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Original Article

Antioxidant Activity of Raw and Roasted Peanut (*Arachis hypogaea* L.) Skins Extracts

Chidiebere Emmanuel Ugwu*, Stephen Monday Suru, Dike C Charles

ABSTRACT

Background: Peanut skins are a by-product from peanut but with low socio-economic value despite having appreciable amounts of bioactive phytochemicals. Studies are on-going to remove some astringent properties in peanut skins and improve its values. In this study, the *in vitro* antioxidant activity of raw peanut skins and roasted peanut skins extracts were determined in hydro alcoholic medium. **Materials and Methods:** The raw and roasted peanut skins powders were each subjected to extraction procedure in 70% methanol for 48 hr. The phytochemical screening including total phenolic and flavonoid contents (TPC, TFC) were determined. The antioxidant activity including DPPH radical scavenging, Fe^{2+}-chelating, ferric reducing antioxidant property (FRAP), OH^{-} radical scavenging and reducing power assays were determined and compared against standard antioxidants. **Results:** The results showed significantly higher ($p<0.05$) TPC and total antioxidant capacity in the raw extract compared to the roasted extract. The roasted peanut skins extract showed significantly higher ($p<0.05$) DPPH radical, OH^{-} radical scavenging capacity, Fe^{2+}-chelating and ferric reducing antioxidant property compared to the raw peanut skins extract. Both extracts each produced concentration dependent percentage inhibitions which were significantly lower than the antioxidant standards used. **Conclusion:** The results showed that roasting improved the *in vitro* antioxidant properties of the peanut skins compared to the raw peanut skins. This could add value to its use as functional food component, antioxidant and in animal feed industries.
Keywords: Antioxidant, Peanut skins, Roasting, Phytochemicals, Percentage inhibition.

Chidiebere Emmanuel Ugwu*, Stephen Monday Suru, Dike C Charles

Department of Human Biochemistry, Faculty of Basic Medical Sciences Nnamdi Azikiwe University Awka, Nnewi Campus, Anambra, NIGERIA.

Correspondence

Dr. Chidiebere Emmanuel Ugwu,

Department of Human Biochemistry, Faculty of Basic Medical Sciences Nnamdi Azikiwe University Awka, Nnewi Campus, Anambra, NIGERIA.

E-mail: ce.ugwu@unizik.edu.ng

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INTRODUCTION

Peanut or groundnut (*Arachis hypogaea* L.) is a species in the legume family *Fabaceae*. Peanut fruit is a woody indehiscent legume consisting of the kernel, seed coat (skin) and hulls. Peanut has been part of the human diet since man's history. A majority of world's population has subsisted on whole peanut and products. The kernels are consumed as food and/or used to make a variety of products (peanut butter, peanut oil and confectionery products). Currently, studies have shown the values in its phytonutrient composition that may improve general health and well-being.[1,2]

In Africa, especially Nigeria, peanuts are consumed boiled or roasted with the intact skin. In recent times, appreciation for aesthetic and organoleptic values has fuelled an increasing demand for de-skinned peanut and products at the expense of latent bioactive compounds present therein. Other obstacles to the consumption of whole peanut are the perceived low socio-economic value attached to peanut skin[3] and the reported peanut allergy; a health problem related with peanut intake predominantly in the developed countries.[4,5]

Peanut skin represents a source of health promoting bioactive compounds such as the polyphenols.[6,7] Peanut skin polyphenols, especially procyanidins exert antioxidant effect, promote the proliferation of normal pancreatic beta cells, reduce risk of cardiovascular diseases and cancers.[8,9] In folkloric Chinese Medicine, peanut skin and products are used to treat chronic haemorrhage, sore and bronchitis.[10] An intriguing report is the strong evidence that aqueous extract of peanut skin and its main constituent procyanidin may suppress allergic diseases as against the reported peanut allergy.[11]

Peanut skin extract has been shown to reduce plasma oxidative stress markers and serves as high-energy and protein diets for ruminants when supplemented.[12] They are high in dietary fibers and amino acids such as phenylalanine, glycine and glutamine. Peanut skins are also rich in palmitic, oleic and linoleic acids.[13] They have high content of proanthocyanidins oligomers that may be effective at reducing risk of Crohn's disease and as an ingredient to improve profitability of the peanut industries.[13]

Recent studies have shown that peanut skin extracts contain 28 phenolics and could serve as inhibitor of glycation by protecting against toxicity, oxidation and inflammation and gives new insight into its use.[14,15] Peanut skin extracts and metformin produced

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significant antidiabetic activity in mice by maintain gut microbiota[16] and also improved atherosclerosis induced by high-fat diet in ApoE mice.[17] Topical application of peanut skin anthocyanins protected the skin against damage and may have human application[18] while protocatechuic acid showed anti-wrinkle potentials in human skin subjects.[19] Procyanidin A-type dimers from peanut skin prevented oxidative damage in prostatic cells induced by hydrogen peroxide.[20]

There are attempts in the past to study the effects of processing on the antioxidant properties of peanut skins.[3,21] Many studies have recognized the value-added qualities of peanut skins and /or peanut skin extracts as an antioxidant, functional food and animal feed ingredient, and antibacterial agent.[22] The roasted peanut skins are removed and discarded as waste. Given the wide range of bioactive phytochemicals inherent in PNS, and the search for functional ingredients to improve peanut industries, we studied the antioxidant potentials of raw and roasted peanut skin extracts.

MATERIALS AND METHODS

Chemicals

All reagents used for antioxidant assay were purchased from Sigma Aldrich, Germany and were of analytical grade. They include ammonium molybdate, diphenyl-picrylhydrazyl, ferrous chloride, ferrozine, tris(2-pyridyl)-1,3,5-triazine(TPTZ), ferrous sulphate, methanol, trolox, ascorbic acid, quercetin, and ethylenediaminetetracetic acid (EDTA).

Plant Material

Raw peanuts were purchased from a local market in Auchi, Edo State, Nigeria. Since there are several varieties of peanut available locally and are often mixed together during cultivation, the Kampala variety which is easily identifiable was used in this study.

Roasting and removal of peanut skins

The raw peanuts were shelled by direct peeling with gloved hands. The shelled peanuts were roasted in an oven at 166°C for 7 min.[3] The roasted peanuts were removed from the oven and allowed to cool. The skins were directly peeled with gloved hands and the dried skins obtained were blended into powdered form with an electric blender (Model HR 2001, China). This was stored in an air tight container and kept in a refrigerator.

Preparation of methanol peanut skin extracts

The roasted peanuts skins and raw peanuts skins powders were each subjected to extraction procedures. The hydromethanol short extraction method was adopted as described by Yu *et al*.[23] The methanol extraction was done by cold maceration technique at room temperature. The methanol extracts were obtained by soaking 25g of each peanuts skins powder in a round bottom flask containing 200ml methanol (70%) for 48 hr with constant shaking in an orbital mixer (Model: Denley, 073185 England). At the end of the extraction procedure, the methanol extract was filtered with muslin cloth and then with Whatman filter paper (125mm). The extract was concentrated in a rotary evaporator (Model: TT22, USA) at 65°C. The crude extract obtained was stored in amber container and kept in a freezer until use.

Phytochemical Screening

Qualitative analysis of phytochemical was conducted using routine standard methods. Test for presence of flavonoids and saponins were done according to the method described by Vinatha and Mamidala.[24] Test for presence of tannins, cardiac glycosides and steroids were carried out according to the method of Jack and Okorosaye-Orubite.[25] The presence of alkaloids was as described by Richa and Sharma.[26]

In vitro Antioxidant Assay

Total phenolic content (TPC)

The total phenolic content of the roasted peanut skins and raw peanuts skin extract were determined by the method of Singleton and Rossi[27] as described by Chukwumah *et al*.[28] for peanuts. Ten µl of each sample extract was made up to two ml with deionized water. Folin-Cicalteau reagent (100 µl) was added to the diluted samples. After eight minutes, 300 µl of 7% sodium carbonate was added. The mixture was placed in a water bath at 40°C for thirty minutes. The absorbance was read at 750 nm. The garlic acid standard curve (50-500 µg/mL) was used to determine the total polyphenol content of the sample extracts and was expressed as garlic acid equivalent (GAE)/g. This was calculated thus;

Total phenolic content (TPC) = CV/M

Where C= concentration of extract from the standard curve, V= volume of the extract used,

M= weight of sample extract.

Total Flavonoid Content (TFC)

The total flavonoid content of the roasted peanut skins and raw peanuts skins extracts were determined by the aluminum chloride colorimetric method as described by Kalita *et al*.[29] In this assay, quercetin (6.25-100µg/ml) was used to prepare the standard curve. One (1%) aluminum chloride and potassium acetate solutions were respectively prepared. The stock solution of each extract was prepared by carefully weighting 100mg to 10 ml volumetric flask and making up the volume with methanol. To 0.5 ml of each extract stock solution, 1.5 ml methanol, 0.1ml each of aluminum chloride and potassium acetate solutions and 208ml distilled water were added and thoroughly mixed. The sample blank was similarly prepared by replacing aluminum chloride with distilled water. The absorbance was measured at 415 nm after filtering through Whatman filter paper.

$$\text{Total flavonoid content (TFC)} = \text{RxDF} \times \text{V} \times 100/\text{W}$$

Where R= result obtained from standard curve, DF= dilution factor, V= volume of stock solution, W=weight of extract used in the experiment.

Total antioxidant capacity (TAC) by phosphomolybdenum assay

The TAC of the roasted peanut skins and raw peanut skins extracts were determined by the phosphomolybdenum method as described by Kalava and Menon[30] with little modifications. An aliquot of 0.3 ml of each sample extract (100mg in 10 ml methanol) was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes were caped and incubated at 95°C for 90 min. The tubes were cooled to room temperature and the absorbance of the solution measured at 695 nm against methanol blank. The ascorbic acid standard curve (31.25-1000 mg/mL) was used to determine the total antioxidant capacity of the sample extracts and was expressed as garlic acid equivalent in mg/ml.

Diphenyl-picrylhydrazyl (DPPH) Radical Scavenging Assay

Diphenyl-picrylhydrazyl (DPPH) radical scavenging effects of the methanol extracts of roasted peanut skins and raw peanuts skins extract was determined according to the methods described by Kalita *et al*.[29] and Kalava and Menon.[30] Various concentrations of the extract (12.0, 10.0, 8.0, 6.0, 4.0 and 2.0 mg/ml) was filtered using Whatman number 125 mm filter paper and 4 ml of this was mixed with 1 ml of DPPH radical reagent. Exact serial concentrations of ascorbic acid were used as standard. The mixtures were shaken and incubated for 30 min at room temperature. The absorbance was measured at 517 nm using UV-visible

spectrophotometer (model 752, China). The percentage inhibition was calculated thus: $[(A_0-A_1)/A_0] \times 100$.

Where A_0 = absorbance of blank (DPPH reagent without extract), A_1 = absorbance of test.

Ferrous Ion Chelating Effect

The ability of the extracts to chelate ferrous ions was determined according to the method described by Kalita *et al.*[29] Various concentrations of the methanol extracts (12.0, 10.0, 8.0, 6.0, 4.0 and 2.0 mg/ml) was each filtered. Two mills each of the filtrate was added to a solution of 0.05 ml ferrous chloride ($FeCl_2$). This was followed by the addition of 0.2 ml of ferrozine. The mixture was shaken and incubated at room temperature for 10 min. The absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine-Fe^{2+} complex formed was calculated as: $[(A_0-A_1)/A_0] \times 100$.

Where A_0 = absorbance of blank, A_1 = absorbance of the test.

Ferric Reducing Antioxidant Properties (FRAP) Assay

The FRAP assay was used to determine the reducing capacities of the extracts according to the method described by Kalava and Menon.[30] The FRAP reagent was prepared by adding 25ml of 10mM HCI(3.4ml HCI in 1000ml distilled water), and 25 mM $FeCI_3.6H_2O$ (5.41g of $FeCL_3.6H_2O$ in 1000 ml distilled water) and 25ml of 300ml mM acetate buffer (10g of sodium acetate pH3.6). This was prepared fresh and incubated at 37°C for 30 min. Nine hundred µl of FRAP reagent was mixed with 90 ml of distilled water and 30 ml of various concentration of the extracts (12.0, 10.0, 8.0, 6.0, 4.0 and 2.0 mg/ml). The reaction mixture was incubated at 37°C for 30 min in an incubator (model DNP-9052A, China) and the absorbance was measured at 593 nm using UV-visual spectrophotometer (model 752, China). The percentage inhibition of ferrous–TPTZ complex formed was calculated using: $[(A_0-A_1)/A_0] \times 100$.

Where A_0 = absorbance of blank, A_1 = absorbance of the test.

Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging activity of the extracts was each evaluated according to the method described by Kalava and Menon.[30] The reaction mixture(3ml) was made up of 1ml of 1.5 mM $FeSo_4$, 0.7ml of 6mM hydrogen peroxide, 0.3ml of 20 mM sodium salicylate and 1ml of various concentrations of the extract (12.0, 10.0, 8.0, 6.0, 4.0 and 2.0 mg/ml). The mixture was shaken vigorously and incubated for 1hr at 37°C using incubator (model DNP-9052A). After the incubation, the absorbance of the hydroxylated salicyclate complex was measured at 562 nm spectrophotometrically. The scavenging activity of hydroxyl radical effect was calculated as follows: $[1-(A_1-A_2)/A_0]$ X100.

Where A_1 = absorbance in the presence of the extract, A_2 = absorbance without sodium salicylate, A_0 = absorbance of blank (absorbance without extracts).

Reducing Power of the Extract

This was done as described by Kalava and Menon.[30] Briefly, 2.5ml of different concentration of the methanol extract, 2.5ml phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide were mixed and incubated at 50°C for 20 min. The mixture was centrifuged at 5000g for 10 min after addition of 2.5ml of trichloroacetic acid(10%). 2.5ml of aliquot of supernatant was mixed with 2.5ml of deionized water and 0.5ml of 0.1% ferric chloride. This was allowed to stand for 10 min for incubation and the absorbance was measured at 700nm against blank. The percentage inhibition was calculated using: $[(A_0-A_1)/A_0] \times 100$.

Statistical Analysis

The results were analyzed using Statistical Package for Social Sciences version 21. The results were expressed as mean ± SD of triplicate readings. Statistical difference between the mean was determined using ANOVA and students *t*-test. Results were presented in tables and bar chats. The acceptable level of significance was $p<0.05$.

RESULTS

Phytochemical Screening of raw Peanut Skins and Roasted Peanut Skins Extracts

Qualitative phytochemical screening was done on the methanol extracts of the raw peanut skins and roasted raw peanuts skin (Table 1). The results showed the presence of tannins, flavonoids, phenols and absence of saponins in both extracts but slight presence of α-glycosides and terpenoids in the raw peanut skin extract. There was also copious presence of flavonoids and phenols in both extracts. The total phenolic content of the raw peanut skins extract and roasted peanut skins extracts was 50.0±1.4 mg/GAE/g and 30.0±3.5mg/GAE/g respectively. The results also showed that the total flavonoid content in both extracts varied between 3.25±6.30 and 2.00±3.56 mg/QE/mg of extract dry weight for the raw peanuts skin and roasted peanut skins (Table 2).

In vitro Antioxidant Studies

Phosphomolybdenum assay for total antioxidant capacity

The total antioxidant capacity of both the raw peanut skins extract and roasted peanut skins extract is presented in Table 2. The extracts studied significantly exhibited a reduction of the molybdenum (VI) to molybdenum (V). The raw peanuts skins extract showed significantly higher total antioxidant capacity relative to the roasted raw peanuts skins extract ($p<0.05$).

Table 1: Quantitative phytochemical screening of the peanuts skins extracts.

Constituent	Roasted peanuts skins extract.	Raw peanuts skins.
Tannins	++	++
Saponin	-	-
Flavonoids	++	++
Steroids	++	-
α-glycosides	-	+
Phenols	++	++
Terpenoids	++	+

++ = very positive, + = slightly positive, - = negative.

Table 2: Total phenolics, flavonoid contents and total antioxidant capacity of the peanuts skins extract.

Extract	Total phenol content (mg/GAE/g)	Total flavonoid content (mg/QE/mg of extract)	Total antioxidant capacity (mg/ml)
Roasted peanuts skins.	30.00±3.51[a]	2.00±3.56[a]	62.50±2.34[a]
Raw peanuts skins.	50.00±1.40[b]	3.25±6.30[a]	80.01±3.24[b]

Results are mean ± SD of triplicate readings. Values with different alphabetical superscripts in a column are statistically significant to each other.

Table 3: Iron Chelating and 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activities of roasted and raw peanuts skins methanol extracts.

Concentration (mg/l)	Iron Chelating Effect			DPPH activity		
	Roasted Peanuts Skins	Raw peanuts skins	Standard (EDTA)	Roasted Peanuts Skins	Raw peanuts skins	Standard (ascorbic acid)
12.00	64.50±10.04^{a*}	66.48±2.92^{a}	94.94±0.81	55.21±0.42^{a*}	54.72±1.96^{a*}	90.77±0.19
10.00	63.67±0.17^{a*}	53.25±0.09^{b*}	94.05±0.18	54.69±0.04^{a*}	53.13±0.05^{c*}	88.83±0.52
8.00	60.13±0.37^{a*}	51.85±4.70^{a*}	94.00±0.23	53.91±0.08^{a*}	52.84±0.12^{a*}	86.06±0.28
6.00	56.59±0.19^{a*}	46.93±0.09^{b*}	94.11±0.34	53.30±0.13^{a*}	46.53±0.62^{d*}	81.45±1.97
4.00	55.01±0.56^{a*}	43.89±1.58^{a*}	93.95±0.39	52.64±0.16^{a*}	45.63±0.33^{b*}	72.17±0.51
2.00	53.09±0.30^{a*}	40.44±0.86^{b*}	92.15±3.21	55.51±5.04^{a}	44.91±0.12^{a*}	44.31±0.21

Results are mean ± SD of triplicate readings. Values with different alphabetical superscripts in a row are statistically significant to each other. Values with superscripts * in a row are statistically significant to the standard.

DPPH Radical Scavenging Activity

The *in vitro* 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of the extract was determined and compared to the ascorbic acid standard (Table 3). A positive DPPH result indicates that the methanol extracts have the potential to scavenge free radicals. The ability of the raw peanut skins and roasted peanut skins extracts to scavenge DPPH significantly varied in a concentration dependent pattern. The percentage inhibition of the raw peanut skins extract and the roasted raw peanut skins extracts were found to be maximum at 54.72% and 55.21% respectively while the ascorbic acid standard inhibited DPPH maximally at 90.77%. The results also show that the ability of the extracts to scavenge DPPH were significantly lower to ascorbic acid (p<0.05) at all the concentrations. In order word, to achieve a similar degree of DPPH scavenging activity, the amount of methanol extracts needed were significantly higher than that required for ascorbic acid. The results also indicated that the ability of the raw peanut skins extract to scavenge DPPH was significantly lower to the roasted raw peanut skins (p<0.05). The extracts showed the potential to quench DPPH depicting that they both have antioxidant scavenging ability.

Chelating Activity of Fe^{2+} Ions

The ferrous ion chelating activity of the methanol raw peanut skins and roasted raw peanut skins extracts and EDTA standard were also studied (Table 3). The EDTA was the positive iron chelator in the assay and inhibited 94.94% of Fe^{2+} ions at the highest concentration of 12.0mg/l. The raw peanut skins extract showed 66.48% Fe^{2+} ions chelating ability while that of the roasted raw peanut skins extract was 64.50% at 12.0 mg/l. The roasted raw peanut skins extract displayed significantly higher Fe^{2+} ions chelating ability relative to raw peanut skins extract (p<0.05). Compared with the standard, the ability of the extracts to chelate Fe^{2+} ions was significantly lower to EDTA standard (p<0.05). The results also displayed a concentration dependent increase in percentage Fe^{2+}ions chelating abilities of the extracts.

Hydroxyl Radical Scavenging Assay

The results of the ability of the different concentrations of the raw peanut skins and roasted peanut skins to scavenge hydroxyl radical produced by Fenton reaction is depicted in Table 4. There was a concentration dependent increase in the ability of both extracts to scavenge hydroxyl radical. The ability of the raw peanuts skins extract to scavenge hydroxyl radicals ranged from 18.10% (2.0 mg/l) to 40.80% (12.0 mg/l) while that of roasted extracts ranged from 19.03% (2.0mg/l) to 62.88% (12.0 mg/l). On the other hand, the ability of quercetin to scavenge hydroxyl radical ranged from 54.84% (2.0 mg/l) to 85.58% (12.0 mg/l) which was significantly higher to both extracts (p<0.05). The hydroxyl radical scavenging activity of the roasted extract was higher than that of the raw peanut skins extract but significant at 8.0mg/l and 10.0 mg/l.

Reducing power

The reductive ability of the extracts and the standard ascorbic acid is shown in Table 4. The reductive capacities of both raw peanut skins and roasted peanut skins extracts showed marginal but high reductive capacity ranging from 79.57% (2.0 mg/l) to 81.84% (12.0 mg/l) while the reductive ability of ascorbic acid standard ranged from 44.38% (2.0 mg/l) to 90.77% (12.0 mg/l) respectively. The results showed that the raw peanut skins extract exhibited significantly lower reductive ability at 8.0 and 10.0 mg/l which was significantly lower compared to the standard ascorbic acid which showed 90.72% inhibition. Unlike other parameters the extracts did not produce a concentration dependent reductive ability.

Ferric Reducing Antioxidant Power (FRAP assay)

The capacity of the raw peanut skins and roasted extracts to reduce ferric ion (FRAP assay) was investigated against the standard (trolex) (Table 5). While the ferric reducing power of the raw peanut skins extract ranged from 21.4% (2.0 mg/l) to 31.2% (12.0 mg/l) that of the roasted peanut skins extract ranged from 2.13% (2.0mg/l) to 44.23% (12.0 mg/l) which were both significantly lower to the standard antioxidant trolex (p<0.05). The results showed that the raw peanuts skins extract produced a significantly higher ability to reduce ferric iron at lower concentrations of the extract relative to the roasted peanut skins extract. The results showed concentration dependent activities by both extracts.

DISCUSSION

Polyphenol compounds have essential function in preventing lipid peroxidation and are also involved in antioxidant activity.[30] The polyphenol content of the peanut skins extract has been reported to differ widely.[31] Peanut skins are abundant in phenolic compounds, and their antioxidant potential has been reported in two recent reviews.[22,32] The polyphenol content in the raw peanut skin and roasted peanut skins extracts were 50.0±1.40 mg/GAE/g and 30.00±3.51 mg/GAE/g respectively. Nepote *et al*.[33] reported that the content of total phenol was higher than 50mg per gram peanut skin in all the different extraction medium used. The report of Yadav *et al*.[6] showed that the total phenol content in the peanut skin ranged between 14.5 and 101.7mg GAE/g dry skin while another study reported the total phenol content in raw peanut skin to be 95.59 mg/kg.[3] Some reports have shown that roasted peanut skin has higher phenol content than raw peanut skin.[6,28] Peanut skins phenolic compounds have demonstrated antioxidant activities and potential food components and value added utilization in animal feed

Table 4: Reducing power and hydroxyl scavenging activities of roasted and raw peanuts skins methanol extracts.

Concentration (mg/l)	Reducing power Effect			Hydroxyl scavenging effects		
	Roasted Peanuts Skins	Raw peanuts skins	Standard (Ascorbic acid)	Roasted Peanuts Skins	Raw peanuts skins	Standard (quercetin)
12.00	81.84±0.07^{a*}	81.84±0.07^{a*}	90.72±0.78	62.88±1.31^{a*}	40.80±2.17^{a*}	85.58±0.32
10.00	81.23±0.01^{a*}	80.61±0.02^{c*}	88.83±0.61	47.55±1.30^{a*}	37.43±0.87^{b*}	75.05±0.71
8.00	81.12±0.06^{a*}	80.97±0.05^{c*}	86.05±0.12	45.71±0.44^{a*}	31.59±1.31^{b*}	71.78±0.31
6.00	80.09±0.16^{a}	80.35±0.03^{a*}	81.39±0.42	38.52±1.68^{a*}	29.46±0.86^{b*}	60.94±0.81
4.00	79.78±0.01^{a*}	80.9±0.01^{c*}	72.15±0.34	22.39±3.89^{a}	27.38±3.15^{a*}	56.24±0.23
2.00	79.78±0.04^{a*}	79.57±0.42^{a*}	44.38±0.10	19.03±1.73^{a*}	18.10±1.30^{a*}	54.84±0.19

Results are mean ± SD of triplicate readings. Values with different alphabetical superscripts in a row are statistically significant to each other. Values with superscripts * in a row are statistically significant to the standard.

Table 5: Ferric Reducing antioxidant Power activities of roasted and raw peanuts skins methanol extracts.

Concentration (mg/l)	Reducing power Effect		
	Roasted Peanuts Skins	Raw peanuts skins	Standard (Trolox)
12.00	44.23±0.25^{a*}	31.18±0.63^{c*}	89.39±3.01
10.00	29.48±0.25^{a*}	29.13±0.25^{a*}	81.43±0.14
8.00	26.82±3.01^{a*}	27.62±0.37^{a*}	73.98±0.87
6.00	8.88±0.25^{a*}	26.11±0.25^{c*}	70.23±0.58
4.00	4.71±0.88^{a*}	25.49±0.13^{c*}	64.35±1.90
2.00	2.13±0.25^{a*}	21.41±0.63^{c*}	60.74±0.14

Results are mean ± SD of triplicate readings. Values with different alphabetical superscripts in a row are statistically significant to each other. Values with superscripts * in a row are statistically significant to the standard.

industry.[22] One other report on peanut skin showed that 1g dry peanut skin contained between 90-125mg of total phenols while solvent type and skin removal method can significantly affect total phenols.[34] The relatively lower phenolic content observed in this study compared to other studies could be attributed to solvent type, duration of extraction and species differences.

Our results showed that the total flavonoid content and total antioxidant capacity of the raw peanut extract was higher than the roasted peanut skin extract (Table 2). Chukwumah *et al*.[28] observed that the total flavonoid content of raw peanut skin and boiled peanuts respectively were significantly higher than those of the raw peanut without skin and those of all roasted peanuts. They attributed the higher flavonoid content in the raw (with skin) and boiled peanut skin to the presence of proanthocyanidins in the peanut skin.[28,35,36] The phosphomolybdenum method involves the reduction of molybdenum by the antioxidants and the formation of a green molybdenum (V) complex.[30] The antiradical activity of flavonoids and phenols is based on the structural relationship between different parts of their chemical structure.[37]

The methanol extracts exhibited DPPH radical scavenging effect as depicted by the percentage inhibition as the concentration of the extract increased. 1,1-diphenyl-2-picrylhydrazyl (DPPH) is routinely adopted to determine scavenging ability of natural compounds because it is a stable free radical.[30] Therefore higher concentration of antioxidant will produce higher DPPH reduction[38] due to the formation of its nonradical form.[30] Report of Sundararajan and Ilengasan[39] showed that inhibition of DPPH by plant extracts is proportional to their antioxidant content. In this study, scavenging activity of roasted peanut skins extract was significantly higher than the raw peanut skins extract indicating that the roasting process could improve the radical scavenging activity which was determined by the DPPH method. This indicates that the peanut skin could be an alternative source of antioxidant due to their proton donating abilities. Previous reports showed that methanol raw peanut skins extract had higher radical scavenging activity than the peanut hull extract.[33] As the peanut skin is not a high-valued by-product of peanut processing, the use of the roasting process could enhance its economic value as source of antioxidant.

The study determined the chelation of ferrous ions by the methanol extracts of raw peanut skins and roasted peanut skins extracts respectively. The results demonstrated that the methanol extracts possessed a strong potential to chelate iron (II) similar to EDTA standard though significantly lower. Ferrozine can quantitatively form complexes with iron(II) ions and formed the bases for metal chelating activity.[30,40] In the presence of chelating agents, the complex formation is hindered and the formation of red colour in the complex fades. Therefore, the chelating activity of the co-existing chelator is evaluated by measuring the reduction in colour.[30] Different flavonoids have been isolated from mature peanut skins including proanthocyanidins.[28,35] These flavonoids could be involved directly in iron chelating by forming metal-flavonoid complex that has much powerful radical scavenging properties than the free flavonoids.[39] Therefore, the complex formation makes metals unavailable to form complexes with ferrozine. The iron chelating activities of the raw peanut skin and roasted peanut skin extracts can be attributed to the endogenous chelating factors which show that their activities as peroxidation protector may be linked to their iron binding potential.[40] Our results showed that the roasting process significantly affected the iron chelating activity of the roasted extract compared to the raw extract. This notwithstanding, the extracts displayed iron (II) chelating activity and may prevent oxidative damage and also add value to peanut skin.

The hydroxyl radical generation is a very reactive and damaging process to cells, DNA, lipids and almost all biological systems that it comes in contact with through oxidative damage.[40-42] The hydroxyl radical scavenging ability of the raw peanut skins and roasted peanut skins extracts were assessed in this study (Table 4). The results showed that the roasted peanut skin extract demonstrated more effective scavenging activity against hydroxyl radicals than the raw peanut skin extract though significantly lower than quercetin standard. Flavonoids including quercetin and luteolin have been reported to suppress the Fenton reaction and the catalytic wave of the iron ATP/Hydrogen peroxide system.[39-43]

The reducing power activity of the methanol extracts and ascorbic acid were compared. In the reducing power determination, the presence of reductants (antioxidant) in the extract will lead to reducing Fe^{3+}/

ferricyanide complex to the ferrous form[44] which can be monitored at 700nm by measuring the formation of Perl's Prussian blue. From the results obtained from this study, the roasted and the raw peanut skin extracts both showed high reductive activity. In this study, the reductive ability of both extracts were similar to ascorbic acid standard used and could be good source of antioxidant. This implies that both extracts may have electron donating ability and may also possess reductones which reacts and converts free radicals to more stable forms thereby terminating radical chain reactions.[44-46]

The antioxidant capacity of the methanol extracts of raw peanut skins and roasted peanut skins were evaluated by the ability of the antioxidant in the extracts to reduce ferric iron to ferrous in FRAP assay. The ferric to ferrous reduction at low pH causes a coloured ferrous tripyridyltriazine complex to form[47] which can be read at 593nm as the decrease in absorbance is proportional to the concentration of antioxidant.[30,48] The results from the FRAP assay showed that the raw peanut skins and roasted peanut skins extracts have a ferric reducing activity that was concentration dependent although significantly lower to trolox -a water soluble analogue of vitamin E. The significant FRAP result signifies the hydrogen donating ability of the methanol peanut skin extracts.

CONCLUSION

The results showed that roasting improved the *in vitro* antioxidant properties of the peanut skins compared to the raw peanut skins and could improve the value of the peanut skin use as functional food component, antioxidant and in animal feed industries.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Toomer OT. Nutritional chemistry of the peanut (*Arachis hypogaea*). Crit Rev Food Sci Nutr. 2018;58(17):3042-53. doi: 10.1080/10408398.2017.1339015, PMID 28662347.
2. Ros E, Singh A, O'Keefe JH. Nuts: Natural pleiotropic nutraceuticals. Nutrients. 2021;13(9):3269. doi: 10.3390/nu13093269, PMID 34579146.
3. Elsorady MEL, Ali SE. Antioxidant activity of roasted and unroasted peanut skin extracts. Int Food Res J. 2018;25(1):43-50.
4. Leickly FE, Kloepfer KM, Slaven JE, Vitalpur G. Peanut allergy: An epidemiologic analysis of a large database. J Pediatr. 2018;192:223-228.e1. doi: 10.1016/j.jpeds.2017.09.026, PMID 29246346.
5. Muraro A, Sublett JW, Haselkorn T, Nilsson C, Casale TB. Incidence of anaphylaxis and accidental peanut exposure: A systematic review. Clin Transl Allergy. 2021;11(8):e12064. doi: 10.1002/clt2.12064, PMID 34708943.
6. Yadav DN, Yogesh K, Aswani A. Antioxidant activity of peanut (*Arachis hypogaea* L.) skin extract: Application in soybean and mustard oil. Int J Food Proc Technol. 2014;1:26-31.
7. Christman LM, Dean LL, Bueno Almeida CB, Weissburg JR. Acceptability of peanut skins as a natural antioxidant in flavored coated peanuts. J Food Sci. 2018;83(10):2571-7. doi: 10.1111/1750-3841.14323, PMID 30184251.
8. Xiang L, Cao XL, Xing TY, Mori D, Tang RQ, Li J, *et al*. Mixture of peanut skin extract and fish oil improves memory in mice via modulation of anti-oxidative stress and regulation of BDNF/ERK/CREB signaling pathways. Nutrients. 2016;8(5):256. doi: 10.3390/nu8050256, PMID 27136583.
9. Zhang D, Dai C, Zhou L, Li Y, Liu K, Deng YJ, *et al*. Meta-analysis of the association between nut consumption and the risks of cancer incidence and cancer-specific mortality. Aging. 2020;12(11):10772-94. doi: 10.18632/aging.103292, PMID 32487780.
10. Dean LL. Extracts of peanut skins as a source of bioactive compounds: methodology and application. Appl Sci. 2020; 10, 8546 doi: 10.3390/app10238546.
11. Takano F, Takata T, Yoshihara A, Arima Y, Ohta T. Aqueous extract of peanut skin and its main constituent procyanidin A1 suppresses serum IgE and IgG1 levels in mice-immunized with ovalbumin. Biol Pharm Bull. 2007;30:922-7.
12. Saito C, Asano S, Kato C, Kobayashi S, Musha A, Kuribayashi H, *et al*. Nutritional values and antioxidative activities of whole peanuts and peanut skins for ruminant feeds. Anim Sci J. 2016;87(1):54-60. doi: 10.1111/asj.12405, PMID 26249105.
13. Muñoz-Arrieta R, Esquivel-Alvarado D, Alfaro-Viquez E, Alvarez-Valverde V, Krueger CG, Reed JD. Nutritional and bioactive composition of Spanish, Valencia, and Virginia type peanut skins. Journal of Food Composition and Analysis. 2021;98:103816. doi: 10.1016/j.jfca.2021.103816.
14. Fernandes ACF, Vieira NC, Santana ÁL, Gandra RLP, Rubia C, Castro-Gamboa I, *et al*. Peanut skin polyphenols inhibit toxicity induced by advanced glycation end-products in RAW264.7 macrophages. Food Chem Toxicol. 2020;145:111619. doi: 10.1016/j.fct.2020.111619, PMID 32791243.
15. Zhao L, Zhu X, Yu Y, He L, Li Y, Zhang L, *et al*. Comprehensive analysis of the anti-glycation effect of peanut skin extract. Food Chem. 2021;362:130169. doi: 10.1016/j.foodchem.2021.130169, PMID 34102509.
16. Xiang L, Wu Q, Osada H, Yoshida M, Pan W, Qi J. Peanut skin extract ameliorates the symptoms of type 2 diabetes mellitus in mice by alleviating inflammation and maintaining gut microbiota homeostasis. Aging (Albany, NY). 2020;12(14):13991-4018. doi: 10.18632/aging.103521, PMID 32699185.
17. Xu M, Lv C, Wang H, Lu Q, Ye M, Zhu X, *et al*. Peanut skin extract ameliorates high-fat diet-induced atherosclerosis by regulating lipid metabolism, inflammation reaction and gut microbiota in ApoE mice. Food Res Int. 2022;154:111014. doi: 10.1016/j.foodres.2022.111014, PMID 35337573.
18. Li K, Zhang M, Chen H, Peng J, Jiang F, Shi X, *et al*. Anthocyanins from black peanut skin protect against UV-B induced keratinocyte cell and skin oxidative damage through activating Nrf 2 signaling. Food Funct. 2019;10(10):6815-28. doi: 10.1039/c9fo00706g, PMID 31577300.
19. Shin S, Cho SH, Park D, Jung E. Anti-skin aging properties of protocatechuic acid *in vitro* and *in vivo*. J Cosmet Dermatol. 2020;19(4):977-84. doi: 10.1111/jocd.13086, PMID 31389672.
20. Yan F, Chen L, Chen W, Zhao L, Lu Q, Liu R. Protective effect of procyanidin A-type dimers against H_2O_2-induced oxidative stress in prostate DU145 cells through the MAPKs signaling pathway. Life Sci. 2021;266:118908. doi: 10.1016/j.lfs.2020.118908, PMID 33333048.
21. Sobolev VS, Cole RJ. Note on utilization of peanut seed testa. J Sci Food Agric. 2004;1:105-11.
22. Toomer OT. A comprehensive review of the value-added uses of peanut (*Arachis hypogaea*) skins and by-products. Crit Rev Food Sci Nutr. 2020;60(2):341-50. doi: 10.1080/10408398.2018.1538101, PMID 30596253.
23. Yu J, Ahmedna M, Goktepe I, Dai J. Peanut skin procyanidins: Composition and antioxidant activities as affected by processing. J Food Comp Anal. 2006;19:364-1.
24. Vinatha N, Mamidala E. An ethnobotanical study of plants used for the treatment of diabetes in the Waragal district, Andhra Pradesh, India. Biolife. 2013;1(1):24-8.
25. Jack IR, Okorosaye-Orubite K. Phytochemical analysis and antimicrobial activity of the extract of leaves of fleabane (*Conyza sumatrensis*). J Appl Sci Environ Manag. 2008;11(4):63-5. doi: 10.4314/jasem.v11i4.55196.
26. Richa SS, Sharma ML. Phytochemical investigations and anatomical study of three species of Sida. Biolife. 2014;2(2):622-5.
27. Singleton VL, Rossi JA Jr. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965;16:144-58.
28. Chukwumah Y, Walker L, Vogler B, Verghese M. Changes in the phytochemical composition and profile of raw, boiled, and roasted peanuts. J Agric Food Chem. 2007;55(22):9266-73. doi: 10.1021/jf071877l, PMID 17924703.
29. Kalita P, Barman TK, Pal TK, Kalita R. Estimation of total flavonoid content (TFC) and antioxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn. J Drug Deliv Ther. 2013;3(4):33-7.
30. Kalava SV, Menon SG. *In vitro* free radical scavenging activity of aqueous extract from the mycelia of *Volvariella volvacea* (Bulliard Ex fries) Singer. Int J Curr Pharm Res. 2012;4(3):94-0.
31. Munekata PES, Calomeni AV, Rodrigues CEC, Fávaro-Trindade CS, Alencar SM, Trindade MA. Peanut skin extract reduces lipid oxidation in cooked chicken patties. Poult Sci. 2015;94(3):442-6. doi: 10.3382/ps/pev005, PMID 25681473.
32. Christman LM, Dean LL, Allen JC, Godinez SF, Toomer OT. Peanut skin phenolic extract attenuates hyperglycemic responses *in vivo* and *in vitro*. PLOS ONE. 2019;14(3):e0214591. doi: 10.1371/journal.pone.0214591. PMID 30917157.
33. Nepote V, Grosso NR, Guzman CA. Extraction of antioxidant components from peanut skins. Grasas Aceites. 2002;53(4):391-5. doi: 10.3989/gya.2002.v53.i4.335.
34. Yu J, Ahmedna M, Goktepe I. Effect of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. Food Chem. 2005;90(1-2):199-206. doi: 10.1016/j.foodchem.2004.03.048.
35. Lou H, Yuan H, Ma B, Ren D, Ji M, Oka S. Polyphenols from peanut skins and their free radical-scavenging effects. Phytochemistry. 2004;65(16):2391-9. doi: 10.1016/j.phytochem.2004.06.026, PMID 15381013.

36. Karchesy JJ, Hemingway RW. Condensed tannins 4β→8;2→O→7)-linked procyanidins in *Arachis hypogaea* L. J Agric Food Chem. 1986; 34:966-70.
37. Rice-Evans C, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci. 1997;2(4):152-9. doi: 10.1016/S1360-1385(97)01018-2.
38. Annapandian VM, Rajagopal SS. Phytochemical evaluation and *in vitro* antioxidant activity of various extracts of *Leucas aspera* (wild.) Link leaves. Free Radic Antioxid. 2017;7(2):166-71. doi: 10.5530/fra.2017.2.25.
39. Sundaraajan R, Ilengesan R. *In vitro* antioxidant assay of methanol extract of *Buddleja asiatica*. Free Radic Antioxid. 2018;8(1):55-61.
40. Asokkumar K, UmaMaheswari M, Sivashanmugam AT, SubhadraDevi V, Subhashini N, Ravi TK. Free radical scavenging and antioxidant activities of *Glinus oppositifolius* (carpet weed) using different *in vitro* assay systems. Pharm Biol. 2009;47(6):474-82. doi: 10.1080/13880200902817901.
41. Koppenol WH. The centennial of the Fenton reaction. Free Radic Biol Med. 1993;15(6):645-51. doi: 10.1016/0891-5849(93)90168-t, PMID 8138191.
42. Ugwu CE, Suru SM, Dike CC, Okpogba AN. Phytochemical and *in vitro* antioxidant activities of methanol leave extract of *Alternanthera basiliana*. J Pharm Res. 2018;12(6):835-9.
43. Cheng IF, Breen K. On the ability of four flavonoids, baicilum, luteolin, naringenin and quercetin to suppress the Fenton reaction of the iron-ATP complex. Biometals. 2000;13(1):77-83. doi: 10.1023/a:1009229429250, PMID 10831228.
44. Chung YC, Chang CT, Chao WW, Lin CF, Chou ST. Antioxidant activity and safety of 50% ethanolic extract from red been fermented by *Bacillus subtilis* IMR=NK1. J Agric Food Chem. 2002;50(8):2454-8. doi: 10.1021/jf011369q, PMID 11929313.
45. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidantive properties of Xanthen on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem. 1992;40(6):945-8. doi: 10.1021/jf00018a005.
46. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem. 1995;43(1):27-32. doi: 10.1021/jf00049a007.
47. Benzie.
48. Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem. 2006;94(4):550-7. doi: 10.1016/j.foodchem.2004.12.004.

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