EVALUATION OF ANTIOXIDANT ACTIVITY OF SOLUBLE AND BOUND PHENOLICS FROM GROUNDNUT (*ARACHIS HYPOGAEA L.*)

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ABSTRACT

Groundnuts were separated as kernel, skin, and some were directly used as whole nuts, by retaining skin. Soluble phenolics were extracted from three fractions using 70% aqueous acetone. Insoluble-bound phenolics were subsequently extracted into diethyl ether-ethyl acetate fractions after alkaline and acid hydrolysis. Both soluble and bound phenolics were assessed for their total phenolics; total flavonoid contents. Antioxidant activities were evaluated by DPPH radical scavenging activity, phosphomolybdenum method, reducing power, ferrous-ion chelating effect and ABTS cation radical decolorization assay. Soluble phenolics in skin were ~1262.5 mg GAE/100g as compared to ~83.8 and ~121.7 GAE/100g in kernel and the whole nut, respectively. Bound phenolics content of the skin, kernel and the whole nut were ~92.6, ~35.8, ~37.4 GAE/100g, respectively. Soluble and bound phenolic extracts from the skin, kernel and the whole nut also exhibited the good antioxidant activity. Results indicate that groundnuts with skin may serve as a viable functional food ingredient and a source of natural antioxidants.

Keywords:

Groundnuts

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1. Introduction

Natural antioxidants are attracting increased attention due to their potential nutritional and therapeutic value together with their presumed safety. There has been increasing interest in finding the presence and availability of such compounds from plant materials that may possess bioactive properties, in particular antioxidant activity. Phenolic compounds exhibit a wide range of useful properties, such as anti-allergenic, anti-inflammatory, anti-microbial, anti-oxidant, anti-thrombotic, cardio protective and vasodilatory effects (Viswanath et al., 2009). For these reasons, great efforts has been made to characterize the phenols occurring in different plants (Pinelo et al., 2005).

Nuts have been identified as being a rich source of antioxidants (Halvorsen et al., 2002; Wu et al., 2004). Epidemiological studies suggest that frequent consumption of peanuts may reduce the risk of coronary heart disease (Fraser et al., 1992) and certain types of cancers (Awad et al., 2000). The numerous cardioprotective nutrients and bioactives endogenous to peanuts and synergies among these components truly make peanut a desirable plant food (Kris-Etherton et al., 1999). Of the useful bioactives found in groundnut kernels, perhaps the least characterized group to date is the phenolics and their potential contribution in the human body relating to anti-oxidative and anti-inflammatory pathways. The limited compositional data available on phenolic profile of peanut kernel suggests that free phenolics and their derivatives are dominant (Talcott et al., 2005). The finding from the above studies are significant to groundnut research, but our interest lies in the skinless kernel part, the edible portion of groundnut, and whole nut both of which can be dietary source to convey the useful bioactive phenolic compounds to human body.

Phenolic compounds in nuts are present in the free, esterified or bound forms. Phenolic acids may form both ester and ether linkages owing to their bifunctional nature through reactions involving their carboxylic and hydroxylic groups, respectively, which allows phenolic acids to form cross-links with cell wall macromolecules (Yu et al., 2001). Bound phenolics may be released by alkali, acid or enzymatic treatment of samples before extraction (Andreasen et al., 2001).

This study was undertaken to determine antioxidant potential of free phenolics as well as bound phenolic portion of skinless kernel, whole nut and outer skin. The antioxidant activity of whole nut and skinless kernel was determined separately so that it will help to understand better the radical scavenging potential of groundnut with or without skin.

2. Materials and methods

2.1 Materials

Authentic variety of TMV-2 of groundnut (*Arachis hypogaea L.*) seeds were procured from Karnataka State Seeds Corporation Ltd., Mysore, India. Gallicacid, DPPH (1, 1 diphenyl-2-picrylhydrazyl), ABTS (2,2 -azino-bis(3-ethylbenzothia- zoline-6-sulfonate)), Trolox (6-hydroxy2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) , catechin (Sigma Aldrich Chemical Co., Germany). All chemicals were of analytical grade (AR), unless otherwise specified. All experiments were performed using quartz triple distilled water (DW).

2.2. Preparation of defatted groundnut kernel flour

Groundnuts were decorticated and the seeds were flaked in Aktiebolaget, Kvar nmskiner, Malmo flaking machine, Type No.6725 keeping 2mm distance between the counter rotating drums of dimensions 60cm length x 45cm outer diameter at 400rpm. The flakes were extracted with n-hexane for 5-6 times (1:1) such that the final fat content was less than 1% as determined by soxhlet method (AOAC, 2005). The defatted flakes were air-dried to remove traces of hexane and the dried material was powdered in a Brabender Quadrumat Senior Automatic Pilot Mill, Buisberg, Germany. Good practices were observed during sample preparation to ensure sanitary conditions to prevent contamination.

2.3. Extraction of free phenolics

2.3.1. Shaking Method

Phenolic compounds were extracted from defatted groundnut flour using different aqueous organic solvents in a temperature regulated water bath. The extractions were repeated, and the supernatants were combined and solvents were evaporated under vacuum at 40°C with rotavapour. Remaining aqueous samples were frozen and then lyophilized in vacuo with a freeze drier. Extracts obtained were tightly sealed and were stored in amber vials at 4°C until assayed. Different parameters for shaking mode of extraction such as, solvent, solid: solvent ratio, temperature, shaking period, number of extractions were also optimized.

2.3.2. Column method

15 grams of defatted groundnut flour was loaded in a glass column of 60 cm length and 3cm diameter. The column was tapped to get a uniform packing. When the bed volume was equilibrated with solvents, the stopper was closed and kept for 24 hrs. After 24 hrs, the extract was eluted out and desolventised using a rotavapour. Remaining aqueous samples were frozen

and lyophilized. Extracts obtained were tightly sealed and were stored in amber vials at 4°C until assayed.

2.3.4. Soxhlet method

5 grams of defatted groundnut flour was weighed into a thimble and introduced inside the soxhlet condenser. Solvents approximately 50 ml, was added and the soxhlet was allowed to run for 6-8 hrs. Solvent was evaporated in rotavapour after extraction and remaining aqueous sample was freeze dried. Extracts obtained were tightly sealed and stored in amber vials at 4°C until assayed.

2.4. Extraction of bound phenolics

The bound phenolic acids in the residue were isolated . The residue of groundnut kernel, whole nut and skin obtained after extraction of soluble phenolics was hydrolyzed with 2M NaOH at room temperature for 4 hours with stirring under nitrogen. The resulting slurry was acidified to pH 2 with 6M HCl, centrifuged and the supernatant was extracted five times with n-hexane to remove fatty acids, which are released during alkaline hydrolysis. The resulting water phase was again extracted five times with diethyl ether and ethyl acetate (1:1, v/v) and subsequently desolventised to dryness at room temperature in a rotary evaporator (Krygier et al., 1982).

2.5. Total Phenol Estimation

The total phenolic content (TPC) of the extracts was determined spectrophotometrically using the Folin-Ciocalteu total phenol method (Spanos et al., 1990) with slight modifications. Gallic acid standard solutions were prepared at 20, 40, 60, 80, 100 mg/L concentrations.

The extract and gallic acid standards (0.4 ml) were transferred to 15 ml test tubes. 0.4 ml of 2N Folin-Ciocalteu's phenol reagent and 3.6 ml of distilled water was added to each test tube and mixed using a vortex mixer. After 5 minutes of incubation, 4 ml of 7% (w/v) Na_2CO_3 in distilled water was added and mixed thoroughly. Absorbance of samples was determined using a spectrophotometer at 765 nm, after 90 minutes of incubation in dark against a blank. The concentration of total phenolic compounds in the extract was determined from gallic acid standard curve. All samples were assayed in triplicates. TPC was expressed as milligram gallic acid equivalent per 100g (mg GAE/100g) of dry groundnut samples.

2.6. Total flavonoid estimation - $AlCl_3$ colorimetric assay

The total flavonoid content (TFC) was determined using the method described by Kim et al., 2003 with slight modifications. 0.5 ml of aliquot of the extract dissolved in 4 ml of DW was

mixed with 0.3 ml of 5% (w/v) $NaNO_2$ and was allowed to react for 5 minutes. Subsequently, 3 ml of 1% $AlCl_3$ was added to the reaction mixture and allowed to stand for 6 minutes. Finally, 2 ml of 1M NaOH solution was added to total reaction mixture and made up to 10 ml and mixed well. Tubes were kept in dark at room temperature for 15 minutes followed by centrifugation for 5 minutes at 4000g. The absorbance was read at 510 nm against a blank prepared in a similar manner by replacing the extract with distilled water. The TFC of the samples were calculated from standard curve, and expressed as milligram of catechin equivalent (CE) per 100g (mg CE/100g) of defatted meal.

2.7. Antioxidant assays

2.7.1. DPPH free radical scavenging assay

The free radical scavenging activity of extracts was measured using the method described by Brand-Williams et al. (1995) with some modifications. 0.1mM solution of DPPH (1, 1 diphenyl-2-picrylhydrazyl) was prepared in absolute methanol. An aliquot of 150µl of extract solution was added to 3 ml of the reagent. The decrease in absorbance was measured at 517 nm after 30 minutes of incubation in dark, until the reaction reached a plateau.

The percentage inhibition activity was calculated from the equation:

$(1 - A_1/ A_0) \times 100$

Where A_0 is the absorbance of Control, A_1 is absorbance of extract.

2.7.2 Determination of Total antioxidant capacity

The total antioxidant activity of the extract was evaluated by the phosphomolybdenum method (Prieto et al., 1999). The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/ Mo (V) complex at acidic pH. 0.1 ml of extract was combined with 1 ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 minutes. Then the absorbance of the solution was measured at 695 nm, after cooling to room temperature using a spectrophotometer against a blank. The antioxidant activity of the samples was expressed in mili Molar (mM) equivalents of ascorbic acid (AA).

2.7.3. Reducing power

The reducing power of extracts was determined according to the method of Oyaizu (1986) . Different concentrations of the extract (1 ml) were mixed with 2.5 ml, 0.2M pH 6.6 phosphate buffer (PB) and 2.5 ml of 1% potassium ferricyanide [K_3 Fe $(CN)_6$]. The mixture was mixed

well and incubated at 50°C for 20 minutes. A portion (2.5 ml) of 10% trichloroacetic acid was added to the mixture, which was centrifuged at 6000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Increase in absorbance of the reaction mixtures indicates a higher reducing power.

2.7.4 Fe^{2+} Chelating Activity

Fe^{2+} chelating activity was measured according to a method of Hsu et al. (2006) with a few modifications. The reaction mixture (2.15 ml) contained 500µL of different concentrations of extracts, 50µL $FeCl_2$ (2mM) and 1.6 ml of deionised H_2O. The mixture was shaken vigorously and left at room temperature for 5 minutes; 100µL of ferrozine (5mM in methanol) were then added, mixed and left for another 5 minutes to complex the residual Fe^{2+}. The absorbance of the Fe^{2+}-ferrozine complex was measured against a blank. The chelating activity of the extracts for Fe^{2+} was calculated as:

Chelating Rate = $[1- (A_1-A_2)/A_0] \times 100$

A_1 – Abs of Control (without extract)

A_2 – Abs in presence of extract

A_0 - Abs of blank (without ferrozine)

2.7.5. ABTS radical cation decolorization assay

ABTS radical- scavenging activity of the extract was determined according to procedure described by Re et al. (1999). In this test, the relative capacity of antioxidants in samples to scavenge the $ABTS^+$ radical was measured. ABTS (2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) and potassium persulfate (7mM ABTS and 2.45 mM potassium persulfate final concentration) were dissolved separately in water, combined and made up to volume in a 10 ml volumetric flask. The mixed solution was transferred to an amber coloured bottle, covered with aluminium foil and allowed to stand at room temperature for 12-16 hours in dark. Before usage, this solution was diluted to get an absorbance of 0.7± 0.020 at 734 nm with phosphate buffered saline (PBS), pH 7.4 or absolute ethanol. The spectrophotometer was blanked with PBS/ethanol. The final reaction mixture of standard group was made up to 1 ml with 950 µl of $ABTS^+$ reagent and 50 µl of standards. Similarly, in the test group, 1 ml of reaction mixture comprised 950 µl of $ABTS^{\cdot+}$ reagent and 50 µl of extract solution. The reaction

mixture was vortexed and absorbance was recorded after 30 min at 734 nm. Percentage of radical remaining was calculated according to the following equation:

ABTS remaining = (1- Abs_0 /Abs_t) x 100

Where Abs_0 is the absorbance of a control without any radical scavenger and Abs_t is the absorbance of the ABTS radical remaining in the presence of antiradical scavenger.

2.7.6. Statistical analysis

Statistical analysis were performed using scientific graphic and analysis computer software Origin Pro (version 7) supplied by Origin Lab corporation, Northampton, MA, USA and results were expressed as Mean ± standard error of three experiments.

3 Results and discussion

3.1 Optimization of conditions for preparation of crude phenolic extracts.

Different solvent systems have been used for the extraction of polyphenols from plant materials (Chavan et al., 2001). Zuo et al. (2002) had suggested that the extraction method chosen must enable complete extraction of the compounds of interest and must avoid their chemical modifications. Sun and Ho (2005) has also reported the use of water, aqueous mixtures of ethanol, methanol and acetone to extract compounds from plants. There is also literature supporting the use of boiling water for the extraction of polyphenols from green, black and mate teas (Filip and Ferraro, 2003; Lee and Ong, 2000). Turkmen et al., (2006) in his work has also concluded that that there is no standard protocol which can be followed to extract maximum polyphenols from plant matrices. Thus, in our work preliminary studies were conducted to determine the best solvent and an appropriate method for the maximum extraction of crude phenolics from groundnuts. The solvents selected during initial studies were hot water, 50% and 70% (v/v) of ethanol, methanol and acetone, 50% ethyl acetate, 50% acetonitrile. Among all the above mentioned solvents, 70% acetone showed highest yield (~13.2%) for defatted groundnut kernel and also highest value among the soluble phenolic content (~99.63 mg GAE/100g).

To further optimize extraction with acetone, we performed extractions with a range of aqueous acetone concentrations viz, 30, 50, 70, 90% (v/v) and also absolute acetone. It was noted that different concentrations of aqueous acetone did not have any significant effect on the extraction yield or total soluble phenolics. But, absolute acetone was found to be poor solvent,

giving least yield and minimum soluble phenolics content (~20.36 mg GAE/100g). Chavan et al., 2001 has reported that aqueous acetone (70%) with or without acid was more efficient than absolute acetone for recovery of maximum amount of condensed tannins from peas, which is in agreement with our findings.

A similar study to determine the best solvent for extraction of phenolic compounds from lentil seeds using 80% acetone, methanol and ethanol at 80°C (Amarowicz et al., 1995). Acetone extracted markedly greater amounts of phenolic compounds compared with methanol and ethanol systems.

After optimization of solvent as 70% acetone, other conditions for shaking mode of extraction were optimized and the optimized conditions for maximum extraction of crude phenolics by shaking method is as following: solvent – 70% acetone; solid to solvent ratio – 1:10; Temperature – 40°C; Period of shaking – 1 hour; Number of extraction – three.

When parameters for shaking extraction were optimized, column method and soxhlet method were also carried out, to check the effectiveness of these as a method to obtain the crude phenolic extracts. The yield obtained for column method and soxhlet method were ~12.3% and ~8.8% respectively, which was lesser than the yield obtained for shaking method. In addition, soxhlet method was not found to be favourable because, of higher temperatures involved which may affect phenolics stability or cause phenolic denaturation. Turkmen et al. (2006) had suggested that solvents used for polyphenol extraction also had a significant effect on its scavenging activities. Thus, initially antioxidant assays were performed with acetonic, ethanolic and methanolic extracts.

DPPH radical has generally been used in model systems to investigate the scavenging activities of antioxidant compounds. DPPH scavenging capacity and reducing power of three different solvent soluble phenolics extracts of groundnut kernel were determined. All three extracts exhibited strong antioxidant activity against DPPH radical in a concentration dependent manner. The EC_{50} value (defined as the concentration of extracts required to lower the initial radical concentration by 50%) was extrapolated from dose-dependent curves. The EC_{50} value obtained for 70% acetonic, ethanolic and methanolic extracts were 0.85, 2.4, 2.55 mg/ ml respectively. Acetonic extracts gave 50% inhibition of DPPH radical at a lower concentration than ethanolic and methanolic extracts.

In reducing power assay, an increase in absorbance, indicates higher reductive potential. A concentration of 0.5 mg/ ml acetonic extract gave more absorbance at 700 nm than 0.5 mg/ ml of ethanolic and methanolic extract. Thus phenolic compounds in acetonic extract have a more reductive potential. Based on these preliminary studies, 70% acetone was selected as the extraction solvent for the preparation of crude phenolic extracts in all subsequent studies.

3.2. Yield, total phenolics and total flavonoid content of groundnut extracts

3.2.1. Yield

The yield of soluble and bound phenolic extracts from groundnut kernel, whole nut and skin are given in Table 1. Yield obtained for soluble phenolics is more when compared to bound phenolics for all the three groundnut extracts.

3.2.2. Total phenol content

The total phenol content of soluble and bound fractions of groundnut extracts was determined using Folin- Ciocalteu assay. The TPC value expressed as milligrams of gallic acid equivalents (GAE) per grams of dry weight of different portions of groundnut are shown Table 1. In this method, under alkaline conditions, phenolic groups are deprotonated, leading to the formation of phenolate ions, which reduce the phosphotungstic-phosphomolybdic complex in the reagent to a blue colour, which is measured at 765 nm. The soluble phenolics content of the skin was found to be significantly higher than that of whole nut and kernel. It was also found that the soluble phenolics content was higher than that of the bound phenolics in all fractions. According to Chukwumah et al. (2009) the higher TPC of skin may be attributed to the presence of phenolic compounds such as proanthocyanidins. In general, phenolic compounds are concentrated in the outer layers, namely hull (pericarp), seed coat (Chandrasekara and Shahidi, 2011). Thus it may be worth pointing out that during preparation or consumption of groundnut as food, if the outer skin is retained, would preserve much of the phenolics and consumers may be able to gain its benefits.

Sales and Resurreccion (2010) had concluded in their work that raw peanuts had 84.0 mg GAE/100g. This very well co-relates with our findings. Chukwumah et al. (2009) had studied the effect of peanut skin colour and its relation to total phenolic content. In this work, the TPC of the different peanut cultivars ranged from 94.4- 228.4 mg GAE/100g peanut with an average of 143.5 mg GAE/100g. Of the 27 cultivars studied, 12 cultivars had a TPC between 100-120 mg GAE/100g.

3.2.3. Total flavonoid content (TFC)

Table 1 also presents the TFC of different portions of groundnut used in this study as determined by aluminium (III) chloride colorimetric assay. Flavonoids are the most studied group of phenolic compounds and are known to possess antioxidant, anticancer, antiallergic, anti-inflammatory, antineuro-inflammatory, and gastroprotective properties. Flavonoids form a pink-coloured complex with aluminium (III) through the 4- keto and neighbouring hydroxyl groups or through adjacent hydroxyl group in the B ring. The flavonoid content of phenolic extract from skin, whole nut and kernel were expressed as mg CE/100g. In general, all soluble extracts had higher TFC than their corresponding bound extracts. Yvonne et al. (2009) in their work had also determined the flavonoid content of some 27 cultivars of peanuts, whose values ranged from 27.6-139.9 mg CE/100g with an average content of 56.4 mg CE/100g.

3.3. Antioxidant Assays

3.3.1. DPPH radical scavenging activity of soluble and bound groundnut extracts

DPPH assay is a preliminary test to examine the antioxidant potential of extracts. This assay has been widely used to test the free radical scavenging ability of various samples (Shimoji et al., 2002). DPPH, a stable nitrogen centered free radical, has been used to evaluate natural antioxidants or their radical quenching capacities because they require a relatively short time compared to other methods. The method is based on the reduction of the absorbance of methanolic DPPH solution at 517 nm in the presence of a proton donating substance, due to the formation of the diamagnetic molecule by accepting an electron or hydrogen radical (Soares et al., 1997). In the present study the extracts from soluble and bound phenolics from groundnut skin, kernel and whole nut were evaluated for radical scavenging activities against DPPH. The radical scavenging activity, in terms of EC_{50} of soluble and bound phenolic from all the fractions of groundnut is given in Table 2 & 3. The soluble and bound phenolic extracts of the skin exhibited a higher DPPH radical scavenging activity. For soluble phenolics from groundnut skin extracts a significant increase of scavenging activity was observed at the concentration range 0.02-0.1 mg/ ml, and the scavenging reached a maximum plateau at round 90% from 0.1-0.2 mg/ ml. For kernel extract, increase in scavenging activity was observed at concentration range 0.5-3.0 mg/ ml and scavenging reached a maximum plateau at around 92% from 3.0-4.0 mg/ ml. For whole nut extract, increase was observed at concentration range 0.2-1.0 mg/ ml and reached a maximum plateau at 93% from 1.0-2.0 mg/ ml. Similarly bound phenolics extracts from

groundnut skin, kernel and whole nut showed a significant DPPH scavenging activity. For bound phenolics from groundnut skin extracts a significant increase of scavenging activity was observed at the concentration range 0.02-0.15 mg/ ml, and the scavenging reached a maximum plateau at around 90% above 0.2 mg/ ml. For kernel extract, increase in scavenging activity was observed at concentration range 0.5-4.0 mg/ ml and scavenging reached around 70% from 3.0-4.0 mg/ ml. For whole nut extract, increase was observed at concentration range 0.2-2.0 mg/ ml and reached a maximum of 80% above 2.0 mg/ ml. However, it is clear that the DPPH radical scavenging activity of the soluble phenolic extracts were better than the bound phenolic extracts in all fractions, which may be due to their relatively higher phenolics concentration in each. EC_{50} value of BHT evaluated along the samples was noted to be 0.87 mg/ ml.

The results shown above indicate that the skin extract scavenges organic free radicals more effectively than the other groundnut extracts. Similar results were also obtained by Siriwardhana and Shahidi (2002) who evaluated the anti-radical activity of different almond extracts and found that 210, 50, 129 ppm of almond seed, skin, and shell soluble phenolics extracts, respectively, were needed to completely scavenge DPPH radicals. Thus, almond skin reported as the most effective DPPH radical scavenger amongst the other almond products examined. Thus this also indicates the statement made by Scalbert et al. (2005) that generally the outer layers of plants such as the peel, shell and hull contains large amounts of polyphenolic compounds to protect the inner materials. Similarly, Alasalvar et al. (2006) demonstrated that the hazelnut green leafy cover scavenged organic free radicals more effectively than seed/kernel extracts.

3.3.2. Total antioxidant activity by phosphomolybdenum method

The total antioxidant capacity of groundnut skin, kernel and whole nut were evaluated by phosphomolybdenum method. The method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm. The assay is simple and independent of other antioxidant measurements commonly employed. Moreover, it is a quantitative one, since the activity of extracts is expressed as the number of equivalents of ascorbic acid or alpha tocopherol. Total antioxidant activity expressed as mg equivalent of ascorbic acid/g of soluble and bound phenolic extract from skin, kernel, and whole nut is represented in Table 2 & 3. Total antioxidant activity of all the fractions from soluble phenol extract is found to be more or less equal. But, for bound phenolics, skin extract is showing high than whole nut and kernel extract.

3.3.3. Reducing power of extracts

The reductive potential of groundnut extracts is clearly presented in Figure 1-3. Compounds with reducing power are capable of donating electrons and thus reducing the oxidized intermediates of peroxidation by acting as antioxidants. Reductants in the extracts reduce the ferric/ferricyanide complex to the ferrous form. Groundnut extracts tested in the present study exhibited considerable reductive potential.

Siddhuraju et al. (2002) reported that the reducing power of bioactive compounds was associated with antioxidant activity. Samples with higher reductive potential would give a higher absorbance at same concentrations. The reducing power of soluble phenolic extracts of groundnut skin, kernel and whole nut increased with increase in concentration; however, when compared, skin and whole nut extracts gave higher absorbance at a lower concentration than that of kernel extract. The reducing power of bound phenolic extracts also exhibited a similar trend, but lesser than that exhibited by soluble phenolic fractions. Reducing power of ascorbic acid only showed a slightly higher activity when compared with different sample extracts. From the above findings it is clear that the results are well correlated with the amount of the phenolic constituents, which are present in the respective extracts. Thus, soluble and bound phenolics present in groundnut skin, kernel and whole nut extracts are good electron donors and could terminate the radical chain reaction by converting free radicals to more stable products.

3.3.4. Ferrous ion –chelating activity

Table 2 & 3 shows the ferrous ion chelating activity of soluble and bound phenolic extracts from groundnut samples represented as EC_{50}. Ferrous ion is a key transition metal ion responsible for initiation of peroxidation in foods and biological systems. In the body ferrous ion contribute to the generation of hydroxyl radicals via fenton's reaction and lead to the destruction of biomolecules; thus causing disease conditions and ageing. Chelating agents reduce the concentration of metal ions available for catalyzing peroxidation and thus are known to serve as effective secondary antioxidants. In this assay ferrous ions form a purple colour complex with ferrozine and the intensity of which decreases in the presence of chelating agents.

EC_{50} value represented in the table shows the concentration of groundnut phenolic extracts in mg/ ml required to achieve 50% chelation of ferrous ion available in the assay medium. The soluble and bound phenolic extracts from all the three groundnut fractions exhibited quite significant chelating activity. For soluble phenolics from skin, a significant increase of

scavenging activity was observed at the concentration range 0.025-0.10 mg/ ml, and the scavenging reached a maximum plateau at round 95% from 0.16-0.2 mg/ ml. For kernel extract, increase in scavenging activity was observed at concentration range 0.5-4.0 mg/ ml which reached around 90% from 4.5 mg/ ml and above. For whole nut extract, increase was observed at concentration range 0.2-1.0 mg/ ml and reached a maximum inhibition of 90% from 2.0 mg/ ml and above concentrations. Bound phenolic extracts from the three groundnut fractions also showed quite good ion chelating activity. For bound phenolics from skin, a significant increase of scavenging activity was observed at the concentration range 0.02-0.125 mg/ ml, and the scavenging reached constant at round at 0.2 mg/ ml concentration attaining 92%. For kernel extract, increase in scavenging activity was observed at concentration range 0.5-3.5 mg/ ml and scavenging reached a maximum plateau at around 89% from 4.0 mg/ ml and above. For whole nut extract, increase was observed at concentration range 0.2-1.0 mg/ ml and reached a maximum of 90% at 2.0 mg/ ml and above concentrations. EDTA, which was used as an external standard also exhibited high chelating effect with 50% inhibition obtained at 0.22 mg/ ml concentration. Accordingly it is suggested that the ferrous ion chelating effects of these fractions would be beneficial to protect against oxidative damage.

3.3.5. Evaluation of antioxidant capacity of extract by ABTS $^{\cdot+}$ decolorization assay.

This method measures the relative antioxidant ability of the extracts to scavenge the cation radical ABTS$^{\cdot+}$. The ABTS$^{\cdot+}$ generated by potassium persulfate, is presented as an excellent tool for determining the antioxidant activity of hydrogen-donating and chain breaking antioxidants. ABTS$^{\cdot+}$ assay can be used to measure the antioxidant activity of a broad diversity of substances (Re et al., 1999; Rice-Evans et al., 1995). The extracts of groundnut skin, kernel and whole nut exhibited effective ABTS$^{\cdot+}$ cation radical scavenging activity. Table 2 & 3 shows the ABTS$^{\cdot+}$ cation radical scavenging activity of soluble and bound phenolic extracts in groundnut samples expressed as EC_{50}. For soluble phenolic extracts from skin an increase in scavenging activity was observed between 0.02-0.075 mg/ ml and subsequently attained maximum inhibition of cation radical for concentrations higher than 0.1 mg/ ml. Similarly, for bound extracts from skin showed an increase in activity from 0.01-0.15 mg/ ml and reached constant for further concentrations. For soluble phenolic extract from kernel, increase in scavenging activity was observed at concentration range 0.5-4.0 mg/ ml and scavenging reached a maximum of 93% at 4.0 mg/ ml and above. Bound phenolic extract from kernel also showed a similar trend. For

whole nut extract, increase was observed at concentration range 0.2-1.5 mg/ ml and attained a maximum scavenging of about 95% at 1.5 mg/ ml and above concentrations. Bound phenolic extracts from whole nut exhibited good scavenging of $ABTS^{\cdot+}$ cation radical with EC_{50} value of 0.45 mg/ ml.

4. Conclusions

This study suggested that total polyphenol content and antioxidant activity of groundnut extracts is strongly affected by the extracting solvents. The maximum yield of phenolic extract depended on the solvent used and the method followed for extraction. Further, 70% aqueous acetone was found to be the most efficient solvent for polyphenol extraction from groundnut while absolute acetone was found to be a poor solvent. Antioxidant activity also depended on the type of solvent used for extraction, which is clear from the comparison of radical scavenging effect of acetonic, ethanolic and methanolic extracts. Though groundnuts seemed to have a lower content of bound phenolics, it also demonstrated significant antioxidant and radical scavenging activity, which are important when the health benefits are to be considered. These bound phytochemicals may be released in the colon through microflora-assisted digestion and thereafter exert their health benefits. The study also clearly shows that outer skin had significantly higher levels of phenolic antioxidants thus showed better antioxidant activity than kernel. Therefore, retention of maximum amount of skin on the kernel of groundnut is essential for taking advantage of their benefits. Thus, whole groundnuts have great potential in development of nutraceuticals rich in antioxidants and functional food ingredients.

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Table 1- Yield, total phenol content and total flavonoid content in different portions of groundnut samples[1].

Sample	Yield[a]		Total phenol content[b]		Total flavonoid content[c]	
	Soluble Phenol	Bound Phenol	Soluble Phenol	Bound Phenol	Soluble Phenol	Bound Phenol
Skin	30.4±1.15	27.6±1.97	1262.5±1.25	92.58±0.624	343.9±2.27	62.63±2.52
Whole nut	18.73±1.39	4.13±0.26	121.7±2.01	37.41±0.370	31.19±1.98	17.54±0.76
Kernel	15.58±1.31	4.04±0.046	83.8±2.25	35.82±0.578	23.99±1.67	15.12±1.65

[1]Results are expressed as mean± SE (n=3).

[a] Yield expressed in g/100g of deffated sample.

[b] Total phenol content expressed in mg GAE/100g of sample.

[c] Total flavonoid content expressed in mg CE/100g of sample.

Table 2- Antioxidant activities of soluble phenolic extracts from different portions of groundnut [1]

Sample	Total antioxidant activity [a]	DPPH radical scavenging activity[b]	Fe 2+ ion chelating activity [c]	ABTS.+ cation scavenging activity[d]
Skin	399.13±3.78	0.05	0.04	0.04
Whole nut	390.76±4.76	0.95	0.0875	0.45
Kernel	380.67±3.95	1.37	2.0	1.875

[1] Data are expressed as mean± SE (n=3).

[a] Total antioxidant activity expressed as mM equivalent of ascorbic acid.

[b, c, d] expressed in EC_{50} (concentration of extracts required to lower the initial radical concentration by 50%)

Table 3- Antioxidant activities of bound phenolic extracts from different portions of groundnut [1]

Sample	Total antioxidant activity [a]	DPPH radical scavenging activity[b]	Fe 2+ ion chelating activity [c]	ABTS.+ cation scavenging activity[d]
Skin	138.31±5.61	0.075	0.086	0.091
Whole nut	102.0±1.75	1.21	1.10	0.68
Kernel	93.0±1.18	2.90	2.35	2.35

[1] Data are expressed as mean± SE (n=3).

[a] Total antioxidant activity expressed as mM equivalent of ascorbic acid.

[b, c, d] expressed in EC_{50} (concentration of extracts in required to lower the initial radical concentration by 50%).

Legend to Figures

Fig 1: Reducing power of soluble and bound phenolic extract from groundnut skin

- ▪ Soluble phenolics
- • Bound phenolics

Fig 2: Reducing power of soluble and bound phenolic extract from groundnut whole nut

- ▪ Soluble phenolics
- • Bound phenolics

Fig 3: Reducing power of soluble and bound phenolic extract from groundnut kernel

- ▪ Soluble phenolics
- • Bound phenolics

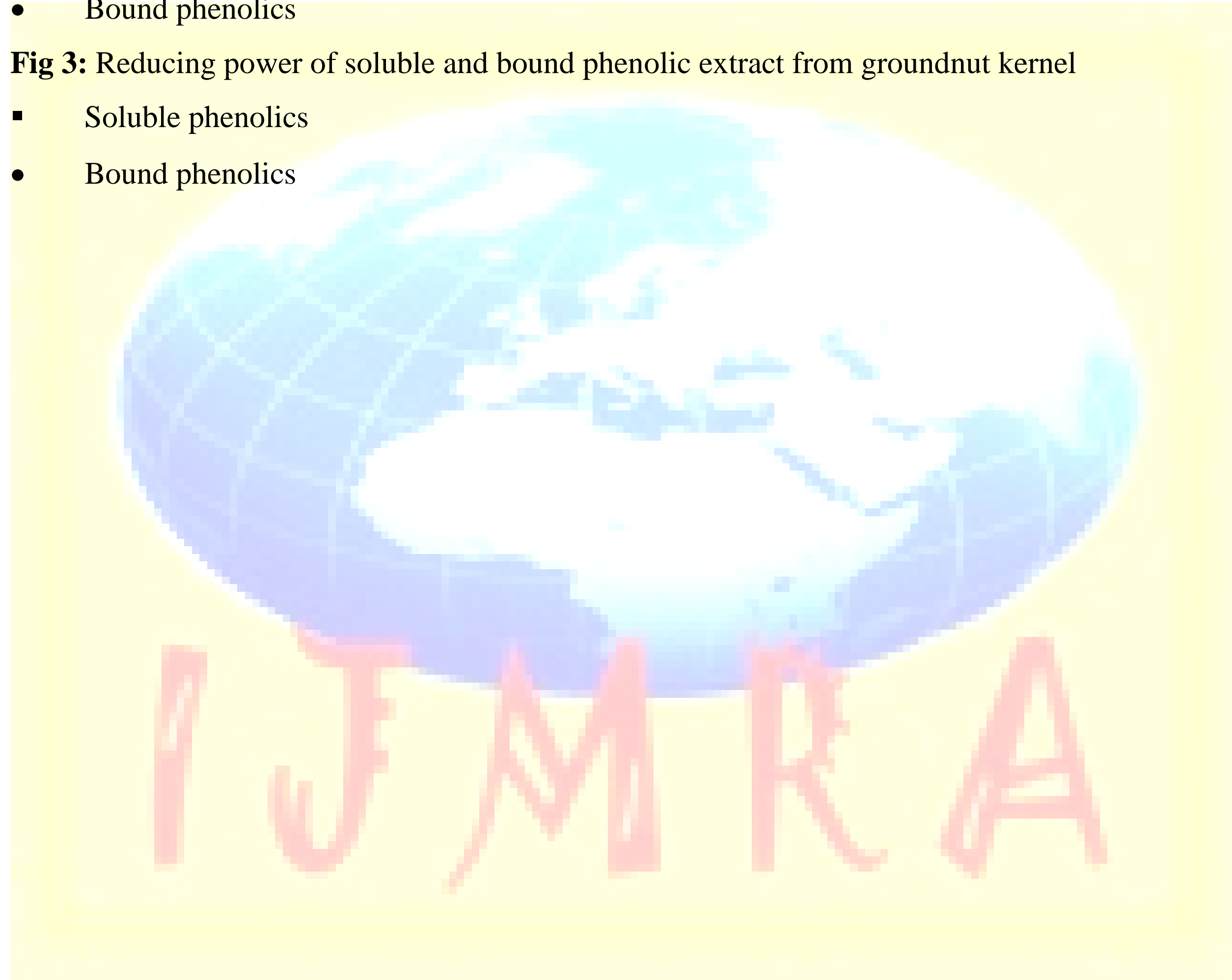

Fig. 1

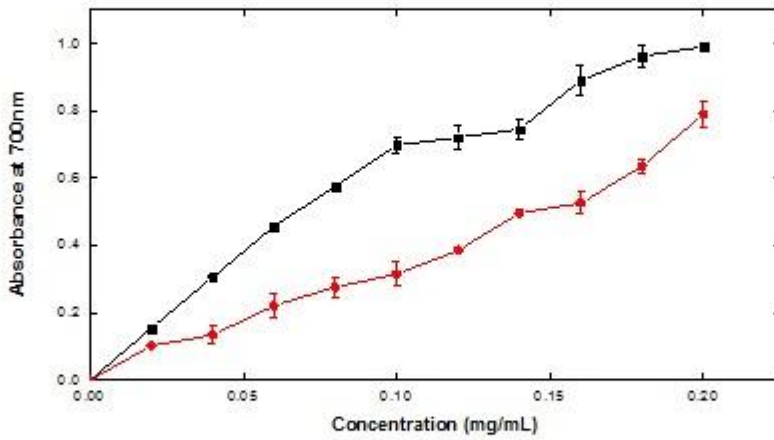

Fig. 2

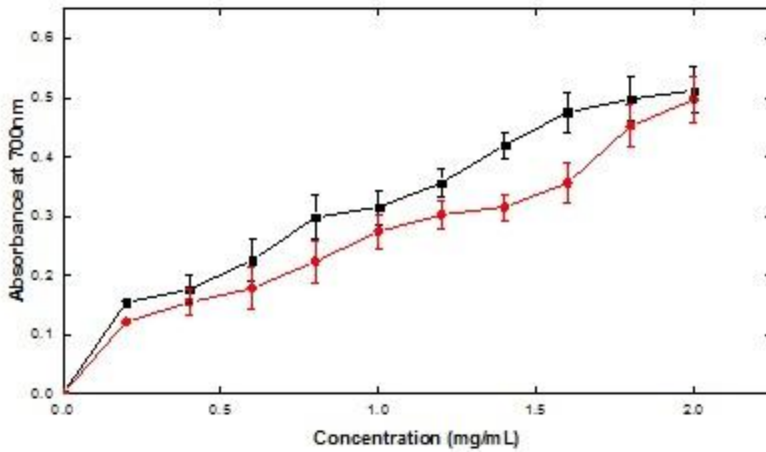

Fig 3.