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Utilization of Grape and Apricot Fruits By-products as Cheap Source for Biologically Active Compounds for Health Promotion

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Abstract

The present study aims to evaluate the antioxidant and anti-cancer activities of nutraceuticals prepared from apricot kernel and grape seeds extracts. Different bioactive compounds were determined in the prepared nutraceuticals (total phenolic compounds, flavonoids, β-carotene, phytosterols and fatty acids). Acute toxicity of these nutraceuticals was evaluated. Apricot kernel showed the highest content of protein and fat, while grape seeds were rich in carbohydrates. Apricot kernel nutraceutical (AKN) showed the highest content of hydrocarbons, while grape seeds nutraceutical (GSN) showed the highest phytosterol content. Stigmasterol was the major phytosterol present in both nutraceuticals. Oleic acid and linoleic acid were the major unsaturated fatty acids present in AKN and GSN, respectively. GSN showed the highest content of phenolic compounds and total flavonoids, while AKN showed the highest content of β-carotene (2.91mg/100g). GSN showed the highest antioxidant activity in all the studied methods (DPPH, reducing power and ferric thiocynate) compared with apricot kernel nutraceutical. Both nutraceuticals showed anti-cancer activity against liver carcinoma cells (HEPG2), breast cancer cells (MCF7) and lung cell cancer (H460). GSN was the most promising in all types of cancer cells. GSN showed complete safety, while AKN was completely safe up to 6 g/kg mice body weight.

Keywords: Grape seeds, Apricot kernel, nutraceuticals, anti-cancer, antioxidant, by-products.

1. Introduction

Agri-food by-products are promising sources of phytochemicals (phenolic compounds, flavonoids, carotenoids and phytosterols) that can be used in the production of nutraceuticals and functional foods for health promotion. Nutraceuticals mention to food possesses medicinal impact on human health [1]. Nutraceuticals and dietary supplements are new era in human health promotion [2]. Increasing evidence suggested that consumption of vegetables and fruits are associated with reduction of the prevalence of non-communicable diseases such as cancer, cardiovascular diseases, hypertension and diabetes [3-7]. Many of bioactive compounds in the fruits are found in parts that are usually discarded during food processing like seeds, peel and pomace, which are considered as agro-industrial by-products. So, using these by-products for preparation of nutraceuticals and functional foods is of great economical interest and adding value for the fruits harvest.

Apricot (*Prunus armeniaca* L.) is one of the most delicious fruits cultivated worldwide. Apricot belongs to genus *Prunus* of Rosaceae family, the fruits contain stone-seed (containing kernel), which is one of the most important food by-product due to high content of dietary protein (14-45%), oil (28-66.7%) and phytochemicals such as phenolic compounds, carotenoids, tocopherols and phytosterols [8,9]. Oil of the kernel is rich source of tocopherols, phytosterols and essential fatty acids so it can be used in cosmetics and medicinal admixtures as well as enrichment food products [10]. It was reported previously that kernel extracts possess anti-inflammatory effects and improve bowel disorders [11]. The meal of the kernel contains up to 70% proteins, so it can be used for preparation of protein isolates for enhancement of nutritional value of food supplements [12].

Grape (*Vitis vinifera* L.) is a famous fruit all over the world, and is widely used in wine and juice industries, which lead to large amounts of by-products, including grape peels and seeds [13]. The seeds contributed by account 5% of weight of the fruits and representing about 40 to 50% of solid wastes that produce during grape process [14].Grape by-products are rich in phytochemicals and bioactive compounds such as phenolic compounds, flavonoids

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(anthocyanin, tannin and quercetin). It was mentioned that these phytochemicals possess antioxidant, anti-inflammatory, anti-cancer and cardio-protective effects [15, 16]. Phenolic compounds are an important non-nutrient with biological activity usually consumed as part of human diet [17]. Food by-products can be used safely as sources of bioactive compounds for preparation of functional foods, food additives and nutraceuticals.

The present study aims to evaluate the antioxidant and anti-cancer activities of nutraceuticals prepared from apricot kernel and grape seeds extracts. Different bioactive compounds were determined in the prepared nutraceuticals (phenolic compounds, flavonoids, β-carotene, phytosterols and fatty acids profile). Also, the acute toxicity of these nutraceuticals has been evaluated.

2. Materials and methods

2.1. Apricot kernel collection. Apricot stone was collected from Edfina factory from Alexandria, washed, dried, and the kernel were splitted from the shell. All kernels were grounded using a household coffee blender and used in extraction.

2.2. *Grape seeds collection.* Grape seeds were prepared from fresh red grape obtained from local markets. The grape seeds were dried in air drying oven at 40 °C and crushed into 100 mesh powders and used in extraction. The powders of both plants were packaged in polyethylene packages and stored at refrigerator till used for analysis.

2.3. *Cancer cells.* Three human tumor-derived cell lines were supplied from National Cancer Institute, Cairo University, Egypt. HEPG2 (liver carcinoma cell), MCF7 (breast cancer cell) and H460 (Lung cancer cell) were the studied cell lines.

2.4. *Chemicals*. Butylated hydroxyl toluene (BHT), β-carotene, linoleic acid, potassium ferricyanide, ammonium thiocyanate and ferrous chloride were purchased from Sigma (USA). Petroleum ether 40-60°C and ethanol were obtained from BDH Chemical Co.

2.5. *Nutritional composition of apricot kernel and grape seeds powder*. Apricot kernel and grape seeds powder samples were sieved through 100-mesh sieve. The samples were analysed for moisture, protein, fat, crude fiber and ash contents using standard AOAC [18] procedure. Carbohydrates were calculated by difference [100-(moisture+ protein+ fat+ crude fiber+ ash)]. Different chemical analysis were carried out in triplicate and averaged.

2.6. *Preparation of plant extract.* The dried powder of apricot kernel and grape seeds were separately subjected to successive extraction in a continuous extraction apparatus (Soxhlet) until exhaustion with petroleum ether (40-60°C) then ethanol for preparation of petroleum ether and ethanol extracts, respectively. The solvent was completely removed by evaporation under reduced pressure at a temperature not exceeding 40°C. Extract were kept in deep-freeze till used.

2.7. *Preparation of nutraceuticals.* For preparation of nutraceuticals petroleum ether and ethanol extracts from apricot kernel or grape seeds were mixed in a ratio 1:1 using Tween 80 as suspending agent.

2.8. *Assessment of hydrocarbons and phytosterols contents in apricot kernel and grape seeds nutraceuticals.* The unsaponifiable fraction of apricot kernel and grape seeds nutraceuticals were prepared according to AOAC [18] to be subjected to GLC analysis of hydrocarbons and phytosterols. Hydrocarbons and phytosterols were analyzed by GLC adopting the following conditions: Column: 10% OV-101 packed column; Stationary phase: Chromosorb W-HP; Detector temperature: 290°C; Injector temperature, 28°C; Carrier gas N_2; flow-rate 30 ml/min; air flow-rate: 300ml/min; H_2 Flow-rate 30ml/min; Detector FID; Chart speed: 0.5 cm/min; Oven program: Initial temperature, 70°C; Final temperature, 270°C; programmed 4°C/min. For 35min at 270°C, total time, 85 min. Identification of hydrocarbons and phytosterols contents of the unsaponifiable matter was carried out by comparison of their retention times with co-injected authentic reference compounds. Quantization was based on peak area integration.

2.9. *Assessment of fatty acids of apricot kernel and grape seeds nutraceuticals.* Fatty acid methyl esters of apricot kernel and grape seeds nutraceuticals were prepared according to AOAC [18] to be subjected to GLC analysis of fatty acids. Assessment of the methyl ester was carried out by injecting 2µl into a

Hewlett Packard HP-system 6890 gas chromatograph equipped with FID. HP-5 capillary column (30 m x 0.32 mm i.d.; 0.25 um film thickness) was used to separate the different methyl esters. The chromatographic analysis conditions were: initial temperature 70 °C with a hold for 1 min, then rose to 120 °C at a rate of 40 °C /min with 2 min hold then the temperature was finally raised to 220 °C at a rate of 4 °C /min with another 20 min hold. The injector and detector temperatures were 250°C and 280 °C respectively. Identification of the fatty acid methyl esters were carried out by direct comparison of retention times of each of the separated compounds with standards of the fatty acid methyl esters analysed under the same conditions. Quantization was based on peak area integration.

2.10. ***Determination of total phenolic compounds in the prepared nutraceuticals.*** Total phenolics were determined colorimetrically in the apricot kernel and grape seeds nutraceuticals using Folin-Ciocalteu reagent [19]. Absorbance was measured at 765 nm using UVPC spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g dietary supplement. The results were expressed as Mean±SD.

2.11. ***Determination of total flavonoids in the prepared nutraceuticals.*** Total flavonoids content in apricot kernel and grape seeds nutraceuticals was determined by the aluminium chloride colorimetric method [20]. The total flavonoids content was calculated from a calibration curve, and the result was expressed as mg quercetin equivalent per g (QE). The results were expressed as Mean±SD.

2.12. ***Determination of β-carotene in the prepared nutraceuticals.*** β-Carotene was estimated by the method of Ranganna [21]. One gram sample was extracted from apricot kernel and grape seeds nutraceuticals with petroleum ether (60–80 °C) and acetone (3:2). The extract was decanted into a 50 ml volumetric flask and the extraction was continued 4–5 times to dissolve all fat-soluble pigments completely. The total volume was adjusted to 50 ml with the extraction medium and the absorbance was noted using a spectrophotometer at 450 nm against the blank. The results were expressed as follows: β-Carotene (mg/100g) = [¼ Absorbance x 13.9 x 10^4 x 100]/ Weight of sample (g) x 560

2.13. ***Evaluation of antioxidant activity by DPPH radical scavenging method in the prepared nutraceuticals.*** Free radical scavenging activity of the prepared nutraceuticals was measured by 1,1-diphenyl-2-picryl hydrazyl (DPPH) according to the method of Shekhar and Anju[22]. In brief, 0.1 mM solution of DPPH in ethanol was prepared. This solution (1ml) was added to 3 ml of different nutraceuticals in ethanol at different concentrations (20, 30, 40, 50, 100, 200, 300, 400, 500 and 1000 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min then; absorbance was measured at 517 nm by using spectrophotometer. Reference standard compound being used were BHT and ascorbic acid in different concentrations (20, 30, 40, 50, 100, 200, 300, 400, 500 and 1000 µg/ml) and experiment was done in triplicate. The percent DPPH scavenging effect was calculated by using following equation: DPPH scavenging effect (%) or Percent inhibition = [A0 - A1 / A0] × 100. Where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard sample.

2.14. ***Evaluation of reducing power assay of the prepared nutraceuticals.*** The reducing power of apricot kernel and grape seeds nutraceuticals was determined according to the method of Oyaizu [23]. Various concentrations of nutraceuticals and BHT as standard (1, 2, 3, 4, and 5 mg/ml) in 1 ml of methyl alcohol were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50°C for 20 min, and then 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for10 min at 1000xg. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. The assay was carried out in triplicate, and the results are expressed as mean ± standard error (SE). Increase in absorbance of sample with concentrations indicates high reducing potential of the samples.

2.15. ***Determination of total antioxidant activity by ferric thiocynate method (FTC) in the prepared nutraceuticals.*** Total antioxidant activity of apricot kernel and grape seeds nutraceuticals was determined by applying ferric thiocyanate (FTC) method, the standard method as described by Kikuzaki and Nakatani [24] was used. A mixture of 4.0 mg nutraceutical in 4ml absolute ethanol, 4.1 ml of 2.5% linolenic acid in absolute ethanol, 8.0 ml of 0.05M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in a vial with a screw cap and then placed in an oven at 40 °C in the dark. To 0.1 ml of this solution was added 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after

addition of 0.1 ml of 0.02M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red color was measured at 500nm in the seventh day. BHT was used as positive controls while the mixture without nutraceutical sample was used as the negative control. All tests were run triplicate, and an analysis of all samples was done in triplicate and averaged. The antioxidant activity of nutraceuticals samples was carried out in triplicate. Calculation of antioxidant activity according to the following equation [25]: Inhibition (%) = 100-[(A1-A0)×100] where, A0 is the absorbance of the control and A1 is the absorbance of the sample.

2.16. ***Anticancer Activity of the prepared nutraceuticals.*** Anticancer Activity of apricot kernel and grape seeds nutraceuticals was tested using the cell line technique according to Cordero et al. [26]. Cells of HEPG2 (liver carcinoma cell), MCF7 (breast cancer cell) and H460 (Lung cancer cell) were plated in 96-multiwell plate (10^4 cells/well) for 24h before treatment to be attached to the wall of plate. The studied dietary supplements were dissolved in dimethyl sulfoxide (DMSO) at 10 mM as a stock solution. Dilutions with culture media were prepared just prior to addition to test plates. Different concentration of the nutraceuticals (0, 0.03, 0.3, 3, 30 and 300 μg/ml) were added to the cell monolayer, triplicate wells were prepared for each individual dose of each plant powder. Plates were incubated for 48h at 37 °C and in atmosphere of 5% CO_2. After 48h, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain, then excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction of cancer cell and plants powder dose was plotted. IC_{50} (concentration which reduces survival of the exposed cancer cells to 50%) was obtained from the curves.

2.17. ***Acute oral lethal toxicity test.*** Acute lethal toxicity test of apricot kernel and grape seeds nutraceuticals was carried out according to Goodman et al. [27]. Adult normal male and female, albino mice of 21–25 g body weight were used. The 24 h mortality counts among equal sized groups of mice (8 animals/group) receiving progressively increasing oral dose levels of the different extracts were recorded.

2.18. ***Statistical analysis***. The experimental results concerning this study were expressed as mean±standard error (Mean ± SE) of the 3 parallel measurements.

3. Results and Discussion

3.1. ***Nutritional composition of apricot kernel and grape seeds.*** Agri-food by-products are a cheap source for many bioactive compounds such as phenolic compounds, flavonoids, polyphenols, sterols and carotenoids [28]. In the present research apricot kernel and grape seeds by-products were studied and their antioxidant and anti-cancer activities were evaluated. Apricot fruits contains 11.7-22.2% stones, which yield 30.7-33.7 % kernels [29].Traditionally apricot kernel was used in Egypt in the preparation of Duqqa, which is an Egyptian condiment consisting of a mixture of herbs (mint), nuts (usually peanut) and spices(coriander and cumin) [30]. Apricot kernel is rich source of protein, oil and fiber [31]. Grape seeds contributes by account 5% of weight of the fruits and representing about 40 to 50% of solid wastes that produce during grape process [14].Grape by-products are rich in phytochemicals and bioactive compounds such as phenolic compounds, flavonoids (anthocyanin, tannin and quercetin) [15].So, a new concept for using agri-food by-products for preparation of nutraceuticals, functional foods and dietary supplements must be followed as way for sustainable development and add-value of foods. Figure1 show the nutritional composition of apricot kernel and grape seeds, respectively. Apricot kernel contain high amount of protein (22.6%) and fat (42.8%) than grape seeds. Grape seeds were found to contain the highest amount of carbohydrate (70.9%) than apricot kernel. It was reported previously by MatthäusandÖzcan [32] that apricot seeds contain higher amount of oil ranged from 45.2 to 53.4 g/100g. Also Alpaslan and Hayta [33] reported that carbohydrates ranged from 17 to 27.9%, protein ranged from 14.1 to 45.3% and oil ranged from 27.7 to 66.7% in apricot kernel. Ash and fiber content in the defatted apricot kernel was 4.82% and 9.17%, respectively [34]. Felhiet al. [35] found that grape seeds contain 6.9% protein, 2.87% fat, 4.5% ash and 82.5% carbohydrate.

3.2. ***Hydrocarbons and phytosterols profile of apricot kernel and grape seeds nutraceuticals.*** Table 1 showed hydrocarbons and phytosterols in apricot kernel and grape seeds nutraceuticals. Apricot kernel nutraceutical showed the highest content of hydrocarbons (85.95%), while grape seeds nutraceutical showed the lowest content of hydrocarbons (73.45%). C21 was the major hydrocarbon in the nutraceuticals of apricot kernel and grape seeds it present by 38.73% and 53.54%, respectively. Grape seeds nutraceutical showed the highest content of phytosterols (11.86%), while

apricot kernel nutraceutical showed the lowest content of phytosterols (6.61%). Stigmasterol was the major phytosterol present in the both nutraceuticals, it present by 4.31% and 8.29% in apricot kernel nutraceutical and grape seeds nutraceutical, respectively. β-Sitosterol was present by 2.3% and 3.57% in apricot kernel nutraceuticaland grape seeds nutraceutical, respectively. The present results are in agreement with the results of Rudzińska et al. [36] who reported that apricot kernel oil contain 9 sterols (campesterol, β-sitosterol, Δ5-avenasterol, 24-methylene-cycloartanol, cholesterol, gramisterol, Δ7-stigmasterol, Δ7-avenasterol and citrostadienol).

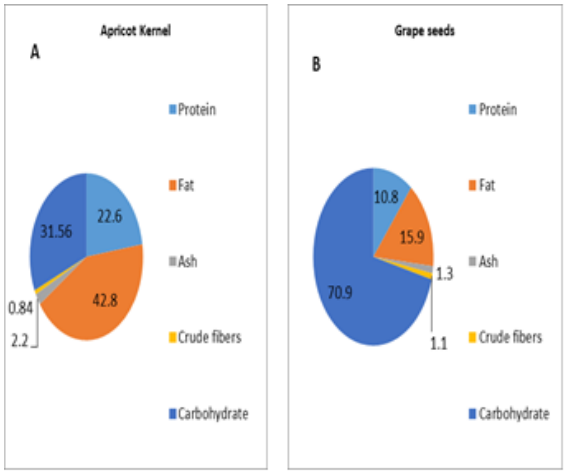

Fig (1): Nutritional composition (g/100g dry sample) of apricot kernel (A) and grape seeds powder (B)

3.3. ***Fatty acids profile of apricot kernel and grape seeds nutraceuticals.*** Table 2 showed the fatty acids present in apricot kernel and grape seeds nutraceuticals. The results revealed that oleic acid was the major unsaturated fatty acid present in apricot kernel nutraceutical; it was present by 51.1%, while linoleic acid was the major unsaturated fatty acid present in grape seeds nutraceutical as 45.1%. Apricot kernel dietary supplement contain 5.1% linolenic acid (ω-3). Palmitic acid was the major saturated fatty acid, in the two nutraceuticalsit present by 5.5% and 8.5% in apricot kernel nutraceuticaland grape seeds nutraceutical, respectively. Grape seeds nutraceuticalcontain total unsaturated fatty acids by 88.35% and total saturated fatty acids by 8.55%. The percentage of total unsaturated fatty acids present in apricot kernel was 73.3%, while the saturated fatty acids were12%. The present results of fatty acids profile of apricot kernel nutraceuticalare in agreement with the results of Matthäus and Özcan [32] who found that oleic acid was the major unsaturated fatty acid in apricot seed oil.

Table (1): GLC analysis of hydrocarbons and phytosterols of apricot kernel and grape seeds nutaceuticals.

Hydrocarbon & Phytosterols	Apricot Kernel	Grape seeds
Hydrocarbon:		
C11	**0.11***	**0.8**
C12	**0.59**	**0.41**
C13	**0.65**	**0.81**
C14	**0.96**	**1.18**
C15	**2.81**	**0.45**
C16	**1.73**	**1.36**
C17	**0.6**	**0.39**
C18	**2.06**	**4.42**
C19	**4.4**	**4.82**
C20	**2.83**	**1.6**
C21	**38.73**	**53.54**
C22	**13.79**	-
C24	**7.00**	-
C26	**2.13**	**0.61**
C27	**3.52**	**0.3**
C28	**2.12**	**1.71**
C29	**1.92**	**1.05**
Total hydrocarbon	**85.95**	**73.45**
Phytosterols:		
Stigmasterol	4.31	8.29
β-Sitosterol	2.3	3.57
Total phytosterols	**6.61**	**11.86**

***: Values are expressed as relative area percentage of total hydrocarbons and phytosterols.**

3.4. ***Phytochemical composition of the prepared nutraceuticals.*** Phytochemical composition of the prepared nutraceuticals is appeared in Figure 2. The total phenolic content of grape seeds and apricot kernel nutraceuticals were evaluated using the method of Folin-Ciocalteu, which is based on the reaction that electrons are transferred from phenolic compounds to the Folin-Ciocalteu reagent in alkaline medium [37]. The total flavonoids content values of grape seeds and apricot kernel nutraceuticals were determined by the AlCl3 colorimetry method according to Chang et al. [20] which is based on the reaction that the 3-hydroxy-4-hydroxyl or 5-hydroxy-4-carbonyl or o-2-phenolic hydroxyl of flavonoids is combined with $Al3^{+}$ to form a red complex under an alkaline condition [20]. Grape seeds nutraceutical showed the highest content of total phenolic (41.4±3.03) and total flavonoids (27.9±0.368) compared with apricot kernel nutraceutical. The highest content of β-Carotene was observed in apricot kernel (2.91±0.057) nutraceutical. Apricot kernel contains phenolic compounds such as quercetin, catechin, epicatechin, p-coumaric acid, caffeic acid, ferulic acid and their esters have been identified in the fruits [38]. Total phenolic in apricot kernel was found to be ranged from 75.47 to 210.63 µg g-1 [9, 39], while Juhaimiet al. [40] found that total phenolic and total flavonoids in apricot kernel were ranged

from 27.18 to 38.51 mg GAE/100 g and 17.33 to 23.56 mg CE/g, respectively. β-carotene present in apricot kernel as 2.78 mg/100 g [9], while percent is ranged from 4.23–6.68 mg/100g in the oil of apricot kernel [34].Grape by-products are rich in phytochemicals and bioactive compounds such as phenolic compounds (gallic acid, hydroxybenzoic acid, ferulic, caffeic and cinnamic acid), flavonoids (anthocyanin, resveratrol, tannin and quercetin) [15, 41, 42]. Total phenolic and flavonoids were observed in grape seeds 392.5 mg of GAE/g and 256.2 mg QE/g, respectively [35].

Table (2): Fatty acids contents of apricot kernel and grape seeds nutaceuticals.

Fatty acids	Apricot Kernel	Grape seeds
Myristic acid: C14 (0)	-	0.05*
Plamitic acid: C16 (0)	6.5	8.5
Oleic acid: C18 (1)	51.1	43.1
Linoleic acid: C18(2)	17.1	45.1
Linolenic acid:C18 (3)	5.1	0.15
Arachidic acid: C20 (0)	5.5	-
Total identified saturated fatty acids	12	8.55
Total identified unsaturated fatty acids	73.3	88.35

***: Values are expressed as relative area percentage of total fatty acids.**

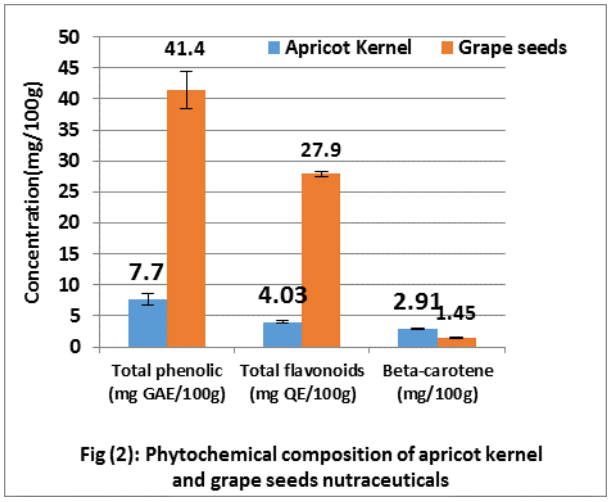

Fig (2): Phytochemical composition of apricot kernel and grape seeds nutraceuticals

3.5. Antioxidant activity by DPPH radical scavenging method in the prepared nutraceuticals. Table 3 showed the results of free radical scavenger activity of ascorbic acid, BHT, apricot kernel and grape seeds nutraceuticals. DPPH scavengingmethod is easy and rapid in accordance with long stability of the free radical DPPH for evaluation of antioxidant activity [43]. In the present study different concentration ranged from 20 to 1000 µg/mlof ascorbic acid, BHT, apricot kernel and grape seeds nutraceuticals were evaluated against DPPH free radical. Ascorbic acid as standard showed the highest inhibition activity compared to BHT and both dietary supplement. Grape seeds nutraceutical observe antioxidant activity in all used concentration higher than BHT. Apricot kernel was the lowest antioxidant against DPPH free radical. The results revealed that grape seeds nutraceutical is very effective treatment against free radical. Also the activity increased with the increment of concentration till be stable at concentration 250 to 1000 µg/ml. The present results are in agreement with the results of Rajendran et al. [44] who found that the scavenging effect of ascorbic acid increased with its concentration. The antioxidant scavenging activity of grape seeds was observed previously [35].

3.6. *Antioxidant activity by ferric thiocynate in the prepared nutraceuticals.* Figure 3 showed the antioxidant activity in ferric thiocynate method of ascorbic acid, BHT, apricot kernel and grape seeds nutraceuticals. In this method the studied nutraceuticals or standards inhibit the oxidation of linoleic acid. Grape seeds nutraceutical showed the highest activity (98.3%) followed by Apricot kernel nutraceutical (96.6%). BHT showed the least antioxidant activity (89.2%).

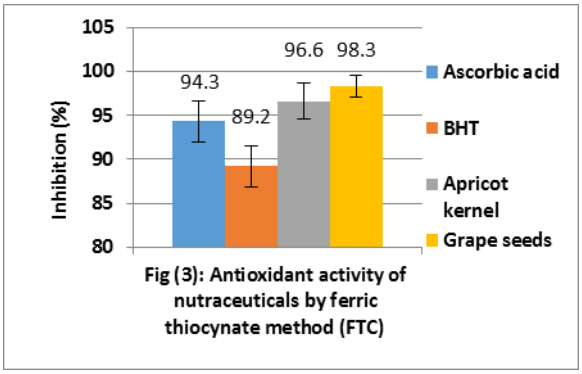

Fig (3): Antioxidant activity of nutraceuticals by ferric thiocynate method (FTC)

3.7. *Reducing power of the prepared nutraceuticals.* Figure 4 showed the reducing power activity of BHT, apricot kernel and grape seeds nutraceuticals. In reducing power method, the antioxidantspresent in nutraceuticals reduced Fe^{3+} from potassium ferrocyanide to Fe^{2+} this lead to the appearance of Prussian blue, which increases with the concentration of the reducing power of the nutraceutical. BHT showed the highest reducing power followed by grape seeds nutraceutical. Apricot kernel nutraceutical showed the lowest reducing power. The present results are in agreement with the results of Weidneret al. [42] who found that grape seeds possess reducing power similar to ascorbic acid. The reducing power of the nutraceutical may be attributed as important indicator of its potential antioxidant activity. The current study revealed that as the

nutraceuticals concentration increases the reducing power increase and vice versa.

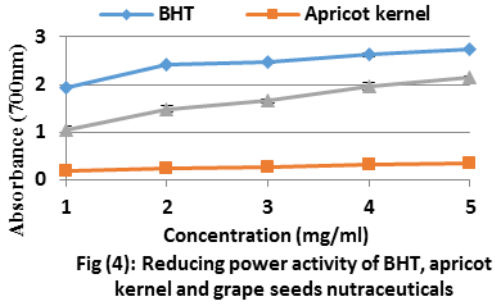

Fig (4): Reducing power activity of BHT, apricot kernel and grape seeds nutraceuticals

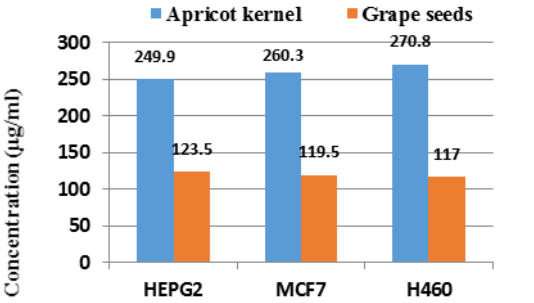

Fig (5): IC_{50} dose of the apricot kernel and grape seeds nutraceuticals under study

3.8. ***Anticancer Activity of the prepared nutraceuticals.*** Figure 5 showed the IC50 dose of apricot and grape seeds nutraceuticals in different types of cancer cells. The results revealed that both nutraceuticals showed anti-cancer activity against liver carcinoma cells (HEPG2), breast cancer cells (MCF7) and lung cell cancer (H460). Grape seeds nutraceutical was the most promising in all types of cancer cells. The IC50 of grape seeds nutraceutical against HEPG2 cells, MCF7 cells and H460 cells was 123.5 µg, 119.5 µg and 117 µg, respectively. The IC50 of apricot kernel nutraceutical against HEPG2 cells, MCF7 cells and H460 cells was 249.9 µg, 260.3 µg and 270.8 µg, respectively.It was reported previously that grape seedspossess anticancer activity through inhibiting cellular proliferation, inducing apoptosis, arresting cell cycleand inhibiting metastatic processes [45]. Apricot kernel extract possess anti-cancer activity against colon cancer (HT-29 cell line) in-vitro [46].

3.9. ***Acute toxicity of the prepared nutraceuticals.*** Acute lethal toxicity test revealed that grape seeds nutraceutical was completely safe up to the highest dose used 12g/kg mice body weight that is corresponding to 93g/70 kg man body weight human when the dose of mice was extrapolated to corresponding estimates in human adopting interspecies dosage conversion scheme [47]. Apricot kernel nutraceutical on the other side was completely safe up to 6 g/kg mice body weight that is corresponding to 46.5g/70 kg man body weight human when the dose of mice was extrapolated to corresponding estimates in human adopting interspecies dosage conversion scheme. The toxicity of apricot kernel may be attributed to the presence of amygdalin, which is a cyanogenic glycoside. Consumption of cyanogenic compounds from some plants such as almond, cherries, plums, peaches and apricot kernel produced acute and sub-acute health problems such as nausea, vomiting, headache and cardiac arrest [48-50].

Table (3): Free radical scavenger activity of ascorbic acid, BHT,apricot kernel and grape seeds nutraceuticals

Concentration (µg/ml)	Ascorbic acid	BHT	Apricot kernel	Grape seeds
20	74.3±0.309	1.35±0.227	-	6.9±0.294
30	79.2±0.287	17.9±0.249	-	29.2±0.205
40	82.1±0.169	50.7±0.850	-	52.2±0.368
50	84.7±0.082	56.9±0.125	-	72.3±0.329
100	86.6±0.205	71.7±0.169	13.4±0.403	76.4±0.368
250	99.0±0.125	88.9±0.047	25.9±0.287	92.7±0.249
500	99.0±0.125	89.0±0.327	30.7±0.339	92.7±0.249
750	99.0±0.125	89.3±0.205	37.5±0.509	92.7±0.249
1000	99.0±0.125	91.1±0.403	48.9±0.432	92.7±0.249

4. Conclusion: Apricot kernel and grape seeds nutraceuticals prepared in the current study possess antioxidant and anti-cancer activities. These activities of the studied nutraceuticals may be attributed to the presence of phytochemicals such as total phenolic, total flavonoids, β-carotene and phytosterols such as β-sitosterol and stigmasterols. The results revealed that grape seeds nutraceutical is the promising one.

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6. Conflicts of interest

The authors declare no conflicts of interests.

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