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ANTIOXIDANT POTENTIAL ACTIVITY AND CYTOTOXICITY EFFECTS OF DIFFERENT PARTS OF PEANUTS (*ARACHIS HYPOGAEA* L.)

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ABSTRACT

Peanut skin and peel, a low economic value by-product of the peanut industry, is rich in potentially health promoting compounds. This work aimed to evaluate the efficiency of different organic solvents such as, methanol, ethanol, acetone, and aqueous extract for extraction of (total phenolics TP, total flavonoids TF and total tannins TT) from the peanut skin and peel. For each solvent, the content of total phenolics, flavonoids, tannins was determined. Antioxidant activity of different extracts was screened using the 2, 2-diphenyl-1-picryl hydrazyl ($DPPH^{\bullet}$) radical scavenging, metal chelating activities, reducing power, and ABTS assays. The results showed that 80% acetone is the best solvent for the extraction of total phenolics (436.88 ± 0.71mg/g DW), flavonoids (340.00 ± 1.11mg/g DW) and tannins (184.63 ± 1.95 mg/g DW) and lower in peanut peel aqueous extract (135.5 ± 1.05 mg/g DW), (163 .15 ± 0.67mg/g DW) and (90.55 ± 3.63 mg/g DW). The results revealed that all extracts of the peanut skin and peel exhibited variable antioxidant activity. The highest $DPPH^{\bullet}$ scavenging activity (IC_{50}=52.18 ± 0.47µg/ml) was achieved with acetone extracts of peanut skin compared to acetone extract of peanut peel (IC_{50}=70.51 ± 0.45µg/ml). Antioxidant as measured by Fe^{2+}-chelating revealed the highest activities with methanolic extract of peanut skin (86.10%) compared to peel extract (74.94%). $ABTS^{\bullet+}$ showed the highest value with peanut skin and peel methanol extract. While the acetone skin extract showed the highest activity by reducing power assay. The present study suggests that a skin peanut acetone extract contained higher antioxidant components and potential as compared to the peel. Peanut skin acetone extract exhibited potential as an anticarcinogenic activity, but needs further investigation. It is concluded that acetone and methanol are polar among the solvent used and play a vital role in the extraction of the plant constituents. Therefore these results suggested that peanut skin might be beneficial as a potent antioxidant and anticancer agents and effectively employed as an ingredient in food applications.

KEY WORDS: Antioxidants, $DPPH^{\bullet}$, Iron chelating, reducing power, $ABTS^{\bullet+}$, anticancer, peanut skin and peanut peel.

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INTRODUCTION

Antioxidants are often added to foods to prevent the formation of free radicals and slow down the oxidation process by inhibiting the initiation, propagation steps, leading to the termination of reaction[30, 31]. Antioxidant can be used to retard formation of reactive oxygen species and help to maintain nutritional quality and shelf-life of foods[19]. Several synthetic antioxidants, namely butylated hydroxytoluene (BHT), butylatedhydroxyanisole (BHA), propyl gallate (PG), and ethyl protocatechuate (EP) have been used in foods to prevent oxidation. However, the use of synthetic antioxidants in food ingredients are restricted by legislation and discouraged due to their perceived toxicity and carcinogenicity [22, 28]. Peanut (*Arachis hypogaea* L.) is one of the major oilseed crops of the world. It is also the important sources of food protein in many countries. Their oil is very easily digested, and for this reason they are useful consumptives. Peanuts not only contain the so-called "good" fat (monounsaturated fat), of minerals and vitamins, fatty acids, fiber and bioactive compounds [16]. The antioxidant activity of peanut skins and peels has been reported [2, 7], but there are little information regarding the relationship between antioxidants, their activity, and anti-prolife properties of peanut skins (PS) and peanut peels (PP).

2. MATERIALS AND METHODS

2.1. Chemicals

$ABTS^+$ (2, 2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)), Folin–Ciocalteau reagents, Gallic acid, Quercetin, $DPPH^{\cdot}$(2, 2-diphenyl-1-picrylhydrazyl), Ferrozine: (3- (2 - pyridyl) - 5, 6- bis- (4-phenylsulfonic acid)-1, 2, 4-triazine, BHT: Butyl Hydroxy toluene and, potassium ferricyanide, were from (Sigma Chemical Co., St. Louis, MO, USA).

2.2. Preparation of plant extract

The peanut (skin and peel) were extracted. Briefly, 10 g of the dried powder from skin and peel were soaked with 100 ml of 80% methanol, 80% ethanol, 80% acetone and water and shaking at room temperature for 48 but they are also high in a variety of helpful antioxidants, or chemicals that shield the damaging effects of free radicals. Peanuts are also a source of helpful biologically active components found in plant foods, such as phytochemicals. Some of the phytochemicals in peanuts include flavonoids and phenolic compounds. Recently, peanuts have gained much attention as functional [12]. By-products of the peanut industry, which include peanut plant skins and peels have also been identified as rich sources of phytochemicals, suggesting that the bioactivity found in fruits and vegetables could possibly be present, although currently these plant parts have little economic value [29]. Recent studies suggest that peanuts consumption might reduce the risk of heart diseases by lowering serum low-density lipoprotein (LDL)-cholesterol level and reduce the risk in the development of type II diabetes[14] and Cancer preventation. The health benefits of peanuts have been attributed to the presence h. The extracts were filtered and the extraction was repeated twice. The resulting of different extracts was used for the determination of total phenolic, flavonoid, antioxidant activity and cytotoxic effects on different human cancer cell line (HePG2, HCT116 and MCF-7).

2.3. Total phenolic content

The total phenolic (TP) of peanut extracts were spectrophotometrically determined by Folin Ciocalteu reagent assay using gallic acid for the preparation of calibration curve (20 – 120 mg/l) according to the method of [33]. A suitable aliquot (1 ml) of each extract or standard solution was added to 25 ml volumetric flask, containing 9 ml of distilled water. One milliliter of Folin Ciocalteu`s phenol reagent was added to the mixture and shaken. After 5 min. 10 ml of 7 % Na_2CO_3 solution were added to the mixture. The solution was diluted to 25 ml with distilled water and mixed. After incubation for 90 min. at room temperature, the absorbance was determined at 750 nm with spectrophotometer (Unicum UV 300) against prepared reagent as blank. A total phenolic content in the samples was expressed as mg

gallic acid equivalents (GAE)/g dry weight. All samples were analyzed in triplicates.

2.4. Total flavonoid content

Total flavonoid (TF) of peanut extracts were spectrophotometrically determined by the aluminum chloride method using quercetin as a standard [48]. One ml of extract or standard solution (quercetin, 20–120 mg/l) was added to 10 ml volumetric flask, containing 4 ml of distilled water. To the flask 0.3 ml 5 % $NaNO_2$ was added and after 5 min 0.3 ml 10 % $AlCl_3$ was added. At 6th min, 2 ml 1M NaOH was added and the total volume was made up to 10 ml with distilled water. The solutions were mixed well and the absorbance was measured against prepared reagent blank at 510 nm by using spectrophotometer (Unicum UV 300). Total flavonoids in sample was expressed as mg quercetin equivalents (QE)/ g fresh weight. Samples were analyzed in triplicates.

2.5. Total tannins content

Total tannins (TT) of different extracts was measured using the Folin-Ciocalteu reagent assay according to [27]. One ml of peanut extracts or standard solution of (tannic 20-120mg/l) was added to 7.5 ml distilled water (dH_2O) then add 0.5 ml of Folin reagent and 1 ml of 35% sodium carbonate solution. The volume was made up for 10 ml with distilled water and absorbance was measured against prepared reagent blank at 775 nm by using spectrophotometer (Unicom UV e300). Total tannins in sample were expressed as mg tannic acid equivalent (TE)/g dry weight. All samples were analyzed in triplicates.

2.6. Antioxidant activity

2.6.1 DPPH$^{\bullet}$ radical scavenging assay

The method described by [8] was used to assess the DPPH$^{\bullet}$ (2, 2-diphenyl-1-picryl hydrazyl) radical scavenging activity of peanut extracts. 0.1 mM of DPPH$^{\bullet}$ in methyl alcohol was prepared and 0.5 ml of this solution was added to 1 ml of peanut extracts at different concentrations (25, 50, 75, 100 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature. Butyl Hydroxytoluene (BHT, Sigma) was used as positive control; and negative control contained the entire reaction reagent except the extracts. Then the absorbance was measured at 515 nm against blank. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capacity to scavenge the DPPH$^{\bullet}$ radical was calculated using the following equation DPPH$^{\bullet}$ scavenging effect (Inhibition %) = [(Ac – A_S / Ac) × 100]

Where Ac was the absorbance of the control reaction and as the absorbance in the presence of the plant extracts.

2.6.2 Ferrous ion chelating activity

Metal chelating effects of ferrous ions was carried out as described by [37]. One ml of peanut extracts, or EDTA solution as a positive control at different concentrations (25, 50, 75, 100 µg/ml) was spiked with 0.1 ml of 2 mM $FeCl_2$- $4H_2O$ and 0.2 ml of 5 mM ferrozine solution and 3.7 ml methanol were mixed in a test tube and reacted for 10 min, at room temperature then the absorbance was measured at 562 nm. Mixture without extract was used as the control. A lower absorbance indicates a higher ferrous ion chelating capacity. The percentage of ferrous ion chelating ability was calculated using the following equation

Iron chelating activity (Inhibition %) = [(Ac – A_S / Ac) × 100]

Where Ac was the absorbance of the control reaction and as the absorbance in the presence of the plant extracts.

2.6.3. Reducing power

The reducing power was assayed as described in [20]. Different concentrations (25, 50, 75, 100 µg/ml) of peanut extracts (1.0 ml) were mixed with 2.5 ml of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 ^{o}C for 20 min. After, 2.5 ml of trichloroacetic acid (10 %) was added to the mixture, centrifuged at 3000 rpm for 10 min. Finally, 1.25 ml from the supernatant was mixed with 1.25 ml of distilled water and 0.25 ml $FeCl_3$ solution (0.1%, w/v). The absorbance was measured at 700 nm. The assays were

carried out in triplicate and the results were expressed as mean values ± standard deviations. Increased absorbance values indicate a higher reducing power. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against the extract concentration. BHT was used as standard

2.6.4. Antioxidant activity by the ABTS$^{\bullet+}$ assay

$ABTS^{\bullet+}$ assay was carried out according to [4]. It was generated by oxidation of $ABTS^{\bullet+}$ with potassium persulphate. $ABTS^{\bullet+}$ was dissolved in deionized water to 7.4 Mm concentration, and potassium persulphate added to a concentration of 2.6 Mm. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12-16 h at room temperature in the dark. The solution was then diluted by mixing 1mL $ABTS^{\bullet+}$ solution with 60 mL methanol to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using the spectrophotometer. Fresh $ABTS^{\bullet+}$ solution was prepared for each assay. Peanut extracts (150 µl) at different concentration (25, 50, 75, 100 µg/ml) were allowed to react with 2850 µl of the $ABTS^{\bullet+}$ solution for 2 h in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer. Results were expressed as in comparison with standard BHT. A bigger antioxidant capacity of the sample exhibited a smaller production of free radicals. Percent activity was calculated using the equation

$\%\ \text{Inhibition} = [(A_0 - A_1)/A_0] \times 100\ \%$

Where: A_0 is the $ABTS^{\bullet+}$ absorbance of the control reaction A_1 is the $ABTS^{\bullet+}$ absorbance in the presence of the sample

2.7. Cytotoxic effect on human cell line (HePG2 – MCF7 – HCT116)

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4, 5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide) to purple formazan [23].

Procedure

All the following procedures were done in a sterile area using a Laminar flow cabinet bio safety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in RPMI 1640 medium for HePG2 - MCF7 and HCT116. The media are supplemented with 1% antibiotic-antimycotic mixture (10,000 U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B), 1% L-glutamine and 10% fetal bovine serum and kept at 37 °C under 5% CO_2. Cells were batch cultured for 10 days, then seeded at concentration of 10x103 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 °C for 24 h under 5% CO_2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (100 – 50 – 25–12.5–6.25–3.125–0.78 and 1.56 µg/ml). After 48 h of incubation, medium was aspirated, 40µl MTT salt (2.5µg/ml) were added to each well and incubated for a further four hours at 37°C under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200µl of 10% Sodium dodecyl sulphate (SDS) in deionised water was added to each well and incubated overnight at 37ºC. A positive control which composed of 100µg/ml was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions [40]. The absorbance was then measured using a micro plate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration in the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula

((Reading of extract / Reading of negative control) -1) x 100

2.8. Statistical analysis

Data were statistically analyzed using Costat statistical package (Anonymous 1989).

3. RESULTS AND DISCUSSION

3.1. Phenolic content

Outer layers of peanuts skin and peel contain large amounts of polyphenolic compounds to protect inner materials. In the present work, the total phenolic content was determined by the Folin–Ciocalteu method, which is considered the best method for total phenolic content (including tannins) determination [10]. The level of phenolic compounds in different solvent extracts (methanol, ethanol, acetone and aqueous) of the skin and peal peanut are shown in Table (1).

Table 1

Total phenolic, total flavonoids and total tannins contents in skin and peel of peanut extracted by different solvent.

Extracts	Phenolics mg/g		Flavonoids mg/g		Tannins mg/g	
	Skin	Peel	Skin	Peel	Skin	Peel
Aqueous	155.50 [a] ± 1.05	8.39 [a] ± 0.07	184.63 [a] ± 1.95	7.96 [a] ± 0.06	90.55 [a] ± 3.63	4.95 [a] ± 0.13
Methanol 80%	229.46 [c] ± 0.36	13.12 [c] ± 0.09	224.81 [b] ± 0.32	14.48 [b] ± 0.23	150.26 [c] ± 1.83	8.45 [c] ± 0.03
Ethanol 80%	222.71 [c] ± 0.36	12.47 [b] ± 0.12	259.93 [c] ± 2.25	14.67 [b] ± 0.11	123.81 [b] ± 1.21	8.17 [b] ± 0.09
Acetone 80%	436.88 [d] ± 0.71	18.22 [d] ± 0.07	340.00 [d] ± 1.11	22.85 [c] ± 0.34	163.15 [d] ± 0.67	9.13 [d] ± 0.07
LSD at 0.05	1.25	0.21	3.31	0.42	4.24	0.18

All values with the same letters are not significantly different at p ≤ 0.05

It noticed that the decreased in solvent polarity, TP, TF, TT content increased in the extract. High content of TP (436.88 mg/g DW), TF (340.00 ± 1.11 mg/g DW) and TT (163.15 ± 0.67 mg/g DW) obtained from peanut skin acetone extract. While, the level of TP content in peanut peal acetone extract (18.22 ± 0.07 mg/g DW), TF (22.85 ± 0.34 mg/g DW) and TT (9.13 ± 0.07 mg/g DW). The highest total phenolic content of 436.88 mg/g DW obtained in this study for the peanut skin acetone extract was at least twice as high as those in previous reports on peanut skins [26, 47]. The increase in total phenolics in peanut skins may be due to the development of Maillard reaction products and/or liberation of phenolic compounds. Several studies reported that processing steps such as heat treatment can liberate the phenolic compounds from residual sources, yielding higher total phenolic content. The total phenolic values (155.50 - 436.88 mg/g DW) obtained for all solvents were found to be significantly higher than in well-known food sources for antioxidants such as green tea (62.10 mg GAE/g,) [42]; black tea (62.00 mg GAE/g,) [21]; grape seeds (32.10–52.70 mg GAE/g,) [44], grape skins (15.00–20.30 mg GAE/g, [44] and blueberries and blackberries (5.60 and 4.90 mg GAE/g, respectively. [46] reported that one gram dry peanut skin contained 0.090–0.125 g total phenolics, and the skin removal methods (such as direct peeling, blanching and roasting) and extraction solvents had significant effects on total extractable phenolics.

3. 2. Flavonoids content

There was a significant difference between flavonoids content of skin peanut acetonic extract and the other extracts ($p \leq 0.05$); but there no significant difference between peanut peel methanolic and ethanolic extracts ($p \leq 0.05$). The highest level has been detected in acetone extract (340.00 mg/g Dw), followed by ethanolic extracts and methanolic (259.93 ± 2.25 and 224.81 ± 0.32 mg/g DW, respectively).

3. 3. Tannins content

Results shown in (Table 1) revealed that a significant difference between tannin contents

of the four solvents extracts ($p \leq 0.05$). Acetone extract exhibited the highest value among the three solvent extracts, it is about (163.15 ± 0.67 mg/g DW), followed by the methanol extract 150.26 ± 1.83 mg/g DW; while the peanut skin aqueous extract has the lowest content (90.55 ± 3.63mg/g DW). Significant differences between solvents at $p \leq 0.05$ were observed for TP, TF, and TT extraction. It has reported by [41] that solvent with different polarity had significant effect on polyphenol content in solvent in hight polarity. This is because of phenolics compounds often associated with other biomolecules (polysaccharides, proteins, terpenes, chlorophyll, inorganic compounds etc,) and a solvent must be found that it is suitable for extracting them) [32, 38].

3.4. DPPH· radical scavenging activity assay

The effect of extracting solvent on the DPPH· radical scavenging activity expressed as IC_{50} µg/ml (concentration µg/ml for 50% inhibition) of peanut skin and peel are shown in (Table 2 and Figure 1). Data presented in Table 2 show the dose-response of IC_{50} of the four extracts of peanut skin and peels, compared with BHT.

Table 2
IC_{50} of DPPH· radical of peanut skin and peel

Extracts	IC_{50} µg/ml	
	Skin	Peel
Aqueous	90.21 [e] ± 0.91	110.84 [e] ± 0.32
Methanol 80%	65.20 [c] ± 0.65	80.79 [c] ± 1.45
Ethanol 80%	74.63 [d] ± 0.36	84.97 [d] ± 1.41
Acetone 80%	52.18 [b] ± 0.47	70.51 [b] ± 0.45
BHT	7.61 [a] ± 0.33	7.61 [a] ± 0.33
LSD at 0.05	0.97	1.77

All values with the same letters are not significantly different at $p \leq 0.05$

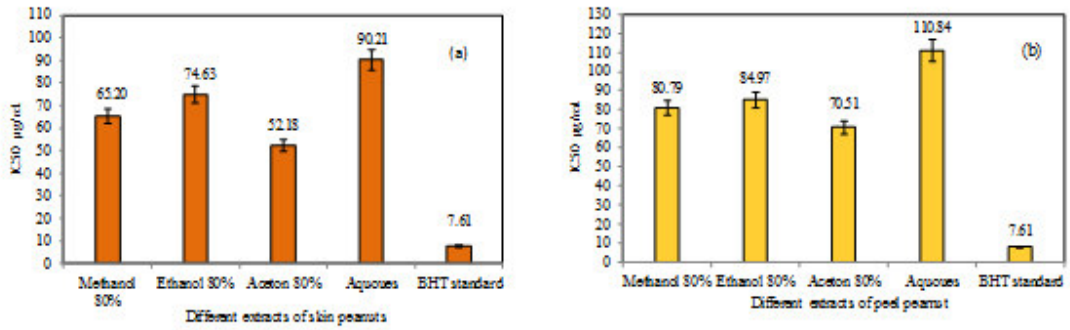

Figure 1
IC_{50} of DPPH· scavenging activity of peanut skin (a) and peel (b) extracts

Peanut skin acetone extract showed higher activity (IC_{50}= 52.18 µg/ml) compared to methanol, ethanol and aqueous extracts (65.20, 74.63 and 90.21 µg/ml) respectively. A similar trend was observed in case of peanut peel extract where acetone extract had the highest activity (IC_{50}= 70.51 µg/ml) compared to methanol, ethanol and aqueous extracts (80.79, 84.97 and 110.84 %) respectively. As reported by [46] the strong antioxidative properties of

peanut extracts could be due to different antioxidant components present in the different part of peanut. The differences in antioxidant activity among the different types of solvent extracts were statistically significant and the values of IC_{50} ranged from 90.21to 52.18 µg/ml for peanut skin and for peanut peel from 110.84 to 70.51 µg/ml. The effect of phenolic compounds on DPPH· is thought to be due to their hydrogen donating ability [11]. The DPPH· radical scavenging abilities of the extracts were less than those of BHT (IC_{50}= 7.61 µg/ml). The higher activity of DPPH scavenging radical may be attributed to the presence of higher levels of total phenolics and flavonoids which play a key role as proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants [15, 45]. In contrast, with the data in the literature it has been found that the extracts obtained using low polarity solvents (acetone 80%) were considerably more effective as radical scavengers than solvents with high polarity .So, the solvent play a vital role in the extraction of the plant constituents due to its ability to dissolve especial group of antioxidant compounds alters and influences the antioxidant activity estimation. It has been found that the acetone extracts of skin and peel were capable of directly reacting and quenching DPPH radical when compared with peanut peel extract [49].

3.5. Fe^{2+}- chelating activity

Foods are often contaminated with transition metal ions which may be introduced by processing methods. Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton chemistry [17]. The metal chelating capacity is expressed by the percentage of inhibition of ferrozine-Fe^{2+}complex formation by different extracts. In this assay both extracts of skin and peel peanut are interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and are able to capture ferrous ion. As shown in Table 3, the methanolic extract exhibit the highest percentage of metal chelating capacity (86.10 % ± 0.27) at concentration 100 µg/ml, compared to that of the other three extracts at the same concentration, which are about 81.78 ± 0.13 for the acetone extract, 76.71% ± 0.21 for the ethanol and (71.17 % ± 0.41) for aqueous one ($p \leq 0.05$). As shown in Table 4, the ethanolic extract of peanut peel exhibit the highest percentage of metal chelating capacity (81.96% ± 0.55), compared to other extracts, which are about 74.94 % ± 0.41for the methanolic extract , 63.11% ± 0.21for the acetone one and 52.23 % ± 0.4 for aqueous extract.

Table 3
Iron chelating activity in skin of peanut extracted by different solvent.

Extracts	Inhibition %			
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Methanol 80%	65.89 ^{d} ± 0.29	72.47 ^{d} ± 0.30	80.79 ^{d} ± 0.34	86.10 ^{d} ± 0.27
Ethanol 80%	45.77 ^{b} ± 0.37	57.52 ^{b} ± 0.51	67.03 ^{b} ± 0.41	76.71 ^{b} ± 0.21
Acetone 80%	51.04 ^{c} ± 0.44	64.63 ^{c} ± 0.44	73.55 ^{c} ± 0.36	81.78 ^{c} ± 0.13
Aqueous	38.22 ^{a} ± 0.51	48.48 ^{a} ± 0.52	59.65 ^{a} ± 0.36	71.17 ^{a} ± 0.41
EDTA	73.29 ^{e} ± 0.29	81.62 ^{e} ± 0.30	85.52^{e} ± 0.47	90.78 ^{e} ± 0.34
LSD at 0.05	0.80	0.79	0.52	0.51

All values with the same letters are not significantly different at $p \leq 0.05$

Table 4
Iron chelating activity in peel of peanut extracted by different solvent.

Extracts	Inhibition %			
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Methanol 80%	42.28 [c] ± 0.22	57.96 [c] ± 0.30	67.12 [c] ± 0.21	74.94 [c] ± 0.41
Ethanol 80%	59.12 [d] ± 0.22	66.09 [d] ± 0.44	73.73 [d] ± 0.34	81.96 [d] ± 0.55
Acetone 80%	25.40 [b] ± 0.44	34.11 [b] ± 0.25	51.91[b] ± 0.43	63.11[b] ± 0.21
Aqueous	12.72 [a] ± 0.51	22.30 [a] ± 0.30	45.30 [a] ± 0.21	52.23 [a] ± 0.40
EDTA	73.29 [e] ± 0.29	81.62 [e] ± 0.30	85.52[e] ± 0.47	90.78 [e] ± 0.34
LSD at 0.05	0.73	0.67	0.68	0.78

All values with the same letters are not significantly different at p ≤ 0.05

3.6. Reducing Power

Reducing power antioxidant activity was expressed as EC_{50} (effective concentration at which the absorbance is 0.5 at 700 nm). Reducing power of peanut skin and peel extracts in comparison with BHT as standard showed in (Figure 2 and Table 5).

Table 5
Reducing power activity of peanut (Skin) and peel

Extracts	EC_{50}	
	Skin	Peel
Aqueous	52.78[e] ± 0.52	150.90[e] ± 0.32
Methanol 80%	30.61[c] ± 1.73	120.32[d] ± 2.95
Ethanol 80%	37.66[d] ± 0.07	93.09[c] ± 0.59
Acetone 80%	21.16[b] ± 0.80	64.03[b] ± 0.40
BHT	11.01 [a] ± 0.32	11.01[a] ± 0.32
LSD at 0.05	1.62	2.69

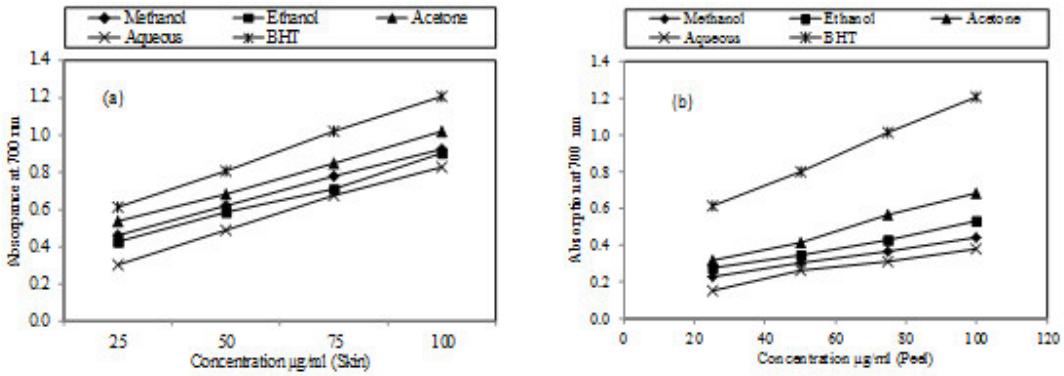

Figure 2
EC_{50} of peanut skin (a) and peel (b) extracts

It was observed that acetone extract of peanut skin and peel had higher reducing power (EC_{50} = 21.16 ± 0.80 and 64.03 ± 0.40 µg/ml), respectively. While the aqueous extract of peanut skin and peel showed the lowest potential activity (EC_{50} = 52.78 ± 0.52 and 150.90 ± 0.32 µg/ml), respectively. The reducing power activity may be due to the presence of reductones as they electron donors and are capable of converting them into a more stable product and terminating the free radical reaction. The reducing power ability of BHT was (EC_{50} = 11.01 ± 0.32 µg/ml). In the present study, peanut skin extracts exhibited the highest reducing power followed by peel extracts. The reducing power of peanut extracts is probably due to the action of hydroxyl group of the phenolic compounds which might act as electron donors. Regarding the reducing power, it has been found that the amount of phenolic compounds was high in acetone extract of peanut skin and there was a tight relationship between the amount of total phenolic content and the reducing power activity. These results were previously recorded [18, 36]. Indicating that the reducing power of bioactive compounds is associated with antioxidant activity. Thus, it is necessary to determine the reducing power of phenolic constituents to elucidate the relationship between their antioxidant effects and the reducing power [6].

3.7. $ABTS^{\cdot+}$ scavenginig activity

The antioxidant capacity of methanol, ethanol, acetone and aqueous extracts of peanut skin and peel were evaluated according to the $ABTS^{\cdot+}$ decoloration method. The results were shown in (Tables 6 and 7).

Table 6
$ABTS^{\cdot+}$ scavenginig activity of peanut skin extracts

Extracts	Inhibition %			
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Aqueous	15.84[a] ± 0.45	21.37[a] ± 0.36	39.33[a] ± 0.30	57.55[a] ± 0.34
Methanol 80%	37.88 [d] ± 0.35	46.75 [d] ± 0.14	77.41 [d] ± 0.42	94.27 [d] ± 0.48
Ethanol 80%	26.39 [b] ± 0.30	35.69 [b] ± 0.41	47.22 [b] ± 0.56	68.24[b] ± 0.42
Acetone 80%	34.08[c] ± 0.24	42.78[c] ± 0.36	59.14[c] ± 0.48	76.31[c] ± 0.41
Trolox	76.98[e] ± 0.38	84.39[e] ± 0.18	87.37[e] ± 0.41	95.14[e] ± 0.41
LSD at 0.05	0.67	1.19	0.90	0.77

Inhibition of generation of the $ABTS^{\cdot+}$ radical cation was the basis of the spectrophotometric methods that had been applied to the measurement of the total antioxidant activities of solutions of peanut skin and peel extracts. Trolox was used as positive control. At the highest concentration (100 µg/ml), the methanolic extract of peanut skin displayed the highest total antioxidant activity (94.27 % ± 0.48) followed by acetone peanut skin extract (76.31 % ± 0.41) (Table 6). While the Total antioxidant capacity of peanut peel extract were evaluated as 75.06 % ± 0.42 and 56.12 % ± 0.24 for acetone and aqueous extracts, respectively

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Table 7
ABTS$^{\cdot+}$ scavenginig activity of peanut peel extracts

Extracts	Inhibition %			
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Aqueous	29.25^{b} ± 0.41	33.53^{b} ± 0.35	43.22^{b} ± 0.24	52.94^{b} ± 0.31
Methanol 80%	33.10^{c} ± 0.30	41.84^{c} ± 0.18	57.33^{d} ± 0.18	75.06^{d} ± 0.42
Ethanol 80%	17.57^{a} ± 0.22	26.90^{a} ± 0.30	38.35^{a} ± 0.31	45.25^{a} ± 0.36
Acetone 80%	33.76^{c} ± 0.24	43.02^{d} ± 0.24	48.94^{c} ± 0.35	56.12^{c} ± 0.24
Trolox	76. 98^{d} ± 0.38	84.39^{e} ± 0.18	87.37^{e} ± 0.41	95.14^{e} ± 0.41
LSD at 0.05	0.67	0.49	0.65	0.66

The aqueous and ethanolic extracts of peanut skin and peel had the lowest potential scavenging activity (57.55 % ± 0.34, and 45.25 % ± 0.36), respectively. In the ABTS$^{\cdot+}$ evaluating system, the scavenging capacity of ABTS$^{\cdot+}$ free radicals in methanolic extract of peanut skin and peel was most obvious, and the aqueous and ethanolic extracts of peanut peel showed the weakest scavenging capacity.

3.8. Cytotoxic effect of peanut skin and peel extract on human cell line HePG2, MCF7 and HCT116

Cancer is a global health problem with high morbidity and mortality and poses both economic and physiological challenges [24, 9]. It is known that different cell lines might exhibit different sensitivities towards the antiproliferative compounds. So, the use of more than one cell line is therefore considered necessary in the detection of antiproliferative compounds. Peanut skin and peel extracts were tested for their anti-cancer activity using three cancer cell lines hepatocellular cell line (HePG2), colon cell line (HCT116) and breast cell line (HCF7), beside normal cell line (human dermal fibroblast cell line). The cytotoxic activity data are presented in (Tables 8 and 9). Acetone extract of peanut skin exhibited a pronounced cytotoxic effect 71.28%, 40.27% and 38.58 %, of HCT116, HePG2 and MCF-7), respectively compared to that of peanut peel ethanol extract 22.44% for HCT116, 13.24 % for HePG2 and 34.27 % for MCF7 human cell line. No reports are available so far on toxicity evaluation of extracts from different peanut parts. The results of our study on cytotoxicity of peanut skin and peel is of much importance because the cell death at low extract concentration points out the chance of active compounds to be present in peanut skin (Table 8).

Table 8
Cytotoxicity effect in skin of peanut extracted by different solvent

Sample	Extracts	Remarks % at 100 ppm		
		HePG2	MCF7	HCT116
Skin	**Aqueous**	10.53	35.26	67.27
	Methanol 80%	30.86	35.19	62.36
	Ethanol 80%	15.95	29.25	52.64
	Acetone 80%	40.27	38.58	71.28
	DMSO	1.00	1.00	1.00
	Negative control	0.00	0.00	0.00

Table 9
Cytotoxicity effect in peel of peanut extracted by different solvent

Sample	Extracts	Remarks % at 100 ppm		
		HePG2	MCF7	HCT116
Peel	Aqueous	0.00	16.53	10.78
	Methanol 80%	5.18	15.06	18.28
	Ethanol 80%	13.24	34.27	22.44
	Acetone 80%	10.57	10.05	19.39
	DMSO	1.00	1.00	1.00
	Negative control	0.00	0.00	0.00

HePG2 : Human hepatocellular carcinoma cell line.
MCF7 : Human Caucasian breast adenocarcinoma.
HCT116: Human Colon Cancer Cells.

The cytotoxicity activity against HCT116 cells may be due to its ability to be used as anti-cancer drug after suitable chemical modifications to reduce normal cell death is also under consideration of future studies. The presence of five phenolic compounds and eight flavonoids in peanut skin extract gallic, protocatechuic, epigallactocatechin, catechin, *β*-resorcyclic (internal standard), caffeic, procyanidin B2**,** epicatechin, epigallocatechingallate, *p*-coumaric ferulic, piceid, epicatechingallate, catechingallate, resveratrol and quercetin has been detected by [11, 13]. It has been reported by [1, 43] that p-hydroxybenzoic acid and resveratrol, chlorogenic acid, and *p*-coumaric acid, ferulic acid and epicatechin were present in the skin of peanuts. Many of these compounds are reported to exhibit anticarcinogenic properties [1, 5, 34, 35, 39].

4. CONCLUSION

This study indicated that the acetone extract of skin possessed the highest phenolic and flavonoid contents than other extracts. Also, it exhibited strong antioxidant capacities in all the assays, which were comparable to the commercial BHT antioxidant. This study suggests that the peanut skin extract can be potentially used as a source of natural antioxidant and anticancer agents.

5. REFERENCES

1. Actis-Goretta L, Romanczyk LJ, Rodriguez CA, Kwik-Uribe C, Keen CL, Cytotoxic effects of digalloyl dimer procyanidins in human cancer cell lines. The Journal of Nutritional Biochemistry, 19: 797- 808, (2008).
2. Alper CM, Mattes RD. Peanut consumption improves indices of cardiovascular disease risk in healthy adults. Journal of the American College of Nutrition, 22:133-141, (2003).
3. Anonymous, A. Cohort Soft Ware Crop. Costat user manual version 3.03, Berkeley CA, USA, (1989).
4. Arnao MB, Cano A, Acosta M, The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry 73: 239–244, (2001).
5. Block G, Patterson B and Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr. Cancer, 18: 1-29, (1992).
6. Brand-Williams W, Cuvelier ME and Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensmittel Wissenschaftund Technologie, 43: 25-30, (2010).
7. Caulfield LE, de Onis M, Blössner M, Black RE. Undernutrition as an underlying cause of child deaths associated with diarrhea, pneumonia, malaria, and measles. The American Journal of Clinical Nutrition, 80: 193-198, (2004).

8. Chu, YH, Chang CL and Hsu HF. Flavonoids content of several vegetables and their antioxidant activity. Journal of the Science of Food and Agricultural. 80: 561–566, (2000).
9. Dossus L, and Kaaks R. Nutrition, metabolic factors and cancer risk", Best Practice & Research Clinical Endocrinology & Metabolism. 22, 551–71, (2008).
10. Engelhardt, U. Flavonoids - analysis. Critical Review in Food Science and Nutrition, 41: 398–399, (2001).
11. Fakhriya S. Taha, Suzanne M. Wagdy and Fatma A. Singer. Comparison between antioxidant activities of Phenolic extracts from different parts of peanut. Life Science Journal, 9(2): 207. (2012).
12. Fransisco M.L.D.L and Resurrection A.V.A. Functional components in peanuts. Critical Reviews in Food Science and Nutrition, 48: 715-746, (2008).
13. Fransisco M.L.D.L and Resurrection A.V.A. Total phenolics and antioxidant capacity of heat treated peanut skin. Journal of Food Composition and Analysis, 22: 16-24, (2009).
14. Fraser GE, Sabate J, Beeson WL and Strathan TM. ``A possible protective effect of nut consumption on risk of coronary heart disease'', Archives of Internal medicine, 152: pp. 1416-1424, (1992).
15. Ghasemzaeh A, Jaafar HZE and Rahmat A. Antioxidant Activities, Total Phenolics and Flavonoids Content in Two Varieties of Malaysia Young Ginger (Zingiber officinale Roscoe). Molecules, 15: 4324-4333, (2010).
16. Griel AE, Eissenstat B, Juturu V, Hsieh G, Kris-Etherton PM. Improved diet quality with peanut consumption. Journal of the American College of Nutrition. 23 (6): 660–668, (2004).
17. Halliwell B, Aeschbach R, Löliger J and Aruoma OI. The characterization of antioxidants. Food and Chemical Toxicology, 33: 601-617, (1995).
18. Hounsome N, Hounsome B, Tomos D, Edwards-Jones G. Changes in antioxidant compounds in white cabbage during winter storage. Postharvest Biology and Technology, 52: 173-179, (2009).
19. Jadhav SJ, Nimbalkar SS, Kulkarni AD, Madhavi DL. Lipid oxidation in biological and food systems. In Food Antioxidants; Madhavi DL, Deshpande SS and Salunkhe, DK, Eds.; Dekker: New York, NY, pp 5-63, (1996).
20. Kuda T, Tsunekawa M, Goto H, and Araki Y. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. Journal of Food Composition and Analysis, 18: 625–633, (2005).
21. Lee K, Kim Y, Lee H and Lee C. Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. Journal of Agricultural and Food Chemistry, 51: 7292-7295, (2003).
22. Madavi DL and Salunkhe DK. Toxicological Aspects of Food Antioxidants. In: Food Antioxidants. (Eds.): D.L. Madav,S.S. Deshpande and D.K. Salunkhe. Marcel Dekker, New York, pp. 267, (1995).
23. Mosmann T. Rapid colorimetric assays for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 65: 55–63, (1983).
24. Moyad MA and Carroll PR. Life style recommendations to prevent prostate cancer, part II: time to redirect our attention? Urologic clinics of North America 31: 301–311, (2004).
25. Nepote V, Grosso NR and Guzman CA. Extraction of antioxidant components from peanut skins. Grasas Y Aceites, 53: 391-395, (2002).
26. Nepote V, Grosso NR and Guzman CA. Optimization of extraction of phenolic antioxidants from peanut skins. Journal of the Science of Food and Agriculture, 85: 33-38, (2005).
27. Polshettiwar SA, Ganjiwale RO, Wadher SJ, and Yeole PG. Spectrophotometric estimation of total tannins in some ayurvedic eye drops. Indian Journal of

Pharmaceutical Sciences. 69: 574-576, (2007).
28. Sang S, Lapsley K, Jeong WS, Lachance PA, Ho, CT and Rosen RT. Antioxidant phenolic compounds isolated from almond skins (Prunus amygdalus Batsch). Journal of Agricultural and Food Chemistry, 50: 2459-2463, (2002).
29. Segura R, Javierre C, Lizarraga MA, Ros E. Other relevant components of nuts: phytosterols, folate and minerals. British Journal of Nutrition, 96 (2): S36–S44, (2006).
30. Shahidi, F. and Amarowicz R. Antioxidant activity of green tea catechins in a β-carotene-linoleate Modeal System. Journal of Food Lipids, 2: 47-56, (1994).
31. Shan B, Cai YZ, Sun M and Corke H. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. Agric. Food Chemistry, 53: 7749-7759, (2005).
32. Siddhuraju P. and Becker K. Antioxidant properties of various extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* lam.) leaves. Journal of Agricultural and Food Chemistry, 51: 2144-2155, (2003).
33. Singleton VL and Rossi JA. Colorimetric of total phenolics with phosphomolibdic – phosphor tungstic acid reagents. American Journal of Enology and Viticulture, 16: 144–158, (1965).
34. Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI and Bahorun T. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. Mutation Research, 579: 2000-2013, (2005).
35. Srinivasan M, Sudheer AR, and Menon VP. Ferulic acid: Therapeutic potential throughits antioxidant property. Journal of Clinical Biochemistry and Nutrition, 40: 92-100, (2007).
36. Sroka Z and Cisowski W. Hydrogen peroxidase scavenging, antioxidant and anti-radical activity of some phenolic acids. Food and Chemical Toxicology. 41, 753-758, (2003).
37. Su C, Chen W, Weng Y and Tseng C. Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. Food Chemistry. 83: 85–92, (2003).
38. Sultana B, Anwar F and Przybylski R. Antioxidant activity of phenolic components present in barks of barks of Azadirachta indica, Terminalia arjuna, Acacia nilotica, and Eugenia jambolana Lam. trees. Food Chemistry, 104: 1106-1114, (2007).
39. Taha FS, Wagdy SM, Hassanein MM and Hamed SF. Evaluation of the biological activity of sunflower hull extracts. Grasas Y Aceites, willappear in issue of February, 2012. DOI: 10.3989 /gya.072111, (2012).
40. Thabrew MI, Hughes RD and McFarlane IG. Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay. Journal of Pharmacy and Pharmacology. 49: 1132–1135, (1997).
41. Turkmen N, Sari F, Velioglu YS. Effect of extraction solvents on concentration and antioxidant activity of black and black mate polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. Food Chemistry, 99: 838-841, (2006).
42. Vinokur Y, Rodov V, Reznick N, Goldman G, Horev B, Umiel N, and Friedman H. Rose petal tea as an antioxidant-rich beverage: Cultivar effects. Journal of Food Science, 71: S42–S47, (2006).
43. Win M, Abdulhamid A, Baharin BS and Anwar F. Phenolic compounds and antioxidant activity of peanuts skin, hull raw kernel roasted kernel flour. Pakistan Journal of Botany, 43: 1635-1642, (2011).
44. Yilmaz Y. Antioxidant activities of grape skin and grape seed polyphenolics and potential use of antioxidants in foods as a functional food ingredient. PhD. Thesis. University of Georgia (2002).
45. Yingming P, Ping L, Hengshan W, Min L, Antioxidant activities of several Chinese medicinal herbs. Food Chemistry, 88: 347–350, (2004).

46. Yu J, Ahmedna M and Goktepe I. Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. Food Chemistry, 90:199-206, (2005).
47. Yu J, Ahmedna M, Goktepe I and Dai J. Peanut skin procyanidins: Composition and antioxidant activities as affected by processing. Journal of Food Composition and Analysis 19: 364–371, (2006).
48. Zhishen J, Mengcheng T, and Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64: 555–559, (1999).
49. Zhou K, Yu L. Effects of extraction solvent on wheat bran antioxidant activity estimation. Lebennsmittel-Wissenschaft und-Technologie, 37: 717–721, (2004).