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Antioxidant and anticancer activities of peanut (*Arachis hypogaea* L.) skin ultrasound extract

K.S.M. Hammad, A.M. El-Roby, and S.M. Galal✉

Department of Food Science, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt
✉Corresponding author: asadgalal@agr.cu.edu.eg

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SUMMARY: This study evaluates the effect of ultrasound-assisted extraction on the extractability of polyphenols from peanut skins (PS) and their antioxidant, and anticancer activities. The extraction was performed with solid/solvent ratios of 1:20 and 1:30 (w/v) at ultrasound intensity ranging from 5.8 to 15.4 W/cm^2 for different extraction times (10, 20, 30 and 40 min). The highest polyphenol yield was 167.46 mg GAE/g dried PS. The most abundant polyphenols were catechin, syringic acid, and vanillic acid. The PS ultrasound extract (PSUE) increased the oxidative stability of sunflower oil by four times its initial level. PSUE possessed high inhibitory activity against MCF-7, HepG-2, HCT-116, and PC-3 cancer cell lines, with IC_{50} ranging from 1.85 ± 0.13 to 6.1 ± 0.43 µg/ml. In addition, the cytotoxicity of PSUE was examined on HFB4 human normal melanocytes using the MTT assay. These results suggest that PSUE can be used as a natural antioxidant and anticancer agent.

KEYWORDS: *Anticancer; Antioxidant; Peanut skin; Polyphenols; Sunflower oil; Ultrasound-assisted extraction.*

RESUMEN: ***Actividades antioxidantes y anticancerígenas del extracto obtenido por ultrasonido de piel de maní (Arachis hypogaea L.).*** Este estudio evalúa el efecto de la extracción asistida por ultrasonido sobre la extractabilidad de los polifenoles de la piel de maní (PS) y sus actividades antioxidantes y anticancerígenas. La extracción se realizó con relaciones sólido/solvente de 1:20 y 1:30 (p/v) a una intensidad de ultrasonido que varió de 5,8 a 15,4 W/cm^2 para diferentes tiempos de extracción (10, 20, 30 y 40 min). El mayor rendimiento de polifenoles fue de 167,46 mg GAE/g de PS seco. Los polifenoles más abundantes fueron la catequina, el ácido siríngico y el ácido vanílico. El extracto de ultrasonido PS (PSUE) aumentó cuatro veces la estabilidad oxidativa del aceite de girasol. PSUE poseía una alta actividad inhibitoria contra las líneas celulares de cáncer MCF-7, HepG-2, HCT-116 y PC-3, con IC50 que oscilaba entre 1,85 ± 0,13 y 6,1 ± 0,43 µg/ml. Además, se examinó la citotoxicidad de PSUE en melanocitos humanos normales HFB4 utilizando el ensayo MTT. Estos resultados sugieren que el PSUE puede usarse como un antioxidante natural y un agente anticancerígeno.

PALABRAS CLAVE: *Aceite de girasol; Anticancerígeno; Antioxidante; Extracción asistida por ultrasonido; Piel de maní; Polifenoles.*

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1. INTRODUCTION

Recent studies indicate that many food wastes are rich sources of bioactive compounds which could be used as nutraceuticals and functional foods (Leichtweis *et al*., 2021). The peanut (*Arachis hypogaea* L.) is an important commercial crop used to produce oil. It is an ingredient in peanut butter, confections, and other finished products. The worldwide production of peanuts with shells in 2020 was 53 million tonnes (FAOSTAT, 2022). PS is the pink-red coat which is produced as waste after peanut kernels are roasted and blanched. It represents 3% of the fruit weight. It has limited industrial applications due to its high level of tannins, bitter flavor, low-calorie level, and poor commercial value. However, it is rich in various bioactive compounds which belong to polyphenols. Composite film containing PS polyphenol extract demonstrated DPPH and ABTS radical scavenging activity (Dai *et al*., 2022).

The oxidation of oils deteriorates the quality characteristics of food during storage. The use of synthetic antioxidants in the food sector is increasingly restricted because they have the potential to cause cancer (Bhadresha *et al*., 2022). This trend is accompanied by the expansion of the use of natural antioxidants such as polyphenols from plant sources. The cytotoxicity of polyphenols from different plant sources has been investigated in cancer cell lines. Olive pomace methanolic extract showed anticancer activity against HepG2, MCF-7, PC3 and HCT116 cell lines (Mahmoud *et al*., 2018). Meanwhile, moringa leaf aqueous extract exhibited the same effect on human lung cancer A549 cells (Bhadresha *et al*., 2022).

Ultrasound-assisted extraction (UAE) is becoming more widely used because of issues with traditional extraction processes. Compared with conventional techniques, the recent ones are more efficient, require less energy, and yield high-quality extracts (Sridhar *et al*., 2021). Khaopha *et al*. (2015) indicated that PS methanolic extract obtained by maceration had an anticancer effect against HeLa, HT29, HCT116 and Jurkat cells.

There is a lack of studies about the antioxidant and cytotoxic activities of PSUE which is rich in polyphenols on colon, breast, and prostate carcinoma cells. The objectives of the present study were maximizing the recovery of polyphenols from PS using ultra-sound assisted extraction, the characterization of the phenolic extract, and evaluating its ability to extend the shelf-life of refined sunflower oil, besides its cytotoxicity on different carcinoma cell lines.

2. MATERIALS AND METHODS

2.1. Materials

Three kg of skins from roasted peanuts were obtained from the local Roasting and Peeling Plant of peanuts, Cairo, Egypt. Completely refined sunflower oil (RBD) without added antioxidants was provided by Cairo Oil and Soap Company (Egypt). Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), Trypan blue dye, 0.25% Trypsin-EDTA solution, L-glutamine, gentamycin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) and all the HPLC standards used for the identification of polyphenols were purchased from Sigma (St.Louis, MO, USA). Fetal Bovine serum (FBS) and Dulbecco's Modified Eagle's medium (DMEM) were purchased from Lonza (Walkersville, USA). HepG-2, HCT-116, MCF-7, and PC-3 cells were obtained from the VACSERA Tissue Culture Unit.

2.2. Preparation and chemical composition of PS

A PS sample (500 g) was ground and sieved in a 20-30 mesh. The chemical composition of PS powder was determined according to AOAC (2012). The Kjeldahl method was used to determine the total nitrogen content. The protein content on a nitrogen basis was measured using a conversion factor of 6.25. Ether extract was obtained using diethyl ether in a Soxhlet system. The ash content was determined by incineration in a muffle furnace at 550 °C. Crude fiber was determined gravimetrically after digestion of the sample with 1.25% sulfuric acid solution and 1.25% sodium hydroxide solution.

2.3. Extraction of polyphenols

Polyphenols were extracted from a roasted peanut skin powder sample with 80% ethanol aqueous solution (v/v). Extraction was performed for 10, 20, 30, and 40 min using a Fisher Sonic Dismembrator (Model 300, USA) and solid/solvent ratios of 1:20 and 1:30 (w/v). Ultrasound power (*P*) and ultrasound intensity (UI) were calculated using the following equations by Vernes *et al*. (2019).

$$Power\ (\mathrm{W}) = \left(\frac{dT}{dt}\right) \times Cp \times M \qquad \text{Eq. (1)}$$

Where (dT/ dt) is the increase of °C/min of 200 ml of aqueous ethanol, *Cp* is the heat capacity of 80% aqueous ethanol (2746 J/kg·°C), and M is the mass (kg) of 80% aqueous ethanol. When the generator was set to 95, 100, 105, and 110 W, the estimated *P* was 16.5, 26, 37.8, and 43.7 W, respectively.
The UI (W/cm^2) was calculated as follows

$$UI = 4P/\pi D^2 \qquad \text{Eq. (2)}$$

where D is the diameter (cm) of the ultrasound probe. Supernatants were saved after extraction and kept at -20 °C until analysis.

2.4. Total polyphenols

The total polyphenol content of the PSUE was determined using the Folin-Ciocalteu method as described by Arnous *et al*. (2002) at 760 nm. Results are expressed as mg gallic acid equivalent (GAE)/g dried PS.

2.5. Total flavonoids

The flavonoid content in the extracts was determined as described by Formagio *et al*. (2014). The absorption of the reaction mixture against the blank was recorded at 510 nm. The analysis was performed in triplicate. Values are expressed as mg quercetin equivalents (QE)/g dried PS.

2.6. Identification and quantification of polyphenols using HPLC

The PSUE with the highest level of polyphenols was concentrated at 40 °C under vacuum using a rotary evaporator. The HPLC analysis of the concentrated extract was carried out according to Kim *et al*. (2006). An Agilent Technologies 1100 series liquid chromatograph equipped with a diode-array detector was used. The Eclipse XDB-C_{18} column (150 mm x 4.6 µm x 5 µm) with a C_{18} guard column (Phenomenex, Torrance, CA) was also used. Before injection, the sample was filtered through an 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI). The injection volume was 20 µl. The mobile phase contained (A) acetonitrile and (B) 2% acetic acid. Gradient flow was conducted at 0.8 ml/min. Peaks were observed simultaneously at 280, 320, and 360 nm. The peaks were identified by UV spectra and retention times and their values were compared with those of the standards.

2.7. Antioxidant assays

2.7.1. DPPH assay

The assay was carried out as described by Brand-Williams *et al*. (1995). An aliquot (0.2 ml) of the extract was mixed with 2.7 ml of DPPH solution (45 mg/L) and the mixture was kept for 30 min in the dark. The absorbance was read at 515 nm using a Vis-UV spectrophotometer. BHT was used as a reference. The following formula was used to calculate the percentage of radical inhibition:

$$Inhibition\ percentage\ = \left[\frac{A0 - A1}{A0}\right] \times 100$$

A_0 denotes absorbance in the absence of sample, while A_1 denotes absorbance in the presence of sample. The IC_{50} value represented the concentration of the extract required to decrease the initial absorbance of the DPPH solution by 50%.

2.7.2. Reducing power assay

The reducing power of various concentrations of the PSUE was measured according to the method described by Chang *et al*. (2002) at 700 nm. From the linear regression analysis, the extract concentration which produced 0.5 absorbance ($IC_{0.5}$) was determined. Results were compared with BHT as a standard.

2.8. Quality characteristics of oil

The acid value and peroxide value of the RBD sunflower oil were determined according to the recommended methods of AOCS (2009). The color of the oil samples was measured with the Lovibond Tintometer (Tintometer Ltd., United Kingdom), and a 5.25-inch cell, according to ISO 15305 (1998).

2.9. Rancimat analysis

The efficiency of the investigated PSUE (concentrated extract) in protecting sunflower oil against accelerated oxidation was carried out using Rancimat 743 (Metrohm, Switzerland) according to AOCS (2009). The concentrated extract was added direct-

ly to the oil samples at the investigated levels. The oil samples enriched with 200 and 400 mg GAE from PSUE (concentrated extract)/kg oil were heated at 110 °C and 120 °C, respectively. Sunflower oil without any added antioxidants was used as a control. BHT was used at a concentration of 200 mg/kg oil. The oxidative stability was expressed by the induction period (h). The airflow rate was set at 20 L/h.

2.10. Determination of anti-cancer activity

The investigated human cancer cell lines and human normal melanocytes (HFB-4) were propagated in DMEM supplemented with 10% heat-inactivated FBS, 1% L-glutamine, and 50 µg/ml gentamycin. All cells were kept at 37 °C in a humidified atmosphere which contained 5% CO_2. The colorimetric method of Mosmann (1983) was used to assess the PSUE's cell cytotoxicity. The absorbance was read at 570 nm with a microplate reader (SunRise, TECAN, Inc., USA). IC_{50} values were calculated from a dose-response curve.

2.11. Statistical analyses

All analyses were carried out in triplicate, except for the Rancimat analysis (two repetitions) and HPLC analysis (single determination). Statistica software (StatSoft Inc., Tulsa, OK, USA) was used to analyze the variance of the results. The results were presented in terms of means and standard deviation.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of the PS

The ether extract, protein, ash, and crude fiber contents of PS were 8.32 ± 0.22, 11.57 ± 0.27, 2.57 ± 0.08, and 48.04 ± 2.14% on a dry weight basis, respectively. These results supported earlier findings of Muñoz-Arrieta *et al*. (2021) and proved that PS is a relatively rich source of protein and could be used in the future for the extraction and purification of this nutritional component. PS composition varies with seed maturity and cultivar.

3.2. Polyphenol extraction yield

The results in Figure 1a illustrate that increasing extraction time to 20 min at the highest solid/solvent ratio increased the yield of polyphenols, after which the yield decreased significantly ($p < 0.05$) at each UI used.

The extracted polyphenols with the lowest solid/solvent ratio and at each UI did not significantly ($p > 0.05$) increase when the extraction duration was extended to 40 min (Figure 1b). The yield in polyphenols increased significantly ($p < 0.05$) with the increase in UI from 5.8 to 9.2 W/cm^2 during extractions lasting 30 and 40 min, regardless of the solid/solvent ratio. However, increasing UI from 9.2 to 15.4 W/cm^2 did not significantly ($p > 0.05$) enhance polyphenol extraction. The decrements of extraction yield of polyphenols at higher ultrasonic power could be due to the decomposition of the components (Wang *et al*., 2018). The maximum polyphenol yield (167.46 ± 0.89 mg GAE/g) was obtained with a relatively high solid/solvent ratio for 20 min at UI of 5.8 W/cm^2 (Figure 1a).

The results of the current study demonstrated that the yield of polyphenols achieved with ultrasonic assistance was superior to that of other researchers (Taha *et al*., 2012) who relied on conventional techniques (41.5 mg GAE/g dry PS). This proved that ultrasound-assisted extraction is an efficient method for extracting polyphenols as reported by Sridhar *et al*. (2021).

The results of this investigation indicated that the highest level of flavonoids reached 321.76 ± 2.26 mg of QE/g of PS. Meng *et al*. (2020) found that the flavonoid content in the PS methanolic extract was 234.33 mg rutin equivalents/g PS. These differences in total polyphenol and flavonoid contents could be attributed to cultivar variations, growing conditions, and extraction techniques.

3.3. Identified phenolic compounds in the PSUE

The phenolic composition of PSUE (concentrated extract, 41.55 mg/ml) was analyzed by HPLC. The results are presented in Figure 2 and Table 1.

The PSUE was characterized by a high level of syringic acid and vanillic acid, followed by protocatechuic and *p*-coumaric acids. Rosmarinic, caffeic, and chlorogenic acids were also identified in the examined extract. The analysis demonstrated that catechin was the most abundant flavonoid compound. This result is in line with the results found by Bodoira *et al*. (2022). Moderate amounts of kaempferol, chrysin, and apigenin were also detected in the extract. Francisco and Resurreccion (2009) examined the polyphenols

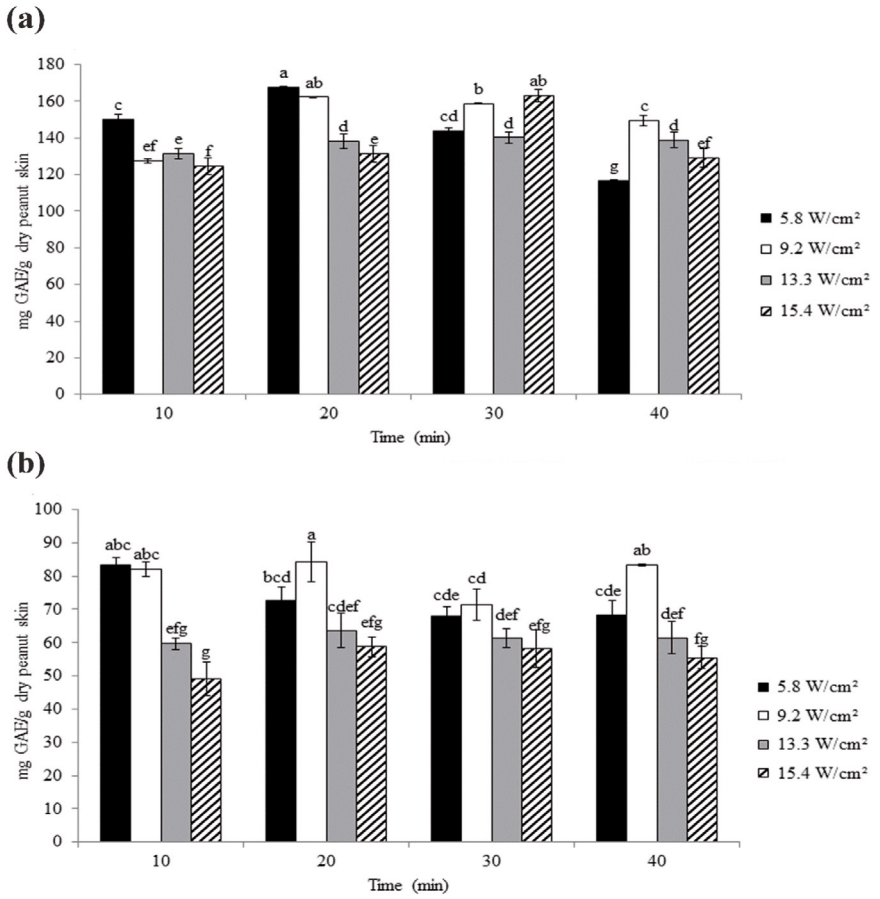

Figure 1. Total phenolic contents (mg GAE/g dry skin) of the ultrasound extract obtained by, (a) peanut skin/aqueous ethanol ratio of 1:20 (w/v) and (b) peanut skin /aqueous ethanol ratio of 1:30 (w/v). Values are means ± standard deviation of three replicates. Bars with different letters indicate significant difference ($p < 0.05$) by Tukey's test

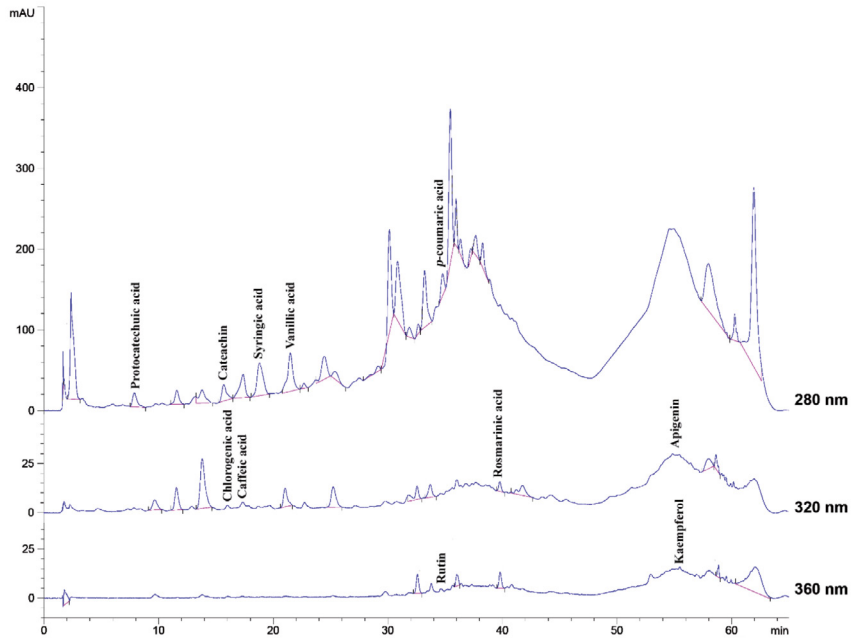

Figure 2. HPLC profiles of phenolic compounds of peanut skin extract simultaneously recorded at 280 nm, 320 nm, and 360 nm. The values refer to a single determination

Table 1. Phenolic and flavonoid compounds of peanut skin ultrasound extract

Identified compounds	Concentration (mg/g dry extract)
Polyphenols	
Gallic acid	ND*
Protocatechuic acid	19.70
ρ-hydroxybenzoic acid	ND*
Gentisic acid	ND*
Chlorogenic acid	1.14
Caffeic acid	1.54
Syringic acid	64.74
Vanillic acid	42.29
Ferulic acid	ND*
Sinapic acid	ND*
ρ-coumaric acid	17.74
Rosmarinic acid	2.92
Cinnamic acid	ND*
Flavonoids	
Catechin	119.98
Rutin	1.36
Apigenin-7-glucoside	ND*
Quercetin	ND*
Apigenin	5.11
Kaempferol	22.30
Chrysin	21.50

* Not detected. The values refer to a single determination

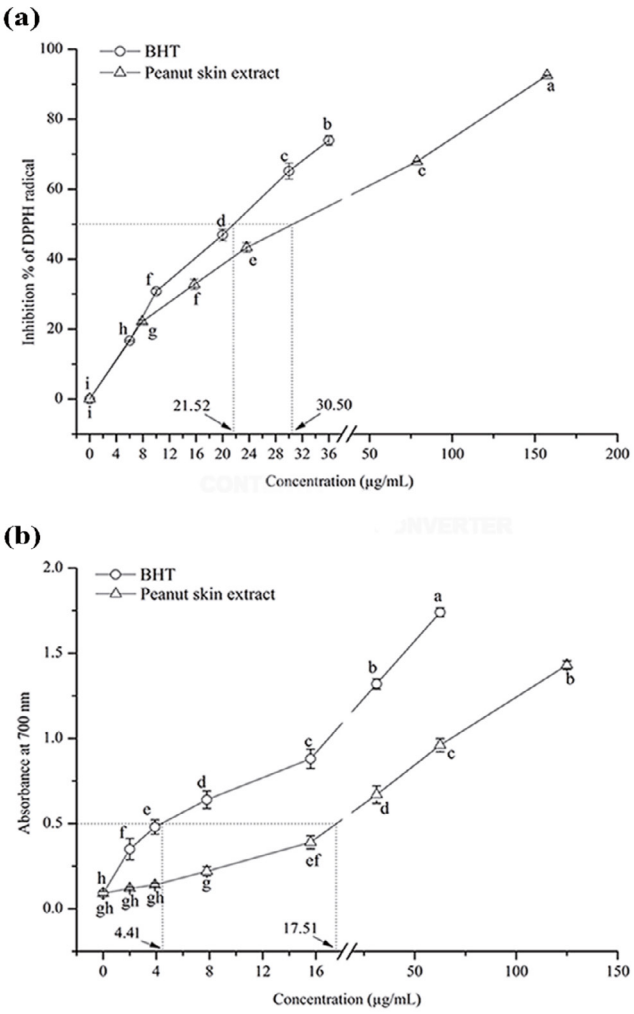

Figure 3. Antioxidant activity of the peanut skin extract compared to BHT as assessed by: (a) DPPH radicals, (b) Ferric reducing power. The results are represented as average values of three replicates ± SD. Bars with different letters indicate significant differences ($p < 0.05$) by Tukey's test

composition of skin extracts from three peanut types (Runner, Virginia, and Spanish). They reported that the discrepancy in the polyphenol levels could be due to the differences in peanut cultivar and skin type.

3.4. Antioxidant activity

The scavenging activity of PSUE against DPPH radicals is shown in Figure 3a.

The results illustrated the positive correlation between PSUE concentration and its activity against DPPH radicals. Compared to BHT, PSUE had a significantly ($p < 0.05$) lower scavenging action on DPPH radicals. PSUE inhibited more than 92% of the DPPH radicals at 157.3 μg GAE/ml. However, the IC_{50} values of PSUE and BHT were 30.5 ± 0.43 μg GAE/ml and 21.65 ± 0.3 μg/ml, respectively. A decrease in the IC_{50} value indicates better antioxidant activity (Brand-Williams *et al*., 1995).

The reduction in ferric ions (Fe^{3+}) from $K_3Fe(CN)_6$ to ferrous ions (Fe^{2+}) by the PSUE is shown in Figure 3b. Increasing extract concentration was accompanied by an increase in reducing power. The results indicated that PSUE had lower reducing power ($IC_{0.5}$=17.51 ± 0.75 mg GAE/ml) than BHT (4.48 ± 0.5 mg/ml). At a concentration of 15.6 μg/ml, PSUE and BHT had reducing power values corresponding to 0.39 ± 0.038 and 0.88 ± 0.057, respectively. These results demonstrate that the PSUE showed concentration-dependent antioxidant activity as reported by Wang *et al*. (2018).

3.5. Oxidative stability of PSUE enriched oil

The acid and peroxide values for the oil were 0.21 ± 0.01 mg KOH/g and 0.73 ± 0.004 meq/Kg, respectively, which supported Codex (2021) requirements for acceptable quality. The results in Table 2 show that the addition of PSUE (concentrated extract) at 200 mg GAE/Kg sunflower oil prolonged the induction period of the oil (tested at 110 °C) by 29%. Meanwhile, enriching oil with BHT at the same level enhanced its stability against oxidation by only 21%.

The addition of 400 mg GAE from PSUE (concentrated extract)/kg oil increased its oxidative stability (measured at 120 °C) by 354%. These results prove that PSUE inhibits lipid oxidation without pro-oxidative effects at a higher concentration.

No significant difference was found in oxidative stability (measured by peroxide value) between sunflower oil samples (control) and those mixed with 0.2% (w/w) of PS ethanol extract, obtained by maceration at room temperature, after 3 days of storage at 60 °C (Larrauri *et al*., 2016). The addition of 1.56 g GAE of PS subcritical fluid extract/kg chia oil increased its oxidative stability to that obtained with TBHQ at 0.2 mg/kg (Bodoira *et al*., 2022).

Color is an important oil quality parameter, which increased as the extract level increased. The addition of PSUE at the highest investigated level produced a more highly colored oil (0.8 ± 0.05 Red/4 Yellow) than the oil prepared with half the concentration of the same extract (0.5 ± 0.05 Red/4 Yellow) or 200 mg BHT/kg oil (0.3 ± 0.00 Red/4 Yellow). However, the color of the studied oil samples was less intense than the red and yellow hues of the bleached sunflower oil (2.5 Red/25 Yellow), according to American trading rules as reported by Guliyev *et al.* (2018).

3.6. Cytotoxic activity of PSUE

The cytotoxicity of the concentrated PSUE (concentrated extract, 41.55 mg GAE/ml) was studied on HepG2, HCT-116, MCF-7, and PC-3 carcinoma cells (Figure 4). The inhibitory activity of the investigated extract against HFB4 human normal melanocytes was also assayed.

The PSUE demonstrated a concentration-dependent reduction in cancer cell viability. The inhibitory activities (IC_{50} value) of the PSUE against HepG-2 (Figure 4a), HCT-116 (Figure 4b), MCF-7 (Figure 4c), and PC-3 (Figure 4d) carcinoma cells were 1.85 ± 0.13, 1.99 ± 0.07, 5.32 ± 0.62 and 6.1 ± 0.43 µg/ml, respectively, compared to 2.93 ± 0.18, 3.5 ± 0.46, 5.9 ± 0.71 and 42.4 ± 2.7 µg/ml for the standard drug. A low IC_{50} value indicates the high sensitivity of a cell line to PSUE or Vinblastine sulphate.

The results showed that PSUE at 31.2 and 15.6 µg/ml had significantly ($p < 0.05$) similar inhibition effects on HepG-2 (Figure 4a) and HCT-116 (Figure 4b) cells as vinblastine sulfate, respectively. It is interesting to note that PSUE at 62.5 µg/ml inhibited 80% of the viability of MCF-7 cells (Figure 4c). The standard drug at 125 µg/ml (Figure 4c) recorded this level of inhibition of the same cells. PSUE at 31.25 µg/ml had a significantly ($p < 0.05$) similar inhibitory effect (~75%) on PC-3 cells as did vinblastine sulfate at 125 µg/ml (Figure 4d).

The PSUE proved more effective than vinblastine sulphate at inhibiting the human cancer cells under investigation. The presence of syringic acid, protocatechuic acid, and catechin might have contributed to the cytotoxic effect of the investigated extract as reported in different studies against cancers of different origins (Elansary *et al*., 2019; Mihanfar *et al*., 2021).

TABLE 2. Induction time and protection factor of sunflower oil enriched with peanut extract at different concentrations

Sample	Induction time (h)	Protection factor [c]
Sunflower oil [a]	3.23 ± 0.28	1.00
Sunflower oil [b]	1.37 ± 0.11	1.00
BHT (200 mg/kg oil) [a]	3.92 ± 0.40	1.21
Extract (200 mg GAE /kg oil) [a]	4.17 ± 0.32	1.29
Extract (400 mg GAE /kg oil) [b]	6.22 ± 0.53	4.54

The values are expressed as mean ± SD of two independent experiments. [a] The induction time was determined at 110 °C. [b] The induction time was determined at 120 °C. [c] Induction time of oil containing antioxidant/Induction time of oil measured at the same temperature.

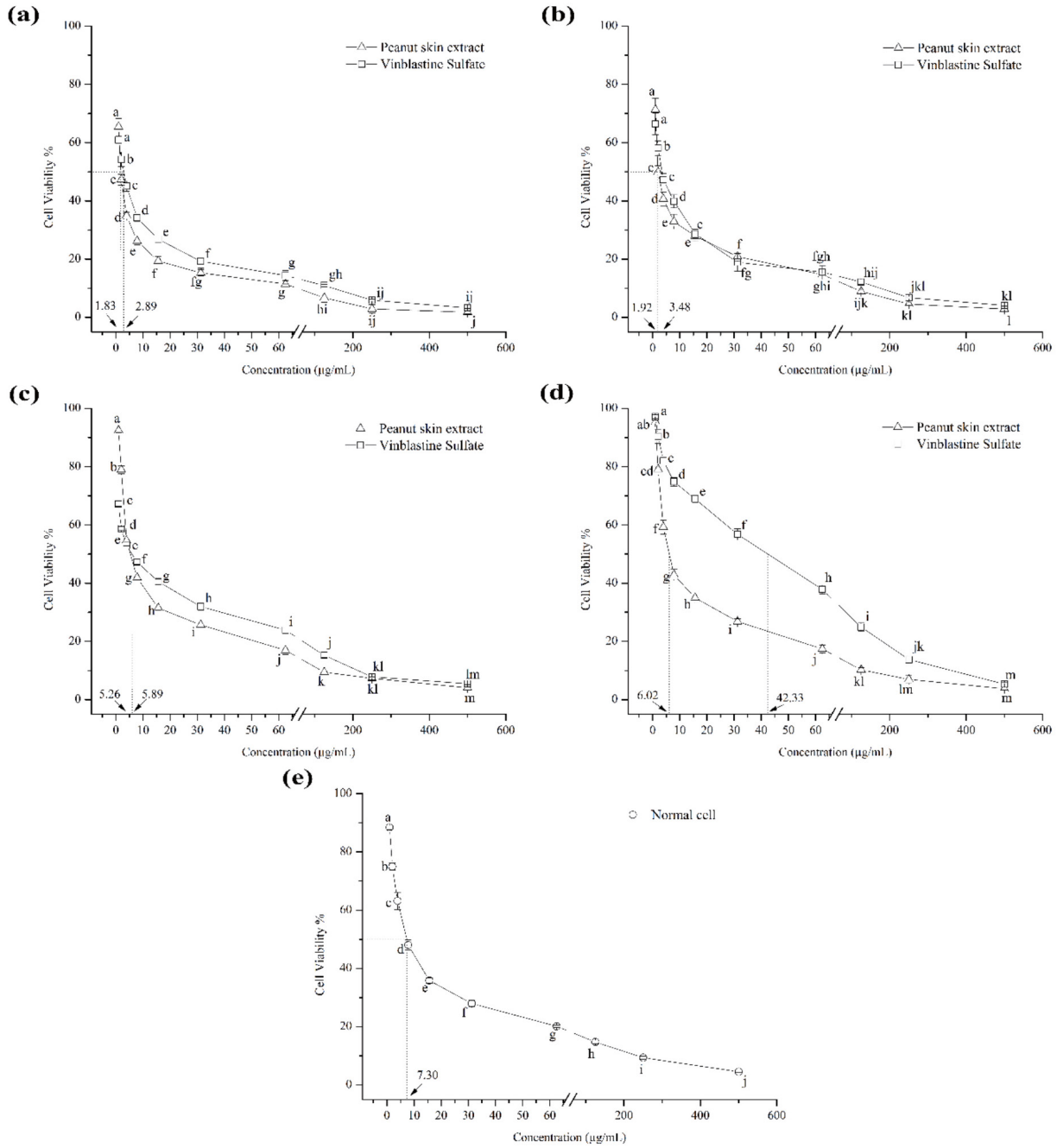

Figure 4. Cytotoxicity effect of peanut skin extract and Vinblastine sulfate concentrations (µg/ml) against (a) HepG-2 hepatocellular cancer cell line, (b) HCT-116 human colon cancer cell line, (c) MCF-7 human breast cancer cell line, (d) PC-3 prostate cancer cell line, and (e) HFB4 human normal melanocytes. The results are represented as average values of three replicates ± SD. Values with different letters indicate significant differences ($p < 0.05$) by Tukey's test

The investigated extract had a low toxicity (CC_{50}= 7.3 ± 0.5 µg/ml) to HFB4 human normal melanocytes (Figure 4e). The selective toxicity levels (IC_{50} values for normal fibroblast cells/IC_{50} value for cancer cells ratio) for HepG-2 and HCT-116 were 3.9 and 3.80, respectively, showing considerable selective ability (> 2) against those cancer cells according to Valderrama *et al*. (2016). Rossi *et al*. (2020) indicated that the PS ethanolic extract did not exert cytotoxicity against human peripheral blood mononuclear cells.

On the other hand, PS methanolic extract was found to have IC_{50} values of 10.9 and 19.3 µg/ml

on HCT-116 and HepG-2 cancer cells, respectively, but did not affect breast carcinoma cells (Taha *et al*., 2012). Furthermore, PS methanolic extract which was obtained by maceration induced the apoptosis of HCT-116 cancer cells (IC_{50} =50.68 μg/ml) (Khaopha *et al*., 2015). They found that MCF-7 cells were less sensitive to the extract (IC_{50} > 90 μg/ml). The results of this study showed that the ultrasound-investigated extract was a more effective anticancer agent than those acquired using other methods from earlier investigations.

Plant-derived medications can advance into clinical trials for further therapeutic development if they are non-toxic to normal cell lines, exhibit cytotoxicity in cancer cell lines, and have high selective toxicity (Rossi *et al*., 2020).

4. CONCLUSIONS

At room temperature, a high yield of polyphenols was successfully extracted from PS with aqueous ethanol using ultrasound. Within the examined ranges, increasing UI and duration had a favorable impact on extraction yields. The incorporation of PSUE into sunflower oil at 200 and 400 mg/kg increased its oxidative stability compared to the samples containing the synthetic antioxidant BHT. The extract proved to have *in vitro* anticancer activity on HepG2, MCF-7, HCT-116, and PC-3 with lower IC_{50} values than those of the standard drug (Vinblastine Sulfate). This suggests that PSUE can be safely used in the preparation of functional foods with antioxidant bioactive ingredients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

AOAC 2012. *Official Methods of Analysis of AOAC International*, 18th ed. AOAC International, Gaithersburg, USA.

AOCS – American Oil Chemist's Society. 2009. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, AOCS Press, Illinois, USA.

Arnous A, Makris DP, Kefalas P. 2002. Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece. *J. Food Compos. Anal*. **15**, 655–665. https://doi.org/10.1006/jfca.2002.1070

Bhadresha, K, Thakore V, Brahmbhatt J, Upadhyay V, Jain N, Rawal R. 2022. Anticancer effect of *Moringa oleifera* leaves extract against lung cancer cell line via induction of apoptosis. *Advances in Cancer Biology - Metastasis* **6**, 100072. https://doi.org/10.1016/j.adcanc.2022.100072

Bodoira R, Martínez M, Velez A, Cittadini MC, Ribotta P, Maestri D. 2022. Peanut skin phenolics obtained by green solvent extraction: characterization and antioxidant activity in pure chia oil and chia oil in water (O/W) emulsion. *J. Sci. Food Agric*. **102**, 2396–2403. https://doi.org/10.1002/jsfa.11577

Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol*. **28**, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Chang LW, Yen WJ, Huang SC, Duh PD. 2002. Antioxidant activity of sesame coat. *Food Chem*. **78**, 347–354. https://doi.org/10.1016/S0308-8146(02)00119-X

Codex 2021. Codex standard for named vegetable oils. Codex Stan 210-1999. Rome: FAO**.**

Dai Q, Huang X, Jia R, Fang Y, Qin Z. 2022. Development of antibacterial film based on alginate fiber, and peanut red skin extract for food packaging. *J. Food Eng*. **330**, 111106. https://doi.org/10.1016/j.jfoodeng.2022.111106

Elansary HO, Szopa A, Kubica P, Al-Mana FA, Mahmoud EA, Zin El-Abedin TKA, Mattar MA, Ekiert H. 2019. Phenolic compounds of *Catalpa speciosa*, *Taxus cuspidata*, and *Magnolia acuminata* have antioxidant and anticancer activity. *Molecules* **24**, 412. https://doi.org/10.3390/molecules24030412

FAOSTAT 2022. Production yearbook. http://faostat.fao.org/site/567/default.aspx#ancor. Accessed on May 2022

Formagio ASN, Volobuff CRF, Santiago M, Cardoso CAL, Vieira MC, Pereira ZV. 2014. Evaluation of antioxidant activity, total flavonoids, tannins and phenolic compounds in *Psychotria* leaf extracts. *Antioxidants* **3**, 745–757. https://doi.org/10.3390/antiox3040745

Francisco MLdL, Resurreccion AVA. 2009. Development of a reversed-phase high performance liquid chromatography (RP-HPLC) procedure for the simultaneous determination of phenolic

compounds in peanut skin extracts. *Food Chem*. **117**, 356–363. https://doi.org/10.1016/j.foodchem.2009.03.110

Guliyev NG, Ibrahimov H, Alekperov J, Amirov FA, Ibrahimova Z. 2018. Investigation of activated carbon obtained from the liquid products of pyrolysis in sunflower oil bleaching process. *Int. J. Ind. Chem*. **9**, 277-284. https://doi.org/10.1007/s40090-018-0156-1

ISO 15305. 1998. Animal and vegetable fats and oils - Determination of Lovibond color. Geneva, Switzerland.

Khaopha S, Jogloy S, Patanothai A, Senawong T. 2015. Histone deacetylase inhibitory activity of peanut testa extracts against human cancer cell lines. *J. Food Biochem*. **39**, 263–273. https://doi.org/10.1111/jfbc.12128

Kim KH, Tsao R, Yang R, Cui SW. 2006. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chem*. **95**, 466–473. https://doi.org/10.1016/j.foodchem.2005.01.032

Larrauri M, Zunino MP, Zygadlo JA, Grosso NR, Nepote V. 2016. Chemical characterization and antioxidant properties of fractions separated from extract of peanut skin derived from different industrial processes. *Ind. Crops Prod*. **94**, 964–971. https://doi.org/10.1016/j.indcrop.2016.09.066

Leichtweis MG, Oliveira MBPP, Ferreira ICFR, Pereira C, Barros L. 2021. Sustainable recovery of preservative and bioactive compounds from food industry bioresidues. *Antioxidants* **10**, 1827. https://doi.org/10.3390/antiox10111827

Mahmoud AE, Fathy SA, Ali MM, Ezz MK, Mohammed AT. 2018. Antioxidant and anticancer efficacy of therapeutic bioactive compounds from fermented olive waste. *Grasas Aceites* **69** (3), e266. https://doi.org/10.3989/gya.0230181

Meng W, Shi J, Zhang X, Lian H, Wang Q, Peng Y. 2020. Effects of peanut shell and skin extracts on the antioxidant ability, physical and structure properties of starch-chitosan active packaging films. *Int. J. Biol. Macromol*. **152**, 137–146. https://doi.org/10.1016/j.ijbiomac.2020.02.235

Mihanfar A, Darband SG, Sadighparvar S, Kaviani M, Mirza-Aghazadeh-Attari M, Yousefi B, Majidinia M. 2021. In vitro and in vivo anticancer effects of syringic acid on colorectal cancer: Possible mechanistic view. *Chem. Biol. Interact*. **337**, 109337. https://doi: 10.1016/j.cbi.2020.109337.

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival. *J. Immunol. Methods*. **65**, 55–63.

Muñoz-Arrieta R, Esquivel-Alvarado D, Alfaro-Viquez E, Alvarez-Valverde V, Krueger C, Reed J. 2021. Nutritional and bioactive composition of Spanish, Valencia, and Virginia type peanut skins. *J. Food Compos. Anal.* **98**, 103816. https://doi.org/10.1016/j.jfca.2021.103816

Rossi YE, Bohl LP, Vanden Braber NL, Ballatore MB, Escobar FM, Bodoira R, Maestri D, Porporatto C, Cavaglieri LR, Montenegro MA. 2020. Polyphenols of peanut (*Arachis hypogaea* L.) skin as bioprotectors of normal cells. Studies of cytotoxicity, cytoprotection and interaction with ROS. *J. Funct. Foods* **67**, 103862. https://doi.org/10.1016/j.jff.2020.103862

Sridhar A, Ponnuchamy M, Kumar PS, Kapoor A, Vo DN, Prabhakar S. 2021. Techniques and modeling of polyphenol extraction from food: a review. *Environ. Chem. Lett.* **19**, 3409-3443. https://doi.org/ 10.1007/s10311-021-01217-8

Taha FS, Wagdy SM, Singer FA. 2012. Comparison between antioxidant activities of phenolic extracts from different parts of peanut. *Life Sci. J.* **9**, 207–215.

Valderrama JA, Delgado V, Sepúlveda S, Benites J, Theoduloz C, Buc Calderon P, Muccioli GG. 2016. Synthesis and cytotoxic activity on human cancer cells of novel isoquinoline quinone-amino acid derivatives. *Molecules* **21**, 1199. https://doi.org/10.3390/molecules21091199

Vernes L, Abert-Vian M, El Maataoui M, Tao Y, Bornard I, Chemat F. 2019. Application of ultrasound for green extraction of proteins from spirulina. Mechanism, optimization, modeling, and industrial prospects. *Ultrason. Sonochem*. **54**, 48–60. https://doi.org/10.1016/j.ultsonch.2019.02.016

Wang T, Guo N, Wang S-X, Kou P, Zhao C-J, Fu Yu-J. 2018. Ultrasound-negative pressure cavitation extraction of phenolic compounds from blueberry leaves and evaluation of its DPPH radical scavenging activity. *Food Bioprod. Process*. **108**, 69-80. https://doi.org/10.1016/j.fbp.2018.01.003