose of 3.0 kGy; the high dose of gamma irradiation (5.0 kGy) caused a significant increase in protein and fiber content (Fig. 1). The results obtained in our study suggest that the dose of 5.0 kGy may be a beneficial method for inhibiting antinutritional compounds in GL, GS and ML. As a result, it was enhancing protein, fiber and carbohydrates availability. The present finding corresponds to reports by Hamza et al. [40], gamma irradiation at levels of up to 10.0 kGy was efficient in inactivating antinutrients like protease inhibitors, lectin, phytic acid, non-starch polysaccharides and oligosaccharides without affecting the nutritional value of food/feed. The benefits of gamma irradiation on the nutritional properties of soy flour were achieved by reducing its antinutritional content and improving functional nutrients [41].

This study showed that dietary proteins, fiber, and carbs found in irradiated GL, GS and ML can be potentially employed to enhance the nutritional and sensory properties of food products. According to Iuga and Mironeasa [42], Grape by-products can be used as functional ingredients in the bread, pastry, and pasta industries without compromising product quality provided they are used in the right ratios. Grape seeds are a potent antioxidant and antimicrobial for the food industry [10]. Grape seeds and grape pomace extracts can be used in the production of active packaging materials so that they can be used in food preservation [43]. Grape by-products can be considered a valuable source of pectin with a wide range of applications in the food industry, e.g., texture/rheology additives and edible film ingredients [44]. Grape seed extracts (GSE) as potential nitrite and nitrate replacements in dry fermented Italian Cinta Senese sausage and Cinta Senese fermented sausage [45, 46].

Total phenolics and flavonoids contents

Polyphenols are bioactive plant secondary metabolites that are found naturally in commonly consumed plant foods. Figure 2A and B) shows the results for the total phenolic content (TPC) and the total flavonoid content (TFC) of GL, GS and ML. The content of phenolic and flavonoids were significantly different in the tested plant by-products. The highest total phenolic content was found in GS (9.75 mg/g DW), followed by GL (7.32 mg/g DW) and the lowest in ML (5.97 mg/g DW). While, ML had a higher total flavonoids

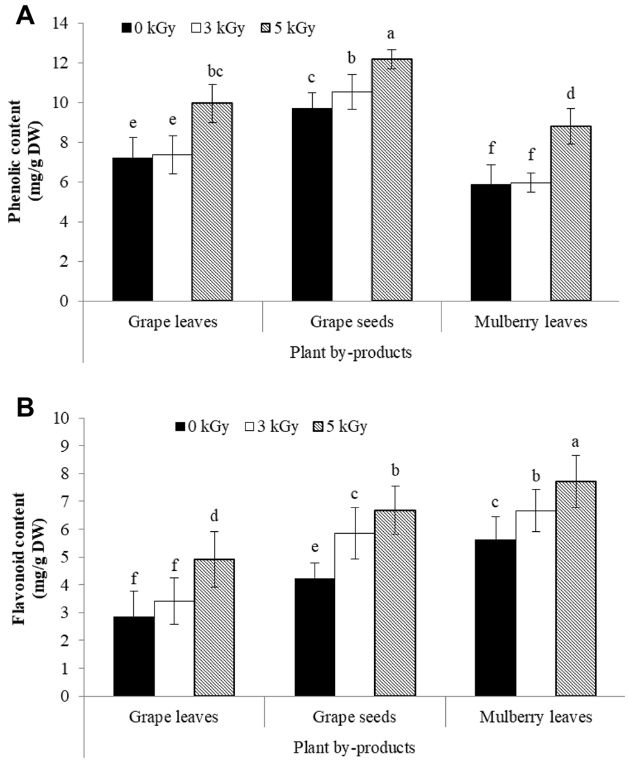

Fig. 2 **A** Effect of γ-irradiation on phenolic contents (mg/g DW) Egyptian grape leaves, grape seeds and mulberry leaves. Bars ± SD ($n = 3$). Different letters indicate statistically significant differences at $p \leq 0.05$. LSD interaction was 0.6289. **B** Effect of γ-irradiation on flavonoid contents (mg/g DW) Egyptian grape leaves, grape seeds and mulberry leaves. Bars ± SD ($n = 3$). Different letters indicate statistically significant differences at $p \leq 0.05$. LSD interaction was 0.6407

content (5.61 mg /g DW) than GS (4.88 mg /g DW) and GL (2.86 mg /g DW). There are significant variances in phenolic contents between the three tested plants. When comparing these results with those described in the literature, Katalinic et al. [47] reported that the total phenol content in leaves of red grape (*Vitis vinifera L.*) ranged from 9.4 to 23.4 g GAE/kg of dry leaves which was less than that of our sample. Also, Matloub [48] reported that the content of total phenols and flavonoids of grape leaves were 380, 94 µg GAE/mg and 107, 21 µg QEs/mg, respectively. Kupe et al. [49] reported that grape seeds are richer sources of total phenolic content than peel and pulp, and total phenolic content in grape seeds of nine varieties was found to be significant (245–207 mg GAE/100 g). The phenol and flavonoid values of these studies were lower than the values obtained by this study, While, high values were shown by Yu et al. [34] the total phenol (TP) content of six China mulberry leaves ranged from 30.4 equivalents (GAE) mg/g DW to 44.7 GAE mg/g DW. It is known that genotype, agronomic and environmental factors can affect on phytochemical production and playing an important role in the synthesis or accumulation of phenolic.

Also, Fig. 2A and B represented the effects of gamma irradiation on TPC and TFC of GL, GS and ML. The results revealed that the increase in TPC and TFC content was due to the doses applied (3 & 5 kGy). In all of the tested samples irradiated at 5.0 kGy, the maximum TPC value was observed as opposed to the lowest in the control. Total flavonoids content (TFC) increased significantly in reaction to gamma irradiation, and the increase followed a TPC-like trend. The maximum content of TFC was detected in irradiated samples at 5.0 kGy. This finding suggests that gamma irradiation is an effective technique to enhance the recovery of phenolics and flavonoids from GL, GS and ML. The results are agreed with Abdelaleema and Elbassionya [50], irradiation process of quinoa flour increased both total phenolic and flavonoid content. In the same concern, El-Beltagi et al. [51, 52] found that gamma irradiation dose levels (2.5–10.0 kGy) enhanced phenolic and flavonoids contents of celery seeds and dates fruits. The antioxidant activity of mulberry and persimmon leaves was enhanced by irradiation, especially at a dose of 1.5 kGy [53]. The current study proved that the dose of 5.0 kGy caused enhance in the polyphenols content of the studied extracts. Therefore, the phenolic compounds of the extracts were identified at this dose by HPLC, as well as the biological activity of these extracts was also studied at this dose and illustrated that (there is a positive relationship between phenolic content and biological activities).

The HPLC analysis of the phenolic compounds in the Egyptian grape leaves, grape seeds and mulberry leaves was identified in Table 1. It is clearly shown that methanolic extracts from tested samples in all varieties exhibited variable patterns of phenolic compounds. Gallic acids, Ferulic acids, Catechin, Chlorogenic acids, Caffeic acids and Coumaric acids were the main phenolic compounds present in the three tested extracts. Protocatechuic aldehyde and Querctin were only recorded in GS, while Syringealdehyde was only recorded in ML. The phenolic compounds of the irradiated extracts at 5.0 kGy were variable compared to the non-irradiated extracts; there was an increase in most compounds, a limited decrease in other compounds, and also the appearance and disappearance of some compounds. In all samples, these variations in profiling analyses may be due to exposure to gamma irradiation that alters/converts certain chemical compounds into each other. The increased phenolic content may also be related to the release of phenolic compounds from a glycosidic component and the breakdown of bigger phenolic compounds into smaller ones due to irradiation treatment [54]. Studies have shown that irradiation treatment can increase the contents of certain phytochemicals and enhance the biological value of some plants [23, 24].

Also, the present study revealed that polyphenols recovered from irradiated GL, GS and ML has the potential to provide a wide range of food products with numerous health benefits. Moreover, these by-products possess

Table 1 Phenolic composition of the Egyptian grape leaves, grape seeds and mulberry leaves

Phenolic compound	Content of phenolic compounds (µg/ml)					
	Grape leaves		Grape seeds		Mulberry leaves	
	0	5 kGy	0	5 kGy	0	5 kGy
Epicatechin	$1.32^{b} \pm 0.5$	$2.4^{a} \pm 0.8$	$9.12^{a} \pm 0.3$	$8.79^{b} \pm 0.4$	$ND^{b} \pm 0.5$	$1.35^{a} \pm 0.2$
Gallic acids	$2.98^{b} \pm 0.1$	$3.34^{a} \pm 0.6$	$3.1^{b} \pm 0.5$	$7.2^{a} \pm 0.5$	$4.7^{b} \pm 0.3$	$5.58^{a} \pm 0.6$
Catechin	$6.6^{b} \pm 0.3$	$7.94^{a} \pm 0.5$	$4.8^{b} \pm 0.8$	$6.63^{a} \pm 0.1$	$1.65^{b} \pm 0.9$	$3.82^{a} \pm 0.4$
Ferulic acids	$1.78^{b} \pm 0.6$	$4.53^{a} \pm 0.3$	$2.04^{b} \pm 0.1$	$3.52^{a} \pm 0.4$	$6.14^{b} \pm 0.2$	$8.21^{a} \pm 0.2$
Chlorogenic acids	$1.66^{a} \pm 0.4$	$1.24^{b} \pm 0.1$	$1.22^{b} \pm 0.4$	$2.4^{a} \pm 0.2$	$7.2^{b} \pm 0.4$	$8.31^{a} \pm 0.1$
Caffeic acids	$3.11^{b} \pm 0.3$	$3.72^{a} \pm 0.2$	$0.9^{b} \pm 0.7$	$1.62^{a} \pm 0.1$	$3.78^{a} \pm 0.8$	$4.15^{a} \pm 0.8$
Coumaric acids	$0.5^{b} \pm 0.1$	$1.15^{a} \pm 0.5$	$0.2^{a} \pm 0.2$	$0.3^{a} \pm 0.5$	$3.56^{a} \pm 0.2$	$3.41^{a} \pm 0.1$
Vanillic acids	$5.45^{a} \pm 0.5$	$5.89^{b} \pm 0.4$	$0.3^{a} \pm 0.4$	$ND^{b} \pm 0.9$	$ND^{b} \pm 0.1$	$0.7^{a} \pm 0.2$
Syringealdehyde	$ND^{b} \pm 0.7$	$0.8^{a} \pm 0.3$	$ND^{a} \pm 0.6$	$ND^{a} \pm 0.1$	$0.9^{a} \pm 0.6$	$1.2^{a} \pm 0.6$
Protocatechuic aldehyde	$ND^{a} \pm 0.9$	$ND^{a} \pm 0.5$	$0.3^{b} \pm 0.1$	$1.57^{a} \pm 0.6$	$ND^{a} \pm 0.5$	$ND^{a} \pm 0.5$
Quercetin	$ND^{a} \pm 0.1$	$ND^{a} \pm 0.8$	$0.4^{a} \pm 0.5$	$ND^{b} \pm 0.8$	$ND^{a} \pm 0.2$	$ND^{a} \pm 0.8$

All values are the mean of three replicates + SD. Mean values followed by different superscript are significantly different at $p > 0.05$ *ND* Not detected

multifunctional properties and could be used as natural antioxidants, antimicrobial and anticancer agents. In the study by Aquilani et al. [46], GSE polyphenol reduced the nitrite residue and suppressed the formation of n–nitrosamines in meat products. Mulberry leaves and grape-derived polyphenols have a wide range of biological activity (pleiotropic), as well as possible health-promoting effects [6, 55], they have anti-inflammatory, anti-amyloidogenic, anti-cholinesterase, anti-amnesic, hypolipidemic, anti-aging agents and immunomodulatory and they are dietary supplements that can help delay the neurocognitive deterioration that occurs with age and Alzheimer's disease (AD) [56, 57]. Mulberry leaves polyphenols reduced acetaldehyde toxicity and oxidative stress-induced apoptosis, suggesting that they could be used to treat alcohol-induced liver disease [58]. Several studies and clinical trials have established the importance of polyphenols in controlling numerous human illnesses, including SARS and MERS, which are related to COVID-19, by enhancing the host immune response to viral infections through multiple biological processes [20].

Antioxidant activity

The DPPH free-radical scavenging activity was used to assess the antioxidant activity of irradiated samples at 5.0 kGy (GL, GS and ML), BHT was used as standard and the results are listed in Table 2. Data showed that all tested extracts have a strong antioxidant activity; the effect was based on the concentration, with the same pattern as BHT. Grape seed extract showed higher antioxidant activity than GL and ML at the same concentrations ($p < 0.05$).

IC_{50} of GL, GS and ML were 156.86, 59.47 and 165.90 µg/ml, respectively. According to IC_{50} grape seed extract showed higher antioxidant activity. Higher antioxidant activity was observed in grape seeds (82.25%) as expected from the high contents of total phenolics. In

Table 2 Antioxidant capacity (% inhibition) and IC_{50} of irradiatedgrape leaves, grape seeds and mulberry leaves at 5.0 kGy

Concentration (µg/ml)	Scavenging activity (%)			
	Grape leaves	Grape seeds	Mulberry leaves	BHT
10	$10.39^{w} \pm 0.960$	$18.19^{t} \pm 0.980$	$5.35^{x} \pm 0.950$	$22.17^{r} \pm 0.980$
20	$12.93^{u} \pm 0.820$	$25.24^{q} \pm 0.995$	$12.25^{v} \pm 1.02$	$29.35^{n} \pm 0.950$
40	$27.24^{p} \pm 1.05$	$43.38^{k} \pm 0.955$	$19.80^{s} \pm 0.970$	$39.46^{L} \pm 0.875$
80	$31.57^{m} \pm 0.905$	$58.85^{f} \pm 0.875$	$28.96^{o} \pm 0.995$	$58.15^{h} \pm 0.970$
160	$51.41^{i} \pm 0.885$	$72.91^{d} \pm 0.975$	$48.22^{j} \pm 1.01$	$91.42^{b} \pm 0.895$
320	$69.61^{e} \pm 0.765$	$83.25^{c} \pm 1.03$	$58.34^{g} \pm 0.970$	$96.22^{a} \pm 1.04$
IC_{50} µg/ml	126.70	54.22	185.90	22.32
LSD AxB	0.1162			

Values are mean ± SD (*n=3*). Different letters indicate statistically significant differences at $p \leq 0.05$

general, as the total phenolics in the seed increased, the antioxidant activities also increased. These results were in agreement with those reported by Guaita and Bosso [59], grape seeds have higher antioxidant activity than grape peels, indicating a link between tannin concentration and antioxidant activity. The hydroacetonic grape seeds extract showed significantly superior content in total phenolic and flavonoid accompanied by the highest DPPH scavenging capability than other tested extracts [48].

The present findings show that irradiated GL, GS and Ml extracts could be alternatives to synthetic additives for preventing lipid oxidation in fresh or functional food products and prevent economic loss for the food processing industry. The addition of grape seeds, grape leaves and mulberry leaves extracts to minced meats prevented rancidity by decreasing the oxidation value (PV and TBRS) without changing the color or odor of the meat, therefore, they can be used as natural antioxidants and as an alternative to chemical antioxidants, e.g. BHT and BHA [60, 61]. The use of grape seeds extracts (GSE) in foods, as a natural antioxidant, could inhibit the production of polycyclic aromatic hydrocarbons (PAHs) and acrylamide, 5-hydroxymethylfurfural (HMF) and other substances that are harmful substances to the human body [62].

In this study, antioxidant activity has been suggested to appear to have a clear positive relationship with the total phenolic material. The increase in the antioxidant activity of irradiated GL, GS and ML at 5.0 kGy can be due to increases in the content of free phenols and flavonoids which, compared to complicated glycosides, exhibit stronger antioxidant properties. The increase in free isoflavone content may be attributed to irradiation-induced conversion of glycosides to aglycones [63]. Research has shown that gamma irradiation improves plant phenolic compounds by stimulating the main enzymes in the phenylpropanoid pathway [64]. In the study by Abdel-Khalek and Younies [24], using 4.0 kGy dose level led to an improvement in the content of total phenols, flavonoids and antioxidants in both Artichoke leave and stem wastes, this may be evidence of the biological values. Farkhad and Hosseini [65], the lower-dose soybean irradiation can possibly increase the total content of phenolics and flavonoids, together with free daidzein and genistein, associated with enhanced antioxidant capacity.

Antibacterial activity

Estimation the antibacterial activity of the methanolic extract of irradiated GL, GS and ML at 5.0 kGy against some bacterial strains was initially determined by the disc diffusion method. These bacterial strains are Gram-positive (*L. monocytoganes* and *S. typhimurium*) and Gram-negative (*P. Aeruginosa* and *E. coli*) as organisms that are commonly found in foodborne diseases. Table 3 displays the effects of the diameters of the inhibition zones. It is possible to note that all extracts exhibited varying degrees of antibacterial activity against all bacterial strains tested, whereas GS extract was more effective in inhibiting at the same tested concentration. The GS followed by GL then ML showed strong antibacterial activity with a diameter of inhibition zone of 26.2, 24.5 and 19.7 mm, against *L. monocytoganes*, respectively as well as 24.4, 21.4 and 17.2 against *S. typhimurium*, respectively. Also, the extracts of GS and GL showed a relatively moderate activity mainly against *P. aeruginosa* (16.7 and 14.7mm) and *E. coli* (18.1 and 16.8mm), respectively, while the extract of ML showed a low activity at the same microorganisms [*E. coli* (11.3 mm) and *P. aeruginosa* (12.5 mm)]. The results are agreed with Ranjitha et al. [66], The GSE was more effective against *S. aureus* (18 mm) followed by *K. pneumonia* (13 mm) and *E. coli* (11 mm) i.e., susceptibility to GSE was high in case of Gram positive bacterium when compared to Gram negative bacteria. In the study by Silvaa et al. [67], Grape seeds extract presented higher antimicrobial activity than peels due to their higher contents of catechin, epicatechin, and trans-resveratrol additionally, Grape seeds extracts has been

Table 3 Antibacterial activity of irradiated grape leaves, grape seeds and mulberry leaves at 5.0 kGy against some food pathogenic bacteria

Food pathogenic bacteria	Halo diameter (mm)		
	Grape leaves	Grape seeds	Mulberry leaves
L. monocytogane	$24.33^{ab} \pm 0.577$	$26.33^{a} \pm 0.577$	$19.67^{cd} \pm 3.06$
S. typhimurium	$21.67^{bc} \pm 2.08$	$24.33^{ab} \pm 0.577$	$17.33^{def} \pm 2.52$
P. aeruginosa	$14.67^{fg} \pm 2.08$	$16.67^{ef} \pm 3.06$	$11.33^{h} \pm 2.52$
E. col	$16.67^{ef} \pm 2.08$	$18.33^{de} \pm 0.577$	$12.67^{gh} \pm 4.04$
LSD A×B	2.781		

Values are mean ± SD (*n=3*). Different letters indicate statistically significant differences at $p \leq 0.05$

DMSO (negative control) was not effective in inhibiting test bacteria, inhibitory activity of reference antibiotic (positive control) was higher than that of plant extracts shown to have antibacterial activities against both Gram-negative and Gram-positive bacteria, and it's have a high concentration of flavonoids, which may help to reduce biofilm formation [68].

These differences in the antibacterial activity in this study, as well as in the other studies in the literature depend on the tested microorganism and the composition in the phenolic compounds of the extracts and on the existence of a synergetic effect between the different polyphenolic compounds with an antiradical role and antimicrobial activity [65]. The present study suggests that gamma irradiation is an effective technique to enhance the recovery of phenolics and flavonoids from GL, GS and ML, and the antibacterial activity has a direct relationship with a total phenolic material. Increasing the antibacterial activity was observed in grape seeds as expected from the high contents of total phenolics. In the same concern, gamma rays at dose 10 kGy have a significant potential to stimulate antibiofilm and antibacterial potency of EOs recovered from clove buds due to an increase in phenolic and flavonoid contents [23].

Minimum inhibitory concentration values (MIC)

By calculating the minimum inhibitory concentration, the efficacy of the plant by-product extracts on the tested bacterial strains was determined (MICs) (Fig. 3). The MICs values obtained from the extract of GS were 25 and 50 µg/ml against *L. monocytoganes* and S. *Typhimurium*, 100 and 200 µg/ml against *P. aeruginosa* and *E. coli*, respectively. Grape leaves extract appear MICs of 50 and 100 µg/ml against *L. monocytoganes* and *S. typhimurium*, 200 and 400 µg/ml against *P. aeruginosa* and *E. coli*, respectively. The minimum inhibitory concentration against *L. monocytoganes* and *S. typhimurium* were 100 and 200 µg/ml and 400 µg/ml against both *P. aeruginosa* and *E. coli* by ML. It was observed in the present study that Gram-positive bacteria (*L. monocytoganes* and *S. typhimurium*) was the most sensitive compared to Gram-negative bacteria (*P. aeruginosa* and *E. coli*) to all tested plant extracts, it is possible to relate the variation in sensitivity among Gram positive and Gram negative bacteria to the morphological differences between these microbes, particularly the variations in cell wall permeability [67]. Similar result was observed in the study of Peixoto et al. [69], MICs against Gram positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*,) were lower than the Gram negative bacteria (*Klebsiella pneumoniae*) by grape seeds extract.

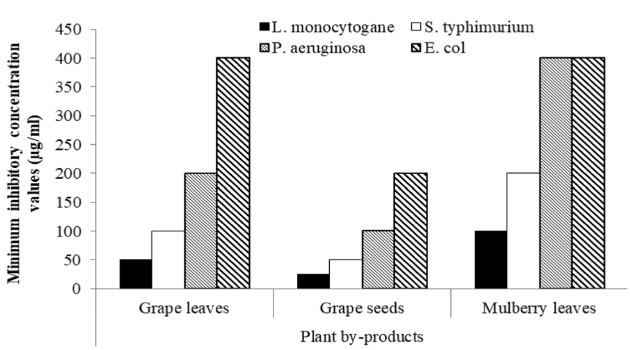

Fig. 3 Minimum inhibitory concentration values (MIC) of irradiated grape leaves, grape seeds and mulberry leaves at 5.0 kGy against some food pathogenic bacteria

The relation between the inhibition zone and the MIC values can be significantly affected by the characteristics of crude extracts, which are a mixture of bioactive constituents that can affect the active components' diffusion capacity and the various levels of intrinsic tolerance, total phenols play an important role in microbial growth, with the number of hydroxyl groups regulating antibacterial action by forming hydrogen bonds with bacteria's membrane protein, resulting in permeability alterations and cell disintegration [70].

Synthetic food additives generate a negative perception of consumers. In the present study, the methanolic extracts of irradiated GL, GS and ML can potentially use alternative preservative agents for controlling microbial pathogen injuries in the food industry. In broth and shrimp, Grape seeds extract- nisin suppressed *Listeria monocytogenes* growth by inhibiting the tricarboxylic acid (TCA) cycle, amino acid biosynthesis [71]. Grape seeds extract was used in food packaging film, its antibacterial effect was significantly enhanced and the shelf life of food was extended [72].

Anticancer activity

After breast and lung cancer, colorectal cancer (CRC) is the third most common malignant neoplasm in the world, although it is more common in underdeveloped countries. Fruits and vegetables are assumed as the main dietary factors supporting cancer prevention [73]. This study examined the cytotoxic activity of the methanolic extract of irradiated GL, GS and ML at 5.0 kGy against colorectal carcinoma cell lines (HCT116 cells). As shown in Table 4, in a concentrate-dependent manner, all studied samples significantly inhibited colon cancer HCT 116 cell growth *in vitro* (cell proliferation is expressed as the mean percentages of viable cells relative to untreated cells).

IC_{50} of GL, GS and ML were 232.24, 168.88 and 480.18 µg/ml, respectively. The highest inhibition activity of HCT 116 cells was seen in GS, compared with GL and ML. In another study, the cytotoxic activity of the GSE was observed against skin cancer cell lines A4321 using MTT assay, the IC_{50} value of the GSE against A431 skin cancer cell line was 480 µg/ml [74]. Grapes and Grape-based products are one type of dietary supplement that has been found to have cancer-fighting properties [75]. Grape stem

Table 4 Percentage inhibition and IC_{50} values of irradiated grape leaves, grape seeds and mulberry leaves at 5.0 kGy on HCT116 cell line

Concentration (µg/ml)	Inhibitory rate (%)			
	Grape leaves	Grape seeds	Mulberry leaves	Ciplatin
100	$12.15^{o} \pm 0.955$	$21.19^{m} \pm 0.970$	$6.24^{p} \pm 0.990$	$33.21^{j} \pm 1.02$
200	$29.58^{k} \pm 0.945$	$37.19^{i} \pm 0.945$	$16.26^{n} \pm 1.06$	$42.43^{h} \pm 0.890$
400	$46.25^{g} \pm 1.02$	$61.92^{d} \pm 0.965$	$23.23^{L} \pm 1.06$	$74.52^{c} \pm 0.955$
800	$61.25^{e} \pm 1.06$	$80.24^{b} \pm 0.965$	$51.27^{f} \pm 0.945$	$96.37^{a} \pm 0.910$
IC_{50} µg/ml	232.24	168.88	480.18	85.90
LSD AxB	0.09133			

Values are mean ± SD (*n=3*). Different letters indicate statistically significant differences at $p \leq 0.05$

extracts reduced cancer cell (Caco-2, MCF-7, and MDA-MB-231) proliferation, triggering death through apoptosis via mitochondrial potential alteration and a decrease in the antioxidant enzyme TrxR1, resulting in an increase in cellular lethality [76].

The current study demonstrates that gamma irradiation at 5.0 kGy is a good way to get more phenolics and flavonoids out of GL, GS, and ML, and that anticancer activity is proportional to total phenolic material, the results are agreed with El-Beltagi et al. [51], the best IC_{50} of oils extracted from celery seeds irradiated at 5.0 kGy were 145 and 124 µg/ml against Lung cancer cell line A549 and MCF-7 Breast cell lines, respectively. Irradiated thyme at 2.0 and 5.0 kGy showed lower toxicity than the control sample (0.0 kGy) on cell lines MCF-7, HeLa and HepG2, whereas irradiated thyme at 10.0 kGy increased their cytotoxicity in the assayed tumor cell lines compared with samples submitted to 2.0 and 5.0 kGy [77].

The recent study indicates that tested plant-by products are regarded as an especially valuable source of powerful anti-proliferative and cytotoxic substances. In various cancer cell lines, many plant extracts and natural products, particularly phenolics with high antioxidant activity, have shown cytotoxic effects [78], several experiments have shown that flavonoids have high cytotoxic and anti-cancer activity, and they cytotoxic activities include cell proliferation inhibition, protein kinase activity inhibition, and apoptosis induction [79].

Conclusions

Recycling irradiated GL, GS and ML can contribute to solving some problems, including the problem of environmental pollution, economic losses and the production of materials that have important nutritional and health benefits. This study proved that the methanolic extracts of irradiated GL, GS and ML at 5 kGy contain many biologically active compounds, mainly polyphenolics, which have been revealed antibacterial, antioxidant, anticancer properties, also, they contain a high degree of crude protein, fiber and carbohydrates, this, in turn, is important in human nutrition and health. These natural by-products may also be considered as nutraceutical products or supplements, allowing for the development of food products with enhanced nutritional value, therapeutic benefits, longer shelf-life and microbial safety. In addition, these by-products could be used by the pharmaceutical industry as auxiliaries in disease treatment. Polyphenolic compounds present in GL, GS and ML might have potential effects against COVID-19 through enhancing the body immunity facing coronaviruses. These all benefits will open up scope for future utilization of fruit and vegetable by-products for therapeutic and nutraceutical purposes in a developing country, especially in Egypt.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent N/A.

Research involving human participants and/or animals No humans or animals were used in this work.

References

1. UN Food and Agricultural Organization (FAO), *Global food losses and food waste—extent, causes, and prevention* (FAO, Rome, 2019)
2. M.F. Bellemare, M. Çakir, H.H. Peterson, L. Novak, J. Rudi, On the measurement of food waste. Am. J. Agric Econ. **99**, 1148–1158 (2017). https://doi.org/10.1093/ajae/aax034
3. M. Jablonský, A. Škulcová, A. Malvis, J. Šima, Extraction of value-added components from food industry based and agro-forest biowastes by deep eutectic solvents. J. Biotechnol. **282**, 46–66 (2018). https://doi.org/10.1016/j.jbiotec.2018.06.349
4. M. Herrero, E. Ibañez, Green extraction processes, biorefineries and sustainability: recovery of high added-value products from natural sources. J. Supercrit. Fluids **134**, 252–259 (2018)

5. N. Mirabella, V. Castellani, S. Sala, Current options for the valorization of food manufacturing waste: a review. J. Clean. Prod. **65**, 28–41 (2014). https://doi.org/10.1016/j.jclepro.2013.10.051
6. R.F.M. Silva, L. Pogacnik, Polyphenols from food and natural products: neuroprotection and safety. Antioxidants **9**(1), 61 (2020). https://doi.org/10.3390/antiox9010061
7. P. Ferreira-Santos, E. Zanuso, Z. Genisheva, C.M.R. Rocha, J.A. Teixeira, Green and sustainable valorization of bioactive phenolic compounds from pinus by-products. Molecules **25**(12), 2931 (2020). https://doi.org/10.3390/molecules25122931
8. M. Carocho, M.F. Barreiro, P. Morales, I.C. Ferreira, P.M. Gomez, Adding molecules to food, pros and cons: a review on synthetic and natural food additives. Compr. Rev. Food Sci. Food Saf. **13**, 377–399 (2014). https://doi.org/10.1111/1541-4337.12065
9. W. Nie, K. Cai, Y. Li, G. Hu, X. Wang, U. Wang, C. Chen, Application of grape seed extract lead to a higer formation of polycyclic aromatic hydrocarbons in roasted pork sausage at the end storage. J. Food Process Preserv. **44**, 1–9 (2020). https://doi.org/10.1111/jfpp.14532
10. T.M. Pfukwa, O.A. Fawole, M. Manley, A. Pieter, P.A. Gouws, U.L. Opara, C. Mapiye, Food preservative capabilities of grape (*Vitis vinifera*) and clementine mandarin (*Citrus reticulata*) by-products extracts in South Africa. Sustainability **11**, 1746 (2019). https://doi.org/10.3390/su11061746
11. M. Faustino, M. Veiga, P. Sousa, E.M. Costa, S. Silva, M. Pintado, Agro-food byproducts as a new source of natural food additives. Molecules **24**(6), 1056 (2019). https://doi.org/10.3390/molecules24061056
12. I. Mourtzinos, A. Goula, Polyphenols in agricultural byproducts and food waste, in *Polyphen Plants*. ed. by R.R. Watson (Academic Press, London, 2019), pp. 23–44. https://doi.org/10.3389/fnut.2020.00060
13. L.D. Shirahigue, S. Regina, C. Antonini, Agro-industrial wastes as sources of bioactive compounds for food and fermentation industries. Food Technol. **50**(4), 1–17 (2020). https://doi.org/10.1590/0103-8478cr20190857
14. A.C. Camargo, A.R. Schwember, R. Parada, S. Garcia, M.R. Júnior, M. Franchin, M.A. Arce, F. Shahidi, Opinion on the hurdles and potential health benefits in value-added use of plant food processing by-products as sources of phenolic compounds. Int. J. Mol. Sci. **19**(11), 1–47 (2018). https://doi.org/10.3390/ijms19113498
15. S. Dhiman, V. Kumar, C.M. Mehta, Y. Gat, S. Kaur, Bioactive compounds, health benefits and utilisation of Morus spp.—a comprehensive review. J. Hortic. Sci. Biotechnol. **95**(1), 8–18 (2020)
16. A. Gryn-Rynko, G. Bazylak, D. Olszewska-Slonina, New potential phytotherapeutics obtained from white mulberry (*Morus alba* L.) leaves. Biomed. Pharmacother. **84**, 628–636 (2016). https://doi.org/10.1016/j.biopha.2016.09.081
17. X. He, J. Fang, Y. Ruan, X. Wang, Y. Sun, N. Wu, Structures, bioactivities and future prospective of polysaccharides from *Morus alba* (white mulberry): a review. Food Chem. **245**, 899–910 (2018)
18. E.W.C. Chan, S.K. Wong, J. Tangah, T. Inoue, H.T. Chan, Phenolic constituents and anticancer properties of *Morus alba* (white mulberry) leaves. J. Integr. Med. **18**, 189–195 (2020). https://doi.org/10.1016/j.joim.2020.02.006
19. Y.A. Attia, M.A. Alagawany, M.R. Farag, F.M. Alkhatib, F. Asmaa, A.F. Khafaga, E. Abdel-Moneim, K.A. Asiry, M. Noura, N.M. Mesalam, E. Manal, A. Shafi, M.E. Mohammed, M.A. Al-Harthi, E. Mohamed, Abd El-Hack, phytogenic products and phytochemicals as a candidate strategy to improve tolerance to coronavirus. Front. Vet. Sci. **7**, 1–18 (2020). https://doi.org/10.3389/fvets.2020.573159
20. A. Khalil, D. Tazeddinova, The upshot of polyphenolic compounds on immunity amid COVID–19 pandemic and other emerging communicable diseases: an appraisal. Nat. Prod. Bioprospecting. **10**, 411–429 (2020). https://doi.org/10.1007/s13659-020-00271-z
21. S. Douar-Latreche, O. Benchabane, N. Sahraoui, Effect of gamma irradiation on the chemical composition and antioxidant activity of *Thymus algeriensis* extracts. J. Essent. Oil-bearing Plants **21**(2), 449–461 (2018). https://doi.org/10.1080/0972060X.2017.1421869
22. P. Loaharanu, M. Ahmed, Advantages and disadvantages of the use of irradiation for food preservation. J. Agric. Environ. Ethics. **4**(1), 14–30 (1991). https://doi.org/10.1007/BF02229144
23. G.N. Hanady, M. Reham, E.Z. Marina, A.A. Amina, Evaluation of chemical composition, antioxidant, antibiofilm and antibacterial potency of essential oil extracted from gamma irradiated clove (*Eugenia caryophyllata*) buds. J. Food Meas. Charact. (2021). https://doi.org/10.1007/s11694-021-01196-y
24. H.H. Abdel-Khalek, B.M. Younies, The nutritive value and biological activity of artichoke wastes as food supplements or adjunctive agents for chemotherapy and radiotherapy. Int. J. Agric Biol. **26**, 527–535 (2021). https://doi.org/10.17957/IJAB/15.1864
25. J. Kobus-Cisowska, M.D. Marcin Dziedzi ´nski, D. Szymanowska, O. Szczepaniak, S. Byczkiewicz, A. Telichowska, P. Szulc, The effects of *Morus alba* L. fortification on the quality, functional properties and sensory attributes of bread stored under refrigerated conditions. Sustainability **12**, 1–16 (2020). https://doi.org/10.3390/su12166691
26. A.O. A. C, *Association official analytical chemists. Official methods of analysis 17th ed.* (Washington, DC., U. S. A, 2010)
27. V. Singleton, R. Orthofer, R.M. Lamuela-Raventos, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods Enzymol. **299**, 152–178 (1999). https://doi.org/10.1016/S0076-6879(99)99017-1
28. S. Jelena Matejić, A.M. Džamić, T. Mihajilov-Krstev, V.N. Ranđelović, Z. Krivošej, P.D. Marin, Total phenolic content, flavonoid concentration, antioxidant and antimicrobial activity of methanol extracts from three Seseli L. taxa. Cent. Eur. J. Biol. 7(6), 1116–1122 (2012). https://doi.org/10.2478/s11535-012-0094-4
29. H.R. Park, E. Park, A.R. Rim, K.I. Jeon, J.H. Hwang, Lee antioxidant activity of extracts from *Acanthopanax senticosus*. Afr. J. Biotechnol. **5**(23), 2388–2396 (2006)
30. National Committee for Clinical Laboratory Standards (NCCLS), *Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A6. Wayne* (National Committee for Clinical Laboratory Standards, Pa, 2001)
31. I. Wiegand, K. Hilpert, R.E.W. Hancock, Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat. Protoc. **3**(2), 163–175 (2008)
32. T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods. **65**, 55–63 (1983). https://doi.org/10.1016/0022-1759(83)90303-4
33. D.B. Duncan, Multiple range and multiple ’F’ tests. Biometrics **11**(1), 1–42 (1955). https://doi.org/10.2307/3001478
34. G. Yu, Y. Fang, J. Liu, Practice and exploration of mulberry leaf edible development and utilization (in Chinese). Bul. Sericult. **49**, 49–50 (2018). https://doi.org/10.3969/j.issn.0258-4069.2018.03.016
35. M.A. Hanaa, M.A. Elshafie, H.A. Ismail, M.E. Mahmoud, Chemical studies and phytochemical screening of grape seeds (*Vitis Vinifera* l.). Minia J. Agric. Res. Develop. **35**(2), 313–325 (2015)
36. M. Elleuch, D. Bedigian, O. Roiseux, S. Besbes, C. Christophe Blecker, H. Attia, Dietary fiber and fiber-rich by-products of food processing: characterization, technological functionality and commercial applications: a review. Food Chem. **2**, 411–421 (2011). https://doi.org/10.1016/j.foodchem.2010.06.077

37. C.M. Ajila, M. Aalami, K. Leelavathi, U.J.S. Prasada Rao, Mango peel powder: a potential source of antioxidant and dietary fiber in macaroni preparations. Innov. Food Sci. Emerging Technol. **11**, 219–224 (2010). https://doi.org/10.1016/j.ifset.2009.10.004
38. S. Perumal, B. Klaus, P.S.M. Harinder, Chemical composition, protein fractionation, essential amino acid potential and antimetabolic constituents of an unconventional legume, gila bean (*Entada phaseoloides* Merrill) seed kernel. J. Sci. Food Agric. **82**(2), 192–202 (2001). https://doi.org/10.1002/jsfa.1025
39. M. Lonnie, E. Hooker, J.M. Brunstrom, Protein for life: review of optimal protein intake, sustainable dietary sources and the effect on appetite in ageing adults. Nutrients **10**, 360 (2018). https://doi.org/10.3390/nu10030360
40. R.G. Hamza, S. Afifi, A.B. Abdel-Ghaffar, I.H. Borai, Effect of gamma-irradiation or/and extrusion on the nutritional value of soy flour. Biochem. Anal. Biochem. **1**, 1–6 (2012). https://doi.org/10.4172/2161-1009.1000118
41. P.M. Devi, M.R. Sahoo, A. Kuna, P. Deb, M.D. Asgupta, N. Prakash, Effect of gamma irradiation on nutritional properties and antinutrient contents of *Citrus jambhiri* Lush. Fruits. J. Pharmacogn. Phytochem. **7**(4), 2833–2836 (2018)
42. M. Iuga, S. Mironeasa, Potential of grape byproducts as functional ingredients in baked goods and pasta. Compr. Rev. Food Sci. Food Saf. **19**, 2473–2505 (2020). https://doi.org/10.1111/1541-4337.12597
43. C.C. Bastante, P. Arjona, M.T. Ponace, L.C. Cardoso, Application of a natural antioxidant from grape pomace extract in the development of bioactive jute fibers for food packaging. Antioxidant **10**(2), 216 (2021). https://doi.org/10.3390/antiox10020216
44. M. Spinei, M. Oroian, The potential of grape pomace varieties as a dietary source of pectic substances. Foods **10**(4), 867 (2021)
45. F. Pini, C. Aquilani, L. Giovannetti, C. Viti, C. Pugliese, Characterization of the microbial community composition in Italian Cinta Senese sausages dry-fermented with natural extracts as alternatives to sodium nitrite. Food Microbiol. **89**, 103417 (2020). https://doi.org/10.1016/j.fm.2020.103417
46. C. Aquilani, F. Sirtori, M. Flores, R. Bozzi, B. Lebret, C. Pugliese, Effect of natural antioxidants from grape seed and chestnut in combination with hydroxytyrosol, as sodium nitrite substitutes in cinta senese dry-fermented sausages. Meat Sci. **145**, 389–398 (2018). https://doi.org/10.1016/j.meatsci.2018.07.019
47. V. Katalinic, S.S. Mozina, I. Generalic, D. Danijela Skroza, I. Ljubenkov, A. Anja, Klancnik, Phenolic profile, antioxidant capacity, and antimicrobial activity of leaf extracts from six *Vitis vinifera* L. varieties. Int. J. Food Prop. **16**(1), 45–60 (2013). https://doi.org/10.1080/10942912.2010.526274
48. A.A. Matloub, Optimization of polyphenol extraction from *Vitis vinifera* L. leaves, antioxidant activity and its correlation with amelioration effect on AlCl3-induced Alzheimer's disease. Arch. Pharm. Sci. Ain Shams Univ. **2**(2), 97–110 (2018). https://doi.org/10.21608/aps.2018.18750
49. M. Kupe, N. Karatas, M.S. Unal, S. Ercisli, M. Baron, J. Sochor, Phenolic composition and antioxidant activity of peel, pulp and seed extracts of different clones of the turkish grape cultivar. Karaerik Plants **10**, 2154 (2021). https://doi.org/10.3390/plants10102154
50. M.A. Abdelaleema, K.R.A. Elbassionya, Evaluation of phytochemicals and antioxidant activity of gamma irradiated quinoa(*Chenopodium quinoa*). Braz. J. Biol. **81**(3), 806–813 (2020). https://doi.org/10.1590/1519-6984.232270
51. H.S. EL-Beltagi, F. Dhawi, A.A. Aly, Chemical compositions and biological activities of the essential oils from gamma irradiated celery (*Apium graveolens* L.) seeds. Notulae Botanicae Horti Agrobotanici Cluj-Napoca **48**(4), 2114–2133 (2020). https://doi.org/10.15835/48412115
52. H.S. EL-Beltagi, A.A. Aly, W. El-Desouky, Effect of gamma irradiation on some biochemical properties, antioxidant and antimicrobial activities of Sakouti and Bondoky dry dates fruits genotypes. J. Radiat. Appl. Sci. **12**(1), 437–446 (2019). https://doi.org/10.1080/16878507.2019.1690799
53. H.M.A. Al-Sayed, M.A. Abdelaleem, H.O. Elkatry, Chemical, technological and biological evaluation of mulberry and persimmon leaves. Arab. J. Nucl. Sci. Appl. **52**(4), 45–63 (2019). https://doi.org/10.21608/AJNSA.2019.5487.1126
54. K. Harrison, L. Were, Effect of gamma irradiation on total phenolic content yield and antioxidant capacity of almond skin extracts. Food Chem. **102**(3), 932–937 (2007). https://doi.org/10.1016/j.foodchem.2006.06.034
55. S.X. Chen, Z.J. Ni, K. Thakur, S. Wang, J.G. Zhang, Y.F. Shang, Z.J. Wei, Effect of grape seed power on the structural and physicochemical properties of wheat gluten in noodle preparation system. Food Chem. 355(1), 129500 (2021). https://doi.org/10.1016/j.foodchem.2021.129500
56. I.H. Borai, M.K. Ezz, M.Z. Rizk, H.F. Aly, M. El-Sherbiny, A.A. Matloub, G.I. Fouad, Therapeutic impact of grape leaves polyphenols on certain biochemical and neurological markers in AlCl3-induced Alzheimer's disease. Biomed. Pharmacother. **93**, 837–851 (2017). https://doi.org/10.1016/j.biopha.2017.07.038
57. G.I. Fouad, M.Z. Rizk, Possible neuromodulating role of different grape (*Vitis vinifera* L.) derived polyphenols against Alzheimer's dementia: treatment and mechanisms. Bull. Natl. Res. Cent. **43**, 108 (2019). https://doi.org/10.1186/s42269-019-0149-z
58. H. Liang, T. Yang, C. Teng, Y. Lee, M. Yu, H. Lee, L. Hsu, C. Wang, Mulberry leaves extract ameliorates alcohol-induced liver damages through reduction of acetaldehyde toxicity and inhibition of apoptosis caused by oxidative stress signals. Int. J. Med. Sci. **18**(1), 53–64 (2021)
59. M. Guaita, A. Bosso, Polyphenolic characterization of grape skins and seeds of four Italian red cultivars at harvest and after fermentative maceration. Foods **8**(9), 395 (2019). https://doi.org/10.3390/foods8090395
60. R.A. Amin, S.N. Edris, Grape seed extract as natural antioxidant and antibacterial in minced beef. PSM Biol. Res. **2**(2), 89–96 (2017)
61. X. Zhao, R. Yang, Y. Bi, M. Muhammad Bilal, Z. Kuang, H.M.N. Iqbal, Q. Luo, Effects of dietary supplementation with mulberry (*Morus alba* L.) leaf polysaccharides on immune parameters of weanling pigs. Animals **10**(1), 1–11 (2020). https://doi.org/10.3390/ani10010035
62. Y.J. Qi, Preparation of procyanidins with different structures and their effects on the formation of acrylamide in foods. Jiangnan Univ. (2019). https://doi.org/10.1016/j.foodchem.2018.01.012
63. P.S. Variyar, A. Limaye, A. Sharma, Radiation-induced enhancement of antioxidant contents of soybean (glycine max merrill). J. Agric. Food Chem. **52**, 3385–3388 (2004). https://doi.org/10.1021/jf030793j
64. P.V. Vardhan, L.I. Shukla, Gamma irradiation of medicinally important plants and the enhancement of secondary metabolite production. Int. J. Radiat. Biol. **93**(9), 967–979 (2017)
65. S.A. Farkhad, A. Hosseini, Efect of gamma irradiation on antioxidant potential, isofavone aglycone and phytochemical content of soybean (Glycine max L. Merrill) cultivar Williams. J. Radioanalyt. Nuclear Chem. **324**, 497–505 (2020)
66. C.Y. Ranjitha, S. Priyanka, R. Deepika, G.P. Smitha Rani, J. Sahana, T.R. Prashith Kekuda, Antimicrobial activity of grape seed extracts. World J. Pharm. Pharm. Sci. **3**(8), 1483–1488 (2014)
67. V. Silvaa, G. Igrejasb, V. Falcoe, T.P. Santose, C. Torresf, A.M.P. Oliveirag, J.E. Pereiraa, J.S. Amarali, P. Poeta, Chemical composition, antioxidant and antimicrobial activity of phenolic compounds extracted from wine industry by-products. Food Control

92, 516–522 (2018). https://doi.org/10.1016/j.foodcont.2018.05.031
68. A.H. Al-Mousawi, S.J. Kaabi, A.J.H. Albaghdadi, A.E. Almull, A. Algon, Effect of black grape seed extract (*Vitis vinifera*) on biofilm formation of methicillin-resistant S*taphylococcus aureus* and *Staphylococcus haemolyticus*. Curr. Microbiol. **77**(2), 238–245 (2020). https://doi.org/10.1007/s00284-019-01827
69. Z. Breijyeh, B. Jubeh, R. Karaman, Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules **25**(6), 2–23 (2020). https://doi.org/10.3390/molecules25061340
70. C.M. Peixoto, M.L. Diasa, M.J. Alvesa, R.C. Calhelhaa, L. Barrosa, S.P. Pinhob, I.C.F.R. Ferreira, Grape pomace as a source of phenolic compounds and diverse bioactive properties. Food Chem. **253**, 132–138 (2018). doi 10.1016/j.foodchem.2018.01.163.
71. F. Blando, R. Rossella Russo, C. Negro, L.D. Bellis, Frassinetti, Antimicrobial and antibiofilm activity against *Staphylococcus aureus* of *Opuntia ficus*-indica (L.) mill. Cladode Polyphen. Extr. Antioxid. **2**(8), 117 (2019). https://doi.org/10.3390/antiox8050117
72. X. Zhao, L. Chen, J. Wu, Y. He, G.H. Yan, Antimicrobial kinetics of nisin and grape seed extract against inoculated *Listeria monocytogenes* on cooked shrimps: survival and residual effects. Food Control **115**, 107278 (2020)
73. Y. Xiong, M. Chen, R.D. Warner, Z. Fang, Incorporating nisin and grape seed extract in chitosan-gelatine edible coating and its effect on cold storage of fresh pork. Food Control **110**, 11–25 (2020). https://doi.org/10.1016/j.foodcont.2019.107018
74. M. Madigan, E. Karhu, The role of plant-based nutrition in cancer prevention. J. Unexplored Med. Data **3**(9), 1–16 (2018). https://doi.org/10.20517/2572-8180.2018.05
75. V. Mohansrinivasan, D.C. Subathra, D. Meenakshi, B. Ananya, N.S. Jemimah, Exploring the anticancer activity of grape seed extract on skin cancer cell lines A431. Braz. Arch. Biol. Technol. **58**(4), 540–546 (2015). https://doi.org/10.1590/S1516-8913201500076
76. Y. Shu, H. Yuan, M. Xu, Y. Hong, C. Gao, Z. Wu, H. Sun, R. Gao, S. Yang, S. Li, J. Tian, J. Zhang, A novel Diels–Alder adduct of mulberry leaves exerts anticancer effect through autophagy-mediated cell death. Acta Pharmacol. Sin. **42**, 780–790 (2021). https://doi.org/10.1038/s41401-020-0492-5
77. E. Pereira, A.I. Pimenta, L. Barros, R.C. Calhelha, A.L. Antonio, S.C. Verde, I.C. Ferreira, Effect of gamma radiation on the bioactivity of medicinal and aromatic plants: *Mentha xpiperita* L., *Thymus vulgaris* L. and *Aloysia citrodora* palau as case studies. Food Funct. **10**, 5156–5161 (2018). https://doi.org/10.1039/c8fo01558a
78. J. Quero, N. Jiménez-Moreno, I. Esparza, J. Osada, E. Cerrada, C. Ancín-Azpilicueta, M.J. Rodríguez-Yoldi, Grape stem extracts with potential anticancer and antioxidant properties. Antioxidants **10**, 243 (2021). https://doi.org/10.3390/antiox10020243
79. F. Grbović, M. Stanković, M. Curčić, N. Djordjević, D. Seklić, M. Topuzović, S. Marković, In vitro cytotoxic activity of *Origanum vulgare* L. on HCT-116 and MDA-MB-231 cell lines. Plants **2**(3), 371–378 (2013). https://doi.org/10.3390/plants2030371