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Antimicrobial, antioxidant and anticancer properties of globe artichoke and grape by-products as a source of the bio-active phenolic compounds

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Abstract

Grape pomace and non-edible parts of globe artichoke were investigated in this study as a rich source of the bioactive compounds. Phenols and flavonoids were determined using HPLC analysis. Biological activities of artichoke and grape by-product extracts were evaluated as antimicrobial, antioxidant and anticancer agents. Artichoke and grape wastes were applied as preservative edible coating films for chicken breast meat and counts of microbial groups were followed during storage. Grape seed extract (GSE) had the highest amounts of phenols (67 mg GAE/g) and flavonoids (46.5 mg QE/g), followed by artichoke floral stem extract contained phenols (62.2 mg GAE/g) and flavonoids (35.31 mg QE/g). Similarly, GSE gave the highest significant DPPH scavenging activity (91.6%) followed by artichoke floral stem extract (89.2%). The tested extracts inhibited the growth of all target microorganisms. The highest inhibition zone diameter was 63.3 ± 1 mm for GSE against *Bacillus subtilis*, while the lowest inhibition zone diameter was 11.6 ± 1.52 mm for artichoke bracts extract (ABE) against *Candida albicans*. For cytotoxicity, GSE caused the greatest inhibition effect against all the treated cell lines (IC_{50}; 20.4 - 53.2 µg/ml), followed by grape seedless pomace extract (GSPE) (IC_{50}; 22.9 - 57.1 µg/ml), whereas ABE had the lowest cytotoxic effect (IC_{50}; 143 - 329 µg/ml). All investigated by-product powders revealed inhibitory effect on the microbial groups causing a decrease in counts or complete disappearance of cells during one week of storage at 4 ± 1 °C for the coated chicken samples. Finally, artichoke and grape by-products represent effective natural food preservatives and sources of pharmaceutical bioactive agents.

Keywords: Globe artichoke, grape, by-products, phenolic compounds, antibacterial, antioxidant, anticancer

1. Introduction

The agro-food processing industries especially fruits and vegetables are considered as the second largest producer of wastes into the environment following the household sewage, causing significant economic, environmental and nutritional problems [1]. These by-products are in forms of leaves, peels, pulps, seeds, roots, and stems, they are rich in bioactive compounds such as polyphenols, flavonoids, carotenoids, proteins, carbohydrates, fibers, lignin, lipids, and other minerals that can be used as a low-cost source to obtain functional ingredients [2]. Moreover, the consumer concerns about the food safety have recently increased, and in this context, there is a rising interest in the use of natural compounds, like plant extracts rich in phenolic compounds, as food preservatives [3]. As well, there is a growing interest in discovering of the new natural antioxidant, antitumor, and antimicrobial compounds in which the plant by-products are rich of these bioactive substances [4].

Grapes (*Vitis vinifera* L.) are considered the most commonly consumed fruits growing worldwide, with approximate annul production of 80 million tons [5]. Egypt is one of the highest countries in Africa and the Middle East in producing grapes which is considered the second important fruit after citrus [6]. Therefore, a huge quantity of grape solid wastes is generated, mainly after processes of pressing as grape pomace (GP) [7]. Grape pomace is a blend of skins, pulp, seeds, and stalks that characterized as a solid material divided into two fractions: seedless pomace

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comprised of pulp, skin, and stem, and grape seeds [8]. This waste is rich in bioactive compounds such as unsaturated fatty acids, dietary fibers, anthocyanins, vitamins, and tannins. Also, it has antioxidant, antimicrobial and antimutagenic properties, depending mainly on the presence of phenolic compounds (phenolic acids, flavonoids, proanthocyanins, epicatechin, catechin, gallic acid, and procyanidins) [9]. Although, GP includes many bioactive substances, its applications other than animal feed and fertilizers are not fully exploited, and still a large quantity of this waste is disposed of as not useful waste representing important environmental and economic issues [5].

Globe artichoke (*Cynara scolymus* L.) is an herbaceous perennial plant belonging to *Asteraceae* family. Traditionally, it is consumed in the Mediterranean diet in different popular recipes (fresh, canned, roasted, or baked) [10]. Artichoke annual production in the Mediterranean region is estimated to reach 770,000 tons. Egypt is ranked as the second highest country for globe artichoke production after Italy, with annual production of 266196 tons in 2014 [11,12]. Artichoke has high nutritional value, being rich in water, minerals, vitamins, and carotenoids as well as the presence of bioactive compounds that has provoked the greater interest, especially phenolic compounds [12]. The industrial processing of artichoke produces a huge amount of non-edible parts such as leaves, external bracts and stems which represent nearly 80 - 85% of the total biomass produced per plant which is usually disposed of as a solid waste [13,14]. Both edible and artichoke by-products have the potential to be used as a source of dietary fibers, inulin and polyphenols [15]. Additionally, the most important phytochemicals found in artichoke waste are phenolic acids, such as mono and di-caffeoylquinic acid derivatives, flavonoids, anthocyanins, sesquiterpene lactones, and inulin [16,17]. Phenolic compounds have an important role in human nutrition due to their functional and food-related pharmacological properties involving antioxidant, anti-bacterial, anti-inflammatory, anti-carcinogenic, anti-diabetic hepatoprotective, and anti-hypercholesterolemic activities [18,19].

Thus, the re-valorization of agro-food processing by-products as bioactive source materials represents a measure to protect the environment as well as helps to create and develop value-added products. Accordingly, the present work aimed to examine the main phytochemical ingredients found in artichoke and grape by-product extracts as well as studying their antimicrobial, antioxidant, and anticancer activities. Finally, investigating the possibility using of these waste extracts as natural food preservatives.

2. Materials and methods

2.1. Chemicals

All used solvents/chemicals were of analytical/HPLC grade and obtained from E-Merck, Mumbai, India. DPPH, phenolic, and flavonoids standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Tested microorganisms

The used target organisms were *Staphylococcus aureus* ATCC 13565, *Bacillus cereus* ATCC 33018 and *Bacillus subtilis* ATCC 6633 as Gram-positive bacteria as well as *Pseudomonas aeruginosa* ATCC 9072, *klebsiella pneumoniae* ATCC 4352, *Escherichia coli* ATCC 25922, and *Salmonella typhimurium* ATCC 14028 as Gram-negative bacteria, while *Aspergillus niger* NRRL 62743 and *Candida albicans* ATCC 10231 as fungi species. The microbial stock cultures were obtained from Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Where, *Campylobacter jejuni* ATCC 700819 was kindly obtained from Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

2.3. Plant materials

Grape seedless pomace and seeds (*Vitis vinifera* L., red grape) were obtained from Al-Ahram factory for drinks, Junklies, Alexandria Egypt Road, Alexandria, Egypt. Grape wastes were air-dried at 30-37°C for three days, ground, sealed in polypropylene bags and stored at 4±1°C until use.

The fresh Globe artichoke (*Cynara scolymus* L.) plants were collected at their full maturity from the farm of the Ministry of Agriculture (Koot Elkloob), Alexandria, Egypt. Artichoke bracts and floral stems were separated from the outer receptacles and then cut into small pieces. Both bracts and stems were separately oven-dried at 40±1°C for two days, ground, sealed in polypropylene bags, and stored at 4±1°C till use.

2.4. Extraction method

One hundred grams of samples powder were mixed with 500 ml of ethanol 80% (v/v) for 3 times with a mechanical stirring at room temperature for 2 h, then allowed to stay in a refrigerator at 4±1°C for 24 h. The extracts were filtered using filter papers (Whatman No.1) and the filtrates were collected [20]. The ethanolic extracts were concentrated in a rotary evaporator (Stuart Rotary Evaporator Model RE300) at 40±1°C and then completely freeze-dried using a

lyophilizer (Snijders Scientific type 2040). Finally, the lyophilized preparations were stored in labelled sterile vials at −20 °C till further use.

2.5. Determination of total phenolic and flavonoid contents

Total phenolic content was determined as described by Wolfe *et al*. [21], the obtained results were expressed as mg gallic acid equivalents (GAE)/g of weight sample. Briefly, samples of each extract solution (200 µl) were transferred to a test tube and then mixed completely with one ml of Folin-Ciocalteu reagent. After mixing for 3 mins, 0.8 ml of 7.5 % $NaCO_3$ was added. The mixture was agitated with a vortex and then allowed to stand in the dark for 30 min, after that was centrifuged at 3300×g for 5 mins. The absorbance of extracts and blank were measured at 765 nm using UV-VIS Shimadzu spectrophotometer 2401PC (Shimadzu, Japan). The determinations were carried out in triplicates.

Flavonoids contents were estimated using the aluminum chloride colorimetric method of Zhishen *et al*. [22] and the results were expressed as mg of Quercetin Equivalent (QE)/ g of dry weight sample. One ml of the extract was added to a ten-ml volumetric flask, containing 4 ml of distilled deionized water and 300 µl of 5 % $NaNO_2$ were added. After 5 min, 300 µl of 10 % $AlCl_3$ were added and allowed to stand for 5 min at room temperature. Then, 2 ml of 1 M NaOH were added, and the total volume was brought up to 10 ml with double distilled water. The solution was mixed thoroughly, and the absorbance was measured against a reagent blank at 510 nm using UV-VIS Shimadzu spectrophotometer 2401PC (Shimadzu, Japan). The determinations were performed in triplicates.

2.6. HPLC analysis of phenolic compounds

Phenolic compounds were quantified using high-performance liquid chromatography (HPLC) system Hewllet Packard (series 1050) equipped with auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm, and quarter HP pump (series 1050). The column temperature was maintained at 35±1ºC. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min., as described by Goupy *et al*. [23]. Phenolic acids standard was dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used for the calculation of phenolic compounds concentration by the data analysis of Hewllet Packared Software.

2.7. HPLC analysis of flavonoid compounds

Flavonoids compounds were determined with the above mentioned HPLC system, but the ultraviolet (UV) detector was set at 333 nm. The column temperature was maintained at 25±1ºC. Gradient separation was carried out with isopropyl alcohol, acetonitrile, and 0.02M sodium dihydrogen phosphate (17:25:58, v/v) and was adjusted to pH 4 with 85% of phosphoric acid was used as a mobile phase. Analysis was run at a flow rate of 0.8 ml/min. according to the method of Loon *et al*. [24]. Flavonoids standards were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used for the calculation of flavonoids compounds concentration by the data analysis of Hewllet Packared Software.

2.8. Antioxidant activity (DPPH radical scavenging activity)

The method used to measure the antioxidant activity of the tested extracts was based on the use of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) as the free radicals according to the technique of Brand-Williams *et al*. [25]. Two ml of the tested extracts were added to 1.0 ml methanolic solution of 0.3 mM DPPH. The mixture was strongly shaken and left in the dark for 30 minutes at room temperature (30 ± 1°C). The control test was prepared by mixing 1.0 ml of methanolic DPPH plus 2.0 ml of methanol. The absorbance of the final solutions was measured at 517 nm using UV-VIS Shimadzu spectrophotometer 2401PC (Shimadzu, Japan). The mean values were calculated from triplicate experiments. Antioxidant activity (radical scavenging activity) of the extracts was expressed as a percentage of inactivated DPPH reagents from the following formula:

% Inhibition = [Abs. 517 of control – Abs. 517 of sample / Abs. 517 of control] ×100.

2.9. Antimicrobial activity of plant extracts

2.9.1. Agar well diffusion assay

The antimicrobial activity of the lyophilized ethanolic extracts was determined using the agar well diffusion method according to the protocol described by Hassan *et al*. [26]. Petri dishes were seeded with 100 µl of suspension containing 10^8 CFU/ml (0.5 Mcfarland standard) of bacteria, 10^6 CFU/ml of yeast, and 10^4 spore/ml on Muller Hinton agar medium for bacteria and potato dextrose agar medium (PDA) for fungi. Wells of 6 mm diameter were punched into agar using a sterile corkborer and 60 µl of the tested extracts were dispensed into the wells. The investigated extracts were applied in a concentration of 100 mg/ml in dimethyl sulfoxide (DMSO 10% v/v). Control wells containing DMSO 10% were used as negative control. Discs loaded with streptomycin (10 µg) and nystatin (100 units) were served as positive standard antimicrobials for bacteria, and fungi; respectively. The plates were

incubated at 37°C for 24 - 48 h for bacteria and at 25°C for 24 - 72 h for fungi. At the end of the incubation period, the antimicrobial activity was assessed by measuring the diameter of the growth zone of inhibition. All treatments were carried out in triplicates and data were presented as the mean of three replicates.

2.9.2. Antibacterial activity against *Campylobacter jejuni*

Campylobacter jejuni was activated by subculturing in Muller Hinton broth medium supplemented with 5% (v/v) sterile defibrinated sheep blood and incubated at 42°C for 48 h under microaerobic conditions (85% N_2, 10% CO_2, 5% O_2) using a Variable Atmosphere Incubator (VAIN) (MACS-VA500) (Don Whitley Scientific, Shipley, UK) to get 10^8 CFU/ml bacterial growth [27]. The antimicrobial activity of ethanolic extracts against *C. jejuni* was determined using agar well diffusion assay as described above with some modifications. Bacterial culture was seeded on Muller Hinton agar medium supplemented with 10% (v/v) sterile defibrinated sheep blood. The tested extracts were applied in a concentration of 100 mg/ml in dimethyl sulfoxide (DMSO 10% v/v) in stepwise volumes 25, 50, 75 and 100 µl/well. The plates were incubated at 42°C for 48 h under microaerobic conditions. The experiments were performed in three replicates.

2.9.3. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Minimal inhibitory concentration for artichoke and grape ethanolic extracts was determined by micro-dilution method according to Andrews [28]. Two-fold serial dilutions of artichoke and grape ethanolic extracts ranging from 10 to 0.02 mg/ml were used. Equal volume of each extract and Muller Hinton broth medium were mixed in sterile microdilution plates (96 wells) to reach the desired concentrations. Then, all tested wells were inoculated with 50 µl of fresh bacterial culture containing 10^8 CFU/ml. The plates were incubated at 37°C for 18-24 h. The lowest concentration of the extract that exhibit no visible bacterial growth was regarded as MIC. Consequently, the MBC was determined by subculturing the test dilutions that had no visual bacterial growth on to the Muller Hinton agar medium (free from plant extracts) and incubated further for 18-24 h. The least concentration that showed no single bacterial colony on the solid medium was considered as MBC.

2.10. *In vitro* anticancer activity (cytotoxicity) of by-product ethanolic extracts

The cytotoxic activity of artichoke and grape ethanolic extracts on cell viability was assessed by neutral red (NR) uptake method [29]. The tested cell cultures were human colon carcinoma (HCT116), human intestinal carcinoma (Caco-2), and mammalian cells from African green monkey kidney (Vero) cell lines. The used cell lines were taken from the Tissue Culture Unit, Holding Company for Biological Products and Vaccines (VACSERA), Giza, Egypt. Cancer cells were cultivated in separate 96-well plates containing Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) at a density of 10^5 cells/well. Post the incubation at 37°C for 48 h, adhered cells were treated with 50 µl of the extract solutions at different concentrations (zero to 500 µg/ml in DMSO) for 48 h. DMEM medium was used as a negative control, where doxorubicin (100 µg/ml) was applied as a positive control (100% inhibition). After that, the supernatant of each well was substituted with 100 µl of fresh medium (without FBS) containing 20 µl NR solution (0.33%) at individual wells. The absorbance (OD) of the well contents was read at 540 and 570 nm using a microplate multi-well reader (BioRad Laboratories Inc., model 3350, Hercules, California, USA). The experiments were repeated by using intact cells as the control and the assays were conducted in triplicates. Growth inhibition rates were calculated using the following equation: Growth inhibition rate (%) = [A-B/A] x 100.

Where: A and B are the absorbance of the supernatant of untreated and treated cell cultures; respectively. Then, the half-maximum inhibitory concentration (IC_{50}) was determined from the plotted graph.

2.11. Using of artichoke and grape by-products as edible coating films for chicken breast meat preservation during storage

2.11.1. Chemical composition of artichoke and grape by-product powders

Dried artichoke bracts, floral stems, grape seedless pomace, and grape seeds were milled with a high-speed laboratory blender and then sieved to obtain the powders. After that, moisture, fat, fiber, protein, and ash contents of artichoke and grape raw samples were measured according to AOAC methods [30]. Total carbohydrates were calculated using the following equation: % carbohydrates = 100 - (% moisture + % protein + % ash + % fat + % fiber) as reported by Elkatry *et al.* [4].

2.11.2. Preparation of coating solutions

The above mentioned powders were suspended in purified hot water (at 60 °C for 12 h) at concentration of 15, 20 and 25% (w/v). The resulting solutions were stirred for 60 min at 40 °C on a hot plate magnetic stirrer (Wiess Gallenkamp, Leicestershire, UK), then filtered to remove any undissolved impurities using muslin sheets. Finally, coating solutions were cooled down to the room temperature before application onto the surface of boneless chicken breast meat [31].

2.11.3. Treatment of deboned chicken breast meat

Boneless chicken breast meat samples were cut into cubes (about 10 g each), then divided into 13 groups, including uncoated group (Control), and 12 coated groups. The control group consists of chicken meat dipped in sterilized distilled water for 5 min. While the coated groups were individually dipped into the coating solutions (15, 20, and 25% for each by-product) for 5 min. All samples were drained for 1 min and then packed in polyethylene bags, tightly closed, and stored at 4 ± 1 °C for 21 days [32].

2.11.4. Microbiological examinations

Microbiological tests were performed at initial, 3, 7, 15 and 21 days of storage. Five grams of the tested samples were removed aseptically from each sample, added to 45 ml of sterile peptone water (1%) and homogenized for 2 min. From the resultant suspension serial decimal dilutions were prepared up to 10^{-6} in the same diluent. Then one ml of each dilution was transferred aseptically to inoculate appropriate media using the pour plate technique or most probable number (MPN) method. The numbers of microbes were estimated as $\log_{10}$ CFU or cells/ g.

a. Total viable bacterial counts were determined on plate count agar medium at 30 ºC for 48 h.
b. Fungi (molds and yeasts) were differentiated visually and estimated on potato dextrose agar medium containing 100 µg/ml each of tetracycline and chloramphenicol (Sigma), which were incubated at 30°C. Yeasts were counted after 2 days and molds after 5 days.
c. Total coliform counts were determined using MacConkey`s broth medium by MPN technique at 37ºC for 24 to 48 h.
d. Total spore forming bacteria were determined on plate count agar medium at 30 ºC for 48 h after the sample dilutions were heated at 80°C in a water bath for 10 min followed by a sudden cooling for 10 min in a cold water bath.

2.12. Statistical analysis

The statistical analysis was carried out using one-way analysis of variance (ANOVA) under a significant level of 0.05 for the obtained results using the statistical program CoStat (Ver. 6.400) and data were treated as complete randomization design according to Steel *et al*. [33]. Least significant difference (LSD) test was applied to determine the significance among means of different samples.

3. Results and discussion

Nowadays, agro-industrial by-products have attracted the attention of the researchers due to their availability, low-costly and sustainability as sources of the broad range of the bioactive compounds. So, the reuse of these wastes can reduce their ecological influences and led to economic benefits because of the production of the added-value products [34,35]. Thus, in this work we present for the first time, a comparative analysis of the main bioactive compounds from artichoke and grape by-products as well as testing their biological activities.

3.1. Total phenolic and flavonoid contents

Data in Table 1 compares the total phenolic content (TPC) and total flavonoid content (TFC) in artichoke and grape by-product ethanolic extracts. Grape seed extract (GSE) had the highest significant amounts ($P < 0.05$) of both chemical groups as 67 mg GAE/g and 46.5 mg QE/g for TPC and TFC, respectively, followed by artichoke floral stem extract (AFSE) of 62.2 and 35.31 mg QE/g for the same chemical groups, respectively. Whereas artichoke bracts extract (ABE) showed the least significant amounts ($P < 0.05$) of TPC and TFC as 33.9 mg GAE/g and 28.1 mg QE/g, respectively. This finding is consistent with that of Butkhup *et al*. [36] who found that the grape seed methanolic extract had the greatest TPC and TFC values in comparison with the grape skin or the whole grape. Lower amounts of TPC and TFC (33.9 mg GAE/g and 15.6 mg QE/g, respectively) were reported by Durante *et al*. [37] for grape seed extract. While Mora-Garrido *et al*. [34] found lower values of TPC (25.6 and 9.36 mg GAE/g) for red and white grape pomace extracts, respectively. For the globe artichoke, our findings are in agreement with those obtained by Shallan *et al.* [12] who observed that higher quantities of TPC and TFC in bracts ethanolic extract comparing with the receptacles ethanolic extract. Also, Ben Salem *et al*. [38] mentioned that the ethanolic extract of globe artichoke leaves comprised the highest levels of TPC and TFC. In this context, Salama and El-Baz [39] reported that the artichoke bracts included higher amounts of the total free phenolic compounds of 14.16 mg/ g, while the artichoke heart contained only 9.06 mg/ g.

3.2. Phenolic profile of artichoke and grape by-product ethanolic extracts

In this work, phenolic compounds profile of artichoke and grape by-product ethanolic extracts

were fractionated, identified, and quantified using the HPLC (Table 2). Twenty-two phenolic compounds were identified in the tested ethanolic extracts. The dominant compounds were gallic acid, pyragallol, chlorogenic, catechol, epicatechol, caffeine, ellagic,

Table 1
Total phenols and total flavonoids (mg/g) of artichoke and grape by-product ethanolic extracts

Constituent \ Extract	GSPE	GSE	ABE	AFSE	$LSD_{0.05}$
Total phenols	58.4 ± 0.44^{c}	67.0 ± 1.0^{a}	33.9 ± 0.34^{d}	62.2 ± 0.26^{b}	1.284
Total flavonoids	30.66 ± 0.15^{c}	46.5 ± 0.41^{a}	28.1 ± 0.36^{d}	35.31 ± 0.19^{b}	0.556

GSPE: grape seedless pomace extract; GSE: grape seed extract; ABE: artichoke bracts extract; AFSE: artichoke floral stems extract; $LSD_{0.05}$: Least significant difference. Values are the mean of three replicates ± SD. Values with the same letters within the row are not significantly different at $P<0.05$

Table 2
Phenolic content in artichoke and grape by-product ethanolic extracts

Compound \ Extract	Retention time (min.)	Concentration (mg/g)			
		GSPE	GSE	ABE	AFSE
Gallic acid	6.93	0.0223	0.1132	0.1123	0.0142
Pyragallol	7.04	0.2243	0.22	0.136	0.3241
4-aminobenzoic	8.21	0.0126	0.00112	0.007	0.0012
Catchine	8.56	0.0121	0.019	0.0303	0.019
Chlorogenic	9.07	0.0892	0.12933	0.4223	0.0432
Catechol	9.52	0.5212	0.3214	0.2111	0.03217
Epicatechol	9.62	0.2001	0.2949	0.003	0.2944
Caffeine	9.78	0.0911	0.1171	0.0319	0.1166
***p*-hydroxybenzoic acid**	9.98	0.0063	0.0023	0.0136	0.0123
Caffic	10.30	0.0221	0.0923	0.0219	0.0166
Vanillic	10.42	0.0041	0.0024	0.0303	0.02241
Coumaric	11.84	0.02631	0.02083	0.0327	0.02088
Ferulic	12.08	0.0761	0.0275	0.009	0.0275
Iso ferulic	12.43	0.0327	0.005	0.0231	0.0623
Ellagic	13.25	0.23217	0.2301	0.0052	0.2433
Vanilla	13.44	0.0231	0.1058	0.173	0.0725
α-coumaric	13.73	0.1001	0.06235	0.0932	0.0019
Benzoic	13.9	0.11413	0.0011	0.0392	0.0788
2,*4*-Dimethoxycinnamic	14.34	0.0222	0.0827	0.021	0.0018
Coumarine	14.54	0.0123	0.0023	0.012	0.002
Salycillic	15.01	0.0552	0.0952	0.136	0.3241
Cinnamic	15.39	0.044	0.044	0.007	0.0012

GSPE: grape seedless pomace extract; GSE: grape seeds extract; ABE: artichoke bracts extract; AFSE: Artichokes floral stem extract

vanilla and salycillic, particularly in GSE, GSPE and AFSE. In a study conducted by Martin *et al*. [40] the GC-MS analysis of grape residues revealed that caffeic, gallic, ferulic and *p*-coumaric acids were identified as phenolic compounds. In another study investigated waste extracts from four grape varieties cultivated in Egypt, the HPLC analysis showed that catechin, gallic, caffeic, vanillic, cumaric, ferulic, cinnamic and chlorogenic acids were the most prevalent phenolic compounds [41]. Recently, seventy-five phenolic compounds were identified and quantified by UHPLC-orbitrap MS^4 characterization of pomace extracts from Prokupac red grape variety [42]. They were distributed into six diverse classes: 1) hydroxybenzoic acids and derivatives, 2) hydroxycinnamic acids and derivatives, 3) flavan 3-ols and proanthocyanidins, 4) flavonols aglycones and glycosides, 5) stilbenoids, and 6) anthocyanins.

Six phenolic compounds were isolated form artichoke by-product extract using the HPLC [43]. These were identified as 3-o-caffeoylquinic acid, caffeic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 1,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid. As well, a total of 17 phenolic compounds consisted of 9 phenolic acids, 6 flavones derivatives, and 2 flavan-3-ols have been identified in the methanolic extract of artichoke floral stem [19]. In Egypt, Shallan *et al*. [12] identified 13 phenolic compounds in the ethanolic extract of artichoke bracts, however the prevailing compounds were catechin, rosmarinic, and apigenin.

3.3. Flavonoid profile of artichoke and grape by-product ethanolic extracts

As an important bioactive phenolic sub-group, flavonoids were also fractionated, identified, and quantified using the HPLC (Table 3). Eleven flavonoid compounds were found in the prepared ethanolic extracts. The prevailing compounds were luteolin, naringin, hisperdine, quertrine, and apigenin which found in higher quantities in GSE and AFSE. In accordance with the present results, a previous study demonstrated that the individual flavonoid content of the red grape cultivar Shiraz by-products (grape skin

and grape seed extracts) was (+)-Catechin, (-)-epicatechin, rutin, naringenin, kaempferol, quercetin, and luteolin [36]. Likewise, nine flavonoid compounds; catechin, epicatechin, epigallocatechin gallate, gallocatechin gallate, epicatechingallate, quercetin, quercetin-3-rhamnoside, kaempferol, and rutin were identified using HPLC in extracts from pomaces of four grape varieties grown in Virginia [44]. In a recent study, artichokes' bracts and stalks have been evaluated for their flavonoid content using HPLC coupled to electrospray time-of flight mass spectrometry (ESI-TOF/MS) analysis [10]. The results emerged that only flavones and their derivatives were detected in artichokes by-products (luteolin-rutinoside, cymaroside, isorhoifolin, apigenin-glucoside, luteolin, apigenin and methylapigenin). In another research conducted on the methanolic extracts of artichoke leaves [17], the flavonoid profile revealed that six compounds were identified (luteolin-glucoside, cymaroside, luteolin-O-deoxyhexoside-hexoside, luteolin-O-hexuronide, luteolin-O-malonylhexoside and luteolin-O-acetylhexoside).

3.4. Antioxidant activity of artichoke and grape by-product ethanolic extracts

Free radicals are unstable molecules that are toxic and risky to the living organisms by causing harmful damages in cells. Hence, neutralization of these compounds is essential to keep the cell vitality [45]. In this work, the antioxidant activities of the artichoke and grape by-product ethanolic extracts were evaluated by the DPPH method and the results are shown in Fig. 1. It was noticed that the GSE gave the highest significant DPPH scavenging activity (91.6%, $P < 0.05$), followed by the AFSE (89.2%), while the ABE was the least active extract (80.7%). Comparable values of antioxidant activity 86.74, 80.10, and 70.13% were reported for grape seed, grape skin and the whole grape methanolic extracts, respectively [36]. However, higher DPPH radical inhibition of 94.06% was achieved by Farhadi *et al.* [46] when developed a methanolic grape skin extract. On the other hand, Biel *et al.* [47] attained only 44% DPPH radical scavenging activity for the artichoke leaf extracts. In accordance with the present results, a recent study conducted on the artichoke stem extract that inhibited the radical DPPH by 80.74% [48]. It is noteworthy to mention that the possible mechanisms suggested for neutralizing unstable molecules; by providing electrons or hydrogen atoms to DPPH and/or interacting with its radicals [49]. In the current work, antioxidant activity was very related to the TPC and TFC results (Table 1). Confirming our findings, Mollica *et al.* [50] presented a relationship between total bioactive compounds (phenolics and flavonoids) and antioxidant effects as performed by correlation analysis. Thus, the antioxidant activity can be considered as one of the most important phenolic compound's bioactivities from artichoke and grape by-products. Generally, the potential antioxidant capacity of phenolic compounds is due to acting as electron and hydrogen donors, metal chelators, free radical scavengers, and quenchers of singlet oxygen [51]. Hence, the specific radical scavenging ability is referred to the O-dihydroxylic structure common in polyphenolic compounds responsible for the stable form of radicals, also contributing in the electron delocalization [50].

3.5. Antimicrobial activity of artichoke and grape by-product ethanolic extracts

Considering the emergent crisis of antibiotic resistance that spreads among bacterial pathogens attention has been paid on obtaining compounds from natural sources such as vegetables and fruits rather than using synthetic antimicrobial compounds [51]. In the present work, the antimicrobial activity of artichoke and grape by-product ethanolic extracts was performed using agar well diffusion technique as an initial qualitative test, and the results are summarized in Table 4. The results demonstrated that the tested extracts inhibited the growth of all target microorganisms, but with different responses. This activity of the tested ethanolic extracts against all tested microorganisms may be implied that the presence of broad-spectrum antimicrobial compounds, which are distributed in these plant extracts. Extracts of artichoke and grape by-product had convergent antimicrobial activity. The highest inhibition zone diameter was 63.3 ± 1 mm for GSE against *Bacillus subtilis*, while the lowest inhibition zone diameter was 11.6 ± 1.52 mm for ABE against *Candida albicans*. Furthermore, Gram-positive bacteria (G+) were more susceptible to the extracts than gram-negative ones (G-). These results are in agreement with those obtained by Butkhup *et al.* [36], Oliveira *et al.*, Sofi *et al.*, and Leal *et al.* [52–54]. Resistance of G- bacteria to antibacterial substances is related to lipopolysaccharides in their outer membrane, which acting as a strong vital barrier against toxic substances due to its high hydrophobicity [52].

Table 3
Flavonoids content in artichoke and grape by-product ethanolic extracts

Extract / Compound	Retention time (min.)	Concentration (mg/g)			
		GSPE	GSE	ABE	AFSE
Luteolin	11.88	0.073	0.2231	0.0536	0.1599
Naringin	12.55	0.1137	0.3364	0.2579	0.3093
Rutin	12.63	0.1039	0.138	0.09702	0.1131
Hisperdine	12.76	0.154	0.2995	0.1273	0.2539
Rosmaric	13.03	0.0983	0.1399	0.0264	0.0935
Quertrine	13.62	0.0944	0.234	0.0943	0.1328
Querctine	15.26	0.04234	0.122	0.04029	0.0739
Narigenine	15.53	0.0219	0.0321	0.01974	0.0339
Hespertin	15.92	0.01318	0.06321	0.01634	0.0442
Kampferol	16.68	0.02935	0.1123	0.01923	0.1032
Apigenin	16.95	0.11826	0.1943	0.1006	0.1599

GSPE: grape seedless pomace extract; GSE: grape seeds extract; ABE: artichoke bracts extract; AFSE: artichoke floral stem extract

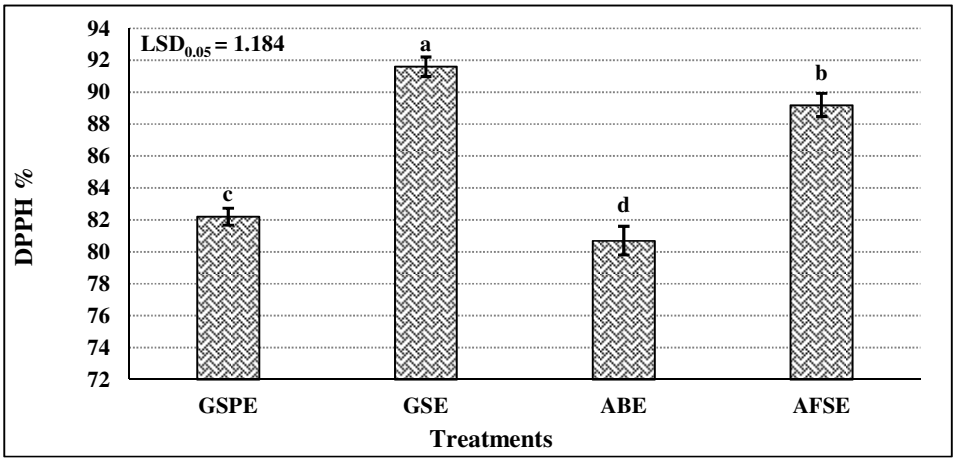

Figure 1: Antioxidant activity of artichoke and grape by-product ethanolic extracts. GSPE: grape seedless pomace extract; GSE: grape seeds extract; ABE: artichoke bracts extract; AFSE: artichoke floral stem extract; $LSD_{0.05}$: Least significant difference. Values are the mean of three replicates ± SD. Columns with the same letters are not significantly different at $P<0.05$

Table 4
Antimicrobial activity of artichoke and grape by-product ethanolic extracts

Treatment / Microorganism	Zone of growth inhibition (mm)				Standard positive control*	$LSD_{0.05}$
	GSPE	GSE	ABE	AFSE		
Gram-positive bacteria:						
***Bacillus cereus* ATCC 33018**	52.3±1.58^{d}	62.2±1^{b}	50.3±1.58^{e}	55.5±2^{c}	63.1±2.18^{a}	2.77
***Bacillus subtilis* ATCC 6633**	56.4±1.58^{c}	63.3±1^{ab}	51.6±1^{d}	58.6±1.58^{b}	64±2.18^{a}	2.43
***Staphylococcus aureus* ATCC 13565**	33.1.3±0.58^{c}	39±1.15^{a}	32.9±1^{d}	35±1^{b}	41.6±2.18^{a}	1.802
Gram-negative bacteria:						
***Escherichia coli* ATCC 25922**	33.3±1.58^{d}	46.1±1^{b}	31.6±1.58^{e}	43.6±1.58^{c}	53.6±2.18^{a}	2.66
***Pseudomonas aeruginosa* ATCC 9072**	36.3±1.58^{bc}	38.6±1.58^{b}	24.6±1.58^{d}	36.3±1.22^{bc}	49.3±2.18^{a}	2.87
***Salmonella typhimurium* ATCC 14028**	38.2±2^{c}	42.0±2^{b}	26±1^{d}	37.3±1.58^{c}	51.3±2.18^{a}	3.169
***klebsiella pneumoniae* ATCC 4352**	23.6±1^{c}	30±1.01^{b}	22.3±1.14^{d}	30±2^{b}	56.7±2.18^{a}	1.205
Fungi:						
***Candida albicans* ATCC 10231**	21±1^{d}	33.2±1.52^{b}	11.6±1.52^{e}	29.3±1.52^{c}	55.3±0.12^{a}	2.17
***Aspergillus niger* NRRL 62743**	19.8±1^{c}	26.3±1.52^{b}	16.3±1.52^{e}	19. 3±1.52^{cd}	59.3±1.14^{a}	2.101

*: Streptomycin (10 µg) for bacteria and nystatin (100 units) for fungi. GSPE: grape seedless pomace extract; GSE: grape seeds extract; ABE: artichoke bracts extract; AFSE: artichoke floral stem extract; $LSD_{0.05}$: Least significant difference. Values are the mean of three replicates ± SD. Values with the same letters within the row are not significantly different at $P<0.05$

However, the tested bacterial strains revealed more sensitivity to the examined extracts comparing with fungal strains. Moreover, among extracts, GSE had the highest significant (P < 0.05) inhibitory effect (inhibition zones; 39 - 62.2 mm for G+ bacteria, 30 - 46.1 mm for G- bacteria and 26.3 - 33.2 mm for fungi). AFSE showed the second larger zone of inhibition diameters came after the GSE (inhibition zones; 35 - 55.5 mm for G+ bacteria, 30 - 43.6 mm for G- bacteria and 19.3 - 29.3 mm for fungi), while ABE exhibited the lowest antimicrobial impact (inhibition zones; 32.9 - 50.3 mm for G+ bacteria, 22.3 - 31.6 mm for G- bacteria and 11.6 - 16.3 mm for fungi). These outcomes probably dependent on the high TPC and TFC contents in both extracts (GSE and AFSE; Table 1). These findings broadly supports the work of other studies that proved the antimicrobial activities of artichoke [12,19,43] and grape [36, 52–54] by-product extracts against pathogenic and food spoilage microorganisms. These results can be again explained by the fact that all these extracts are rich in phenolic compounds. Various studies reported the bactericidal actions of phenolic compounds are due to several factors: (1) changing the cellular morphology; (2) affecting the selective permeability of cytoplasmic membrane by denaturation of membrane proteins; (3) causing alterations in the structure of the cell wall; (4) modifying of protein synthesis; (5) impairing replication of bacterial DNA; (6) interacting with enzymes and substrates; and (7) depriving of metallic ions [51,52,54,55].

As an important food-born pathogen, *Campylobacter jejuni* causes campylobacteriosis, a gastro-intestinal tract infection in humans, characterized by severe diarrhea, abdominal pains, fever, nausea and vomiting that commonly takes for 5 to 7 days. This pathogen can affect any person; however, the symptoms are normally more serious in children, older people, and persons who have health problems. Animal food contamination with fecal or intestinal material representing the main source of infection, especially in poultry products [56]. So, there is a need to control of *Campylobacter* infection without developing of antibiotic-resistant cells as well as keeping the beneficial effects of the host microbiota. For that purpose, using of low doses of natural bioactive phytochemicals to reduce the incidence of *Campylobacter* is an alternative approach [27,57]. In the current work, testing of artichoke and grape by-product ethanolic extracts as anti-*Campylobacter* agents was studied using agar well diffusion methods. Table 5 shows that the GSE again gave the greatest significant suppressive effect (P < 0.05) against *C. jejuni*, followed by AFSE, while ABE had the lowest significant effect. It is clear that the impact of the extracts was volume-dependent per well. Generally, in reviewing the literature, little is known about the effect of artichoke by-product extracts on *Campylobacter* combat [58]. Nevertheless, several reports have demonstrated the influence of grape by-product extracts on controlling of *Campylobacter* spp. [27,57,59,60]. These studies referred the anti-*Campylobacter* effect to the phenolic compounds found in the tested artichoke and grape by-product extracts. Agar well diffusion method is considered as an initial screening technique for the antibacterial activity of the tested substances to offer an indication for further quantitative assessment of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) [44]. In this study, MBC values were identical or greater than MIC values. MIC and MBC of the ABE and GSPE for the tested bacterial strains ranged from 0.616 to 1.85 mg/ml (Table 6). GSE showed the highest antibacterial activity recording 0.205 to 0.616 mg/ml for both MIC and MBC. However, AFSE exhibited moderate antibacterial activity recording 0.205 to 1.85 mg/ml in terms of MIC and MBC.

Table 5
Antibacterial effect of artichoke and grape by-product ethanolic extracts on *Campylobacter jejuni*

Treatments / Volume/well	Zone of growth inhibition (mm)				$LSD_{0.05}$
	GSPE	GSE	ABE	AFSE	
25µl	12.3±0.58^{c}	18±0.01^{a}	10.3±0.58^{d}	16.3±15^{b}	1.63
50µl	19.3±0.58^{c}	25.3±1.1^{a}	17.3±1.1^{d}	21.1±1^{b}	1.88
75µl	29.6±0.58^{c}	36.6±1.15^{a}	20.3±0.58^{d}	31.3±1.1^{b}	1.95
100µl	34.3±0.58^{c}	48.1±1^{a}	27.3±1.5^{d}	39.4±1^{b}	2.13

GSPE: grape seedless pomace extract; GSE: grape seeds extract; ABE: artichoke bracts extract; AFSE: artichoke floral stem extract; $LSD_{0.05}$: Least significant difference. Values are the mean of three replicates ± SD. Values with the same letters within the row are not significantly different at P<0.05

Table 6
Minimum inhibitory concentration (MIC, mg/ml) and minimum bactericidal concentration (MBC, mg/ml) of artichoke and grape by-product ethanolic extracts

Bacterial strain	GSPE			GSE			ABE			AFSE		
	MIC	MBC	MBC/ MIC	MIC	MBC	MBC/ MIC	MIC	MBC	MBC/ MIC	MIC	MBC	MBC/ MIC
***Bacillus cereus* ATCC 33018**	0.616	0.616	1	0.205	0.616	3	0.616	1.85	3	0.205	0.616	3
***Bacillus subtilis* ATCC 6633**	0.616	0.616	1	0.205	0.205	1	0.616	1.85	3	0.205	0.616	3
***Staphylococcus aureus* ATCC 13565**	1.85	1.85	1	0.616	0.616	1	1.85	1.85	1	0.616	1.85	3
***Escherichia coli* ATCC 25922**	0.616	1.85	3	0.205	0.205	1	1.85	1.85	1	0.205	1.85	9
***Pseudomonas aeruginosa* ATCC 9072**	0.616	1.85	3	0.616	0.616	1	0.616	1.85	3	0.205	0.616	3
***Salmonella typhimurium* ATCC 14028**	0.616	1.85	3	0.616	0.616	1	1.85	1.85	1	0.616	1.85	3
***klebsiella pneumoniae* ATCC 4352**	1.85	1.85	1	0.616	0.616	1	0.616	1.85	3	0.616	0.616	1

GSPE: grape seedless pomace extract; GSE: grape seeds extract; ABE: artichoke bracts extract; AFSE: artichoke floral stem extract

Butkhup *et al*. [36] reported lower MIC values for the effect of grape seeds and skins methanolic extracts (0.016 - 0.128 mg/ml) on G+ bacteria, while moderate values were recorded for G- bacteria (0.128 - 0.512 mg/ml). In addition to Xu *et al*. [44] recorded MIC of 4.70 - 18.8 mg/ml and MBC of 9.38 - 37.5 mg/ml for *Listeria monocytogenes* ATCC 7644. Whereas the recorded values for *Staphylococcus aureus* ATCC 29213 were 40.6 - 250 mg/ml (MIC) and 75 - 250 mg/ml (MBC) when tested the antimicrobial activity of pomace extracts from four varieties of grapes. Shallan *et al*. [12] found that the MIC values of artichoke ethanolic extracts against the target bacteria ranged from 0.08 - 0.27 mg/ml. Also Mejri *et al*. [19] stated that the MIC and MBC for AFSE were 1 - 1.5 mg/ml and 1.5 - 2 mg/ml, respectively. Interestingly, in this study, the four tested ethanolic extracts revealed strong bactericidal activity against the target bacteria, with an exception for AFSE against *Escherichia coli* (Table 6). It is known that the efficacy of an antibacterial agent is reliant mainly on MBC to MIC ratio. Thus, an antibacterial agent is regarded as bactericidal if the MBC value is not more than 4 times of the MIC value [26,61].

3.6. *In vitro* cytotoxic effect of artichoke and grape by-product ethanolic extracts

Due to increasing incidence of cancer, the search for novel, efficient and less toxic bioactive compounds remain a priority. Therefore, the cytotoxic effect of artichoke and grape by-product ethanolic extracts against three cell lines HCT116, Caco-2 and Vero was evaluated, and the results are illustrated in Fig. 2 and Table 7. Similar patterns were observed for the four examined extracts against the three treated cell lines (Fig. 7). Also, grape ethanolic extracts (GSE and GSPE) demonstrated superior inhibitory effect for the three cell lines over the artichoke extracts (ABE and AFSE). Obviously, the effect of the extracts on cell lines was dose-dependent. Overall extracts, GSE caused the greatest inhibition effect against all the tested cell lines (IC_{50}; 20.4 - 53.2 µg/ml), followed by GSPE (IC_{50}; 22.9 - 57.1 µg/ml), whereas ABE had the lowest cytotoxic effect on the studied cell lines (IC_{50}; 143-329 µg/ml). Generally, Caco-2 cell line was more sensitive to all the investigated extracts, HCT116 cell line showed a moderate sensitivity, while Vero cell line revealed a slight resistant to the used ethanolic extracts (Table 7). In harmony with the present results, a previous cell line study has confirmed the inhibition of cancer cell invasion by grape seed proanthocyanidins in a dose-dependent manner for concentrations 0, 10, 20, 40 µg/ml [62]. In a recent study, Pérez-Ortiz *et al.* [63] described anti-proliferative characteristics of grape pomace extract at different levels of 5-250 µg/ml on colon cancer cell lines (Caco-2, HT-29) and fibroblasts. Miccadei *et al*. [64] stated that the extract edible part of globe artichoke showed cancer-preventive activity on hepatocellular carcinoma cell line HepG2. Recently, Shallan *et al*. [12] in an experiment conducted on globe artichoke against cancer cell lines, observed that bracts extract gave higher activity against HepG2 and MCF7 (IC_{50}; 0.514 and 0.847 mg/ml, respectively) than receptacles (IC_{50}; 0.661 and 0.1.724 mg/ml, respectively), whilst both extracts had no effect against HC T116 cell lines. This cytotoxic activity may be explained by the fact that all the studied ethanolic extracts are rich in phenolic compounds. For instance, polyphenolic compounds exhibit cancer prevention mechanisms such as inhibition of proteases, phases I and II drug-metabolizing enzymes, and metabolic pathways including angiogenesis, invasion, and metastasis. They also stop receptor-mediated functions, modifies cell-cycle checkpoints and apoptosis [62]. In cell line models, several mechanisms are developed in which

phenolic compounds demonstrated uncommon anti-tumoral activity in breast, bladder, leukemia, prostate, colon, and lung tumors [51]. In this regard, several reports have stated that phenolic compounds had anticancer activity and the mechanisms of inhibition could be summarized as follows: 1) stimulation and enhancement of immune system; 2) promoting tumor cell apoptosis; 3) inhibition of angiogenesis; 4) cell cycle arrest, inhibiting cell proliferation; 5) reducing metastasis and spread; and 6) modulating the redox balance, showing both pro-oxidant and antioxidant action [51,62,65,66].

3.7. Preservative effect of artichoke and grape by-products on chicken breast meat

Poultry products are good sources of nutrients for humans; nevertheless, they also provide satisfactory conditions for microbial growth and hence they have a short shelf-life. To prevent microbial growth in animal origin foods, synthetic preservatives are extensively used in the food industry owing to their low cost and strong antimicrobial effect. However, consumers do not prefer synthetic preservatives due to their negative effect on health. Consequently, the need for natural substances as food preservatives has increased. These natural preservatives inhibit the growth of spoilage microorganisms or food-borne pathogens thereby can extend the shelf-life of chicken meat products [67]. In the present study, to extent the shelf-life period of fresh boneless breast chicken meat during chilling storage, artichoke and grape by-product powders were used to formulate edible coating films. The proximate chemical compositions of artichoke and grape by-product powders are displayed in Table 8. The results reveal that grape seed powder (GSP) had the highest

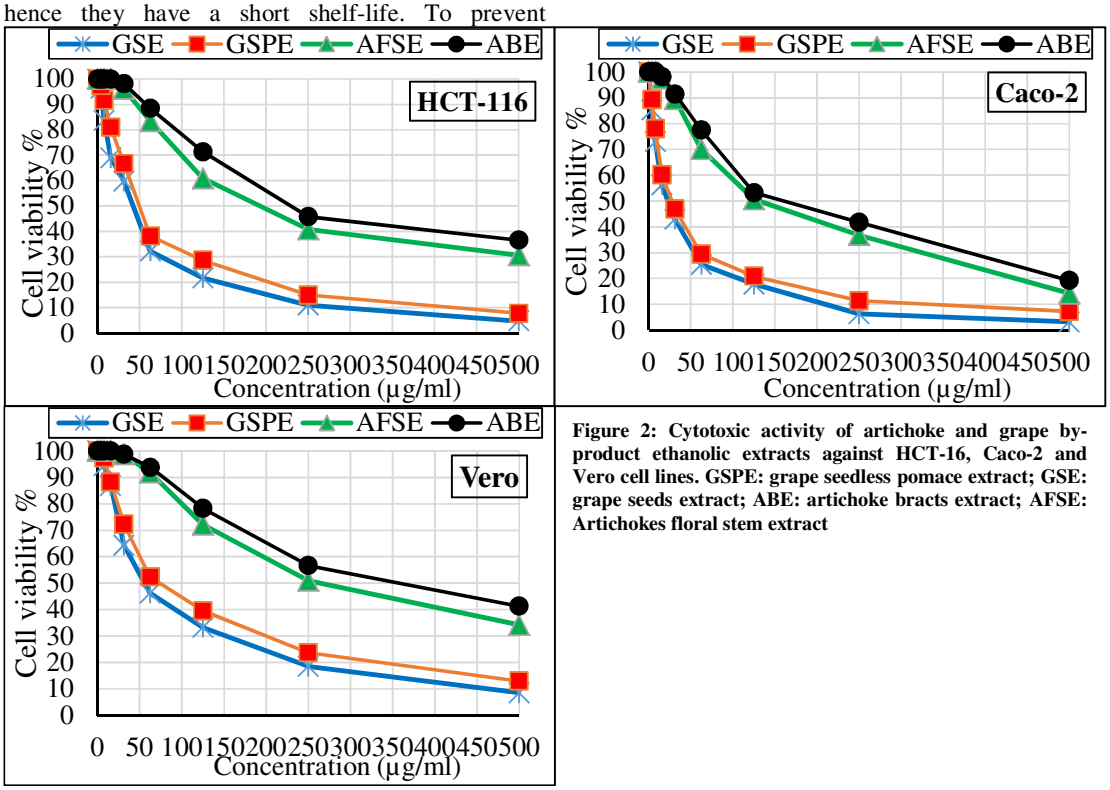

Figure 2: Cytotoxic activity of artichoke and grape by-product ethanolic extracts against HCT-16, Caco-2 and Vero cell lines. GSPE: grape seedless pomace extract; GSE: grape seeds extract; ABE: artichoke bracts extract; AFSE: Artichokes floral stem extract

Table 7
The half-maximum inhibitory concentration (IC_{50}) of artichoke and grape by-product ethanolic extracts against selected cell lines

Extract / Cell line	IC_{50} (µg/ml)			
	GSPE	GSE	ABE	AFSE
HTC-116	46.8 ± 0.8	42.3 ± 0.5	234 ± 5.9	226 ± 3.9
Caco-2	22.9 ± 0.6	20.4 ± 0.3	143 ± 4.1	138 ± 3.2
Vero	57.1 ± 1.2	53.2 ± 1.0	329 ± 9.6	321 ± 7.8

GSPE: grape seedless pomace extract; GSE: grape seeds extract; ABE: artichoke bracts extract; AFSE: artichoke floral stem extract. Values are the mean of three replicates ± SD

Table 8
Proximate chemical composition of artichoke and grape by-product powders

Content / Powder	Composition (g/100 g DW)					
	Protein	**Carbohydrates**	**Ash**	**Moisture**	**Fat**	**Fiber**
GSP	13.8± 0.25^{b}	65.83 ± 0.38^{a}	8.35 ± 0.14^{a}	11.58 ± 29^{a}	2.7 ± 0.34^{c}	6.34 ± 0.18^{c}
GS	15.43 ± 0.42^{a}	58.75 ± 0.58^{b}	6.28 ± 0.21^{b}	10.25± 33^{b}	5.92 ± 0.19^{a}	6.02 ± 0.17^{d}
AB	11.73± 0.47^{d}	57.09 ± 0.43^{c}	3.4 ± 0.28^{d}	6.53± 0.33^{d}	2.27 ± 0.18^{d}	7.92 ± 0.22^{b}
AFS	12.6± 0.55^{c}	55.33 ± 0.52^{d}	5.77 ± 0.2^{c}	8.48± 0.2^{c}	3.24 ± 0.24^{b}	11.013 ± 0.24^{a}
$LSD_{0.05}$	0.681	5.308	0.404	0.555	0.464	0.388

GSP: grape seedless pomace; GS: grape seeds; AB: artichoke bracts; AFS: artichoke floral stem; $LSD_{0.05}$: Least significant difference. Values are the mean of three replicates ± SD. Values with the same letters within the column are not significantly different at P<0.05

significant (P < 0.05) protein and fat contents; 15.43 and 5.92%, respectively. While grape seedless pomace powder (GSPP) contained the greatest significant (P < 0.05) percentages of moisture, carbohydrates, and ash (11.58, 65.83 and 8.35%, respectively). As expected, artichoke floral stem powder (AFSP) demonstrated the highest significant (P < 0.05) dietary fiber content of approximately 11.0%. The composition of the plant origin wastes is highly dependent on the type of waste, plant variety, cultivation conditions, processing method, and many other factors [68]. In a review study conducted on grape pomace valorization [5], the authors reported that the chemical compositions of GSPP were in ranges of 1.73 -9.10%, 3.57 - 14.17%, 1.14 - 13.90%, and 17.28 - 88.70% for ash, protein, fat, and dietary fiber, respectively. The obtained values within this study for GSPP existed in these ranges, except for the dietary fiber content. The levels observed in this investigation are different from those observed by Mora-Garrido *et al*. [34] who quantified the chemical composition of grape pomace powder, which was 7.93, 8.62, 19.53, and 0.55% for protein, lipid, fiber, and ash, respectively. Conflicting data were also reported by Elkatry *et al*. [4] for the chemical composition of GSP. Noriega-Rodríguez et al. [69] attained comparable results for freeze-dried outer bracts of artichoke for lipids and proteins (1.3 and 10%, respectively), but different values for ash and dietary fibers (12 and 53%). In contrast to our findings, Carpentieri *et al*. [70] achieved higher values for carbohydrates and dietary fibers and lower for ash, lipids and proteins when analyzed AFSP. Fiber-rich by-products can be normally utilized in food products as low-cost, non-caloric bulking agents for enhancing water and oil preservation, to improve emulsion or oxidative stability as well as their health benefits. Besides, some fiber compounds in grape and artichoke wastes make chemical bonds with phenolic compounds and, hence, form antioxidant dietary fibers, giving the wastes stronger radical scavenging potential (antioxidant activity) [4,5]. Due to protein shortage as well as the rising costs of products of animal origin, that have triggered research to develop new protein sources from non-utilized by-products [4]. Generally, the chemical analysis of the present study showed that artichoke and grape by-products were rich in basic nutrients, suggesting use of them as functional food ingredients.

The results of microbiological parameters from different samples of breast chicken meat coated with films from artichoke and grape by-products during storage period at initial, 3, 7, 15 and 21 days at 4°C are shown in Table 9. The results presented that in the control treatment, counts of all monitored microbial groups increased with the progress of storage period. It is worth noting that in the control treatment, by the end of storge period the number of total bacterial count surpassed 7 $\log_{10}$ CFU/g the spoilage index of chicken products as reported by Jo *et al*. and El Sheikha *et al*. [71,72]. All investigated by-product powders of artichoke and grape revealed inhibition effect on the four microbial groups causing a decrease in counts or complete disappearance of cells. This effect was dose-dependent with a superiority for GSP. This result may be explained by the fact that all by-products included high contents of TPC and TFC which have strong antimicrobial activity (Table 1). During the storage period, for all by-product treatments, the total bacterial counts decreased with the time until the day 3, after that declined totally below the detection level in sampling time at day 7 and day 15. Then at the end of storage period a few numbers of cells were detected on average of 1.17 $\log_{10}$ CFU/g. Appearance of these cells again might be explained as some bacterial species gained resistance with the time for the bioactive compounds founds in the by-products. Also, fungal group (molds and yeasts) followed similar performance like the total bacterial count. The total spore formers group lasted to the day 3 of storage period, after that were completely disappeared. While total coliform group as an indicator on the presence of pathogenic bacteria were not detected at all starting from the day 3 of storage period. To our knowledge, this is the first report describing the use of artichoke or grape by-products as coating films for chicken breast meat to ensure the

microbial quality during the storage period. Confirming our results, in a study conducted by Sheikh [31] on using Arabic gum and Plantago seeds mucilage as edible coating for chicken boneless breast, Arabic gum at level 25% reduced the total bacterial count from 7.4 to 1.9 $\log_{10}$ CFU/g. Similar effect was observed by Eldaly *et al*. [32] who found the use of chitosan for coating chicken fillets during chilled storage led to a significant reduction ($P < 0.05$) in the total aerobic bacterial count, *Enterobacteriaceae*, and *Staphylococcus* counts along the storage period. Also, Dakheli [73] studied the effect of pomegranate and grape waste extracts on decontamination of poultry carcasses in a slaughterhouse. Both extracts caused a significant ($P < 0.05$) inhibition for total bacterial counts, coliforms, *E. coli* and *S. aureus*. On the contrary, El Sheikha et al. [72] found that with the progress of storage time, the total bacterial count increased significantly ($P < 0.05$) for coated and uncoated chicken breast meat samples, but the numbers remained within the recommended range (< 7 $\log_{10}$ CFU/g). However, only the control sample count approached the spoil limit at the end of storage. This result was obtained when they used a coating film containing carboxymethyl cellulose plus propolis extract to extent the shelf-life of chicken breast meat.

Table 9
Effect of artichoke and grape by-products coating films on microbiological quality of deboned chicken breast meat during storage at 4ºC

Microbial group	Storge period (day)	Microbial count ($\log_{10}$ CFU or cells/g)												
		Uncoated sample	GSPE			GSE			ABE			AFSE		
		Control	15%	20%	25%	15%	20%	25%	15%	20%	25%	15%	20%	25%
Total bacterial count	**0**	4.47	4.26	4.20	4.09	4.19	4.08	3.89	4.40	4.37	4.25	4.35	4.23	4.16
	3	4.82	2.85	2.30	2.21	2.62	2.31	2.15	2.86	2.59	2.60	2.90	2.71	2.54
	7	5.02	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	15	6.43	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	21	7.76	1.25	1.12	ND	1.13	1.02	ND	1.38	1.26	1.13	1.22	1.15	1.08
Spore-forming bacteria	**0**	3.48	3.41	3.32	3.27	3.35	3.26	3.18	3.45	3.44	3.36	3.50	3.46	3.39
	3	3.62	2.76	2.27	2.15	2.54	2.21	2.10	2.80	2.52	2.48	2.81	2.63	2.46
	7	3.79	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	15	4.98	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	21	6.02	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mold and yeasts	**0**	2.95	2.86	2.72	2.61	2.90	2.65	2.59	2.96	2.88	2.81	2.91	2.84	2.79
	3	3.11	2.77	2.65	2.51	2.69	2.53	2.48	2.85	2.72	2.67	2.80	2.71	2.63
	7	3.30	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	15	4.61	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	21	5.18	1.45	ND	ND	1.33	ND	ND	1.68	1.26	ND	1.62	ND	ND
Total coliform	**0**	3.28	3.22	2.99	2.86	3.12	2.87	2.74	3.18	3.09	3.0	3.15	3.04	2.93
	3	4.34	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	7	4.76	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	15	5.15	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	21	6.32	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

GSPE: grape seedless pomace extract; GSE: grape seeds extract; ABE: artichoke bracts extract; AFSE: artichoke floral stem extract; ND: not detected

4. Conclusions

The present study revealed that wastes from artichoke and grape represent a rich source of bioactive compounds, mainly phenolics. Both by-products ethanolic extracts exhibited strong antimicrobial, antioxidant and anticancer activities suggesting their use as effective low-cost raw materials in the pharmaceutical industry. Their powders also extended the shelf-life period of fresh boneless breast chicken meat during chilling storage according to microbiological parameters. Therefore, they can be used as novel antimicrobial agents for application in the food industry as natural preservatives.

5. Conflicts of interest

There are no conflicts to declare.

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