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Nutritional and phytochemical components and *in vitro* antioxidant capacity of raw *Arachis hypogaea* seeds

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Abstract
This study examined the nutritional and phytochemical constituents as well as the *in vitro* antioxidant capacity of the raw form of *Arachis hypogaea* seeds.
Qualitative and quantitative analyses of phytochemical components of the ethanolic extracts of *Arachis hypogeae* seeds were carried out using standard procedures. Proximate analyses of *Arachis hypogaea* seed powder was also carried out using standard analytical methods.
Phytochemical analyses revealed the presence of tannins, saponins, flavonoids, alkaloids and coumarins. The total tannin, phenol, proanthocyanidin and flavonoid content of the seeds were 35.38±0.58mg TAE/g, 28.37±0.69 mgGAE/g, 993.00±23.09 mgAAE/g and 17.00±0.47 mgQE/g extract respectively. Proximate analysis of *Arachis hypogaea* seeds showed that it contained 5.43% moisture, 2.38% ash, 26.26% protein, 46.58% fat, 6.72% fiber and 12.63% carbohydrate. *In vitro* antioxidant capacity studies revealed that peanut extract had a better DPPH scavenging capacity relative to standard vitamin C as evidenced by its lower IC_{50} value of 3.04±0.11 µg mL^{-1} as against 8.01±0.42 µg mL^{-1} obtained for vitamin C.

Keywords: *Arachis hypogeae*, nutritional, phytochemical, antioxidant, flavonoids, resveratrol

Introduction
Peanut or groundnut (*Arachis hypogaea* L.) belong to the legume or "bean" family (Fabaceae). *Arachis hypogaea* is grown in the tropical and sub-tropical areas globally (Al-Snafi, 2014) [4]. Peanuts and its products represent a major part of diet globally (Lopes *et al*., 2011; Al-Snafi, 2014) [23, 4]. Worldwide, it is the fourth most important oil seed crop behind soybean, cottonseed and rapeseed (USDA, 2009) [40]. Peanut seeds produce about 18.6- 20.8% oil (Al-Snafi, 2014) [4]. Studies on different varieties of groundnut have shown that peanut oil contains the following fatty acids; capric, lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic, behenic, and lignoceric (Lopes *et al*., 2011) [23]. Peanut has been reported to contain about 16.2 - 36% protein, about 18% carbohydrates and an ash content of about 3% (Al-Snafi 2014) [4]. Several health benefits have been linked with the consumption of peanuts notably weight management, (Alper and Mattes, 2002) [3], prevention of cardiovascular diseases, (Feldman, 1999) [16], prevention of Alzheimer's disease and cancer inhibition (Awad *et al*., 2000) [7]. These beneficial effects are believed to be largely due to the low levels of saturated fatty acids found in peanuts (Misra, 2004) [26] and the absence of *trans*-fatty acids (Sanders, 2001) [33], richness in mono- and poly-unsaturated fatty acids (Kris-Etherson, 1999). Also believed to be responsible for its beneficial effects are the presence of micronutrients chiefly vitamin E, folate, minerals (potassium, magnesium, and zinc), fiber, and phytochemicals, predominantly resveratrol and a host of several other phenolic compounds (Isanga and Zhang, 2007) [20]. The present study was carried out determine the nutritional and phytochemical constistent as well as the *in vitro* antioxidant capacity of the raw form of *Arachis hypogea* seeds sourced from a local market in Benin-City, Nigeria.

Materials and Methods
1. Materials
Seed
Arachis hypogaea seeds were purchased at Uselu market, Benin City. Some of the seeds were planted for proper identification and verification. The verification of the peanut seed and plant

was done in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo state, Nigeria and assigned a voucher number UBH_A 352.

Chemicals

Ethanol (BDH Chemical, England), L-Ascorbic Acid (Sigma Chemical Co., St Loius, U.S.A), Ferric Chloride (Merck, Darmstadt, Germany) Sodium Dodecyl Sulphate, SDS (Hopkins and Williams Ltd, England), 1,1-Diphenyl-2-Picrylhydrazyl, DPPH (Sigma Chemical Co., St Loius, U.S.A), gallic acid (Sigma Chemical Co., St Loius, U.S.A), tannic acid (Sigma Chemical Co., St Loius, U.S.A), quercetin (Sigma Aldrich, Germany).

Methods

Preparation of *Arachis hypogaea* seed for phytochemical and antioxidant capacity analyses

The peanut seeds were sorted, cleaned and air dried for 48 hours. The raw peanuts were then blend into fine coarse powder using an electric blender (Kenwood, BL 355 350W). Portion of the coarse powder (300g) was soaked in 3.8 liters of 80% ethanol for 72 hours with regular stirring. The extract was then filtered through a sintered funnel and Whatman No. 1 filter paper. The resultant filtrate was concentrated using a rotary evaporator at 50°C. The resultant brownish paste deposited at the bottom of the flask was then dried in an oven at 45°C. The dry extract was then scraped out with a stainless spatula, crushed in a mortar and then kept in glass bottle until required for phytochemical analyses and *in-vitro* antioxidant activity evaluation.

2. Proximate analyses

Proximate analyses on *Arachis hypogaea* seed powder was done by method described by AOAC (2000) [1].

3. Phytochemical Analyses

3(a). Qualitative phytochemical Sreening

The presence of the phytochemicals in groundnut seed were tested for: tannins, phlobatannins and alkaloids by the method described by Trease & Evans, (1989) [39], flavonoids by the method of Harborne, (1973) [18], saponins by the method described by Evans, (2002) [15], terpenoids and cardiac glycosides by the procedure described by Edeoga *et al*., (2005) [14]. Anthraquinones were analysed by the method desribed by Sofowara, (1993) [37].

Quantitative phytochemical Analyses

Quantitaive analyses of the phytochemicals were based on the method described for the total phenol content by Folin and Ciocalteau's method (1927) [17], while that of total flavonoid was based on method Chang *et al*., (2002) [11] aluminum chloride. The method of Sun *et al.* (1998) [38] was adopted in estimation of the proanthocyanidin content of the extract. Total tannin content was determined by the Folin-Denis method (Polshettiwar *et al.*, 2007) [30].

4. *In-vitro* antioxidant capacity analyses

The ability of *Arachis hypogaea* seed extracts to scavenge 1,1–diphenyl–2–picrylhydrazyl (DPPH) radical was determined by the method of Brand-Williams *et al*. (1995) [9]. The method of Ohkawa *et al*. (1979) [28] was used to estimate the thiobarbituric acid reactive substances (TBARS), while the Ferric Reducing Antioxidant Power (FRAP) of the extract was ascertained by the method of Benzie and Strain (1996) [8].

Statistical analysis

Data are expressed as mean ± SD. Statistical analysis was performed using SPSS (16.0).

Results

Proximate Composition

The results of the proximate analyses of *Arachis hypogaea* seeds are presented in Table 1. The findings show that peanut seeds analysed had 5.43% moisture, 2.38% ash, 26.26% protein, 46.58% fat, 6.72% fiber and 12.63% carbohydrate.

Table 1: Proximate composition of *Arachis hypogaea* seeds

Parameter	Proximate composition (%)*
Moisture	5.43±0.11
Fibre	6.72±0.63
Ash	2.38±0.53
Crude Protein	26.26±0.55
Crude Fat	46.58±0.27
Total Carbohydrate	12.63±0.82

*Values of each parameter are expressed as percentage of the total amount of the constituents on dry weight basis. The value of each parameter is presented as mean ± SD (n=3)

Phytochemical Composition of *Arachis hypogaea* seeds

The result of the phytochemical analyses of the ethanol extract of *Arachis hypogaea* seeds is presented in Table 2. All photochemical tested for were present, apart from phlobatannins and anthraquinones. Some phytochemicals were quantified–namely: tannin, total phenols, proanthocyanidin and flavonoids (Table 3) to identify their comparative levels in *Arachis hypogaea* seeds. The results obtained showed that total tannin, phenol, proanthocyanidin, flavonoid content of the seed were 35.38±0.58 mgTAE/g, 28.37±0.69 mgGAE/g, 993.00±23.09mg AAE/g and 17±0.47 mgQE/g extract respectively.

Table 2: Phytochemical screening of Arachis hypogaea seeds

S/N	Phytochemical	Ethanol seed extract
1.	Tannin	+
2.	Phlobatannins	-
3.	Flavonoids	+
4.	Saponin	+
5.	Alkaloids	+
6.	Coumarins	-
7.	Quinone	+
8.	Anthraquinones	-
9.	Steroids	+
10	Cardiac glycosides	+
11.	Terpenoids	+

Where + = present, - = absent

Table 3: Phenolic content of ethanolic extract of *Arachis hypogaea* seeds

Phytochemical	Mass/unit volume
Total phenols	28.37±0.69 mg GAE/g extract
Proanthocyanidin	993.00±23.09 mg AAE/g extract
Total flavonoids	17.00±0.47 mg QE/g extract
Total tannin	35.38 ±0.58mg TAE/g extract

Data are phenolic composition of ethanol extracts of *Arachis hypogaea* seeds and and are expressed as mean ± SD (n = 3)

mgGAE/g extract = mgGallic Acid Equivalent/g extract
mgAAE/g extract = mgAscorbic Acid Equivalent/g extract
mgQE/gextract =Quercetin Equivalent/g extract
mgTAE/gextract =Tannic Acid Equivalent/gextract

Assessment of *in vitro* antioxidant capacities of the ethanolic extract of *Arachis hypogaea* seeds

Table 4 shows the result of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability of peanut seed extract and ascorbic acid. The increase in DPPH free radical scavenging ability was directly proportional to the concentration of the ethanolic extract. The IC_{50} of DPPH quenching abilities of *Arachis hypogaea* seed extract and the standard antioxidant, (vitamin C) was found to be 3.04 µgmL-1 and 8.01 µg mL-1respectively (Table 5).

There was concentration-dependent increase in ferric reducing capacity in terms of absorbance values at 700nm (Table 5). The reducing power of *Arachis hypogaea* seed extract was found to be lower than that of vitamin C. The inhibitory effect of *Arachis hypogaea* extracts and vitamin C on 2-thiobarbituric acid reactive substances (TBARS) production is shown in Table 5. The inhibitory effect of *Arachis hypogaea* seed extract was lower compared to vitamin C.

Table 4: DPPH radical scavenging ability of *Arachis hypogaea* seed extract relative to ascorbic acid (vitamin C)

Concentration of Extract (µg/mL)	Vitamin C (% Inhibition)	Peanut Extract (% Inhibition)
5	94.83±0.63*	74.66±0.84
10	95.25±0.12	90.18±0.22
25	95.93±0.32	92.60±0.76
50	97.19±0.18	94.91±0.55
100	97.20±0.22	95.77±0.14
200	97.67±0.42	96.37±0.18

*Values are expressed as mean ±SD (n=3)

Table 5: Ferric reducing capacity, TBARS inhibition and DPPH IC_{50} of peanut seed extract relative to standard vitamin C

Agent	FRAP (µmole Fe(II)/g extract) $\times 10^3$	TBARS (% Inhibition)	DPPH IC_{50} (µg mL^{-1})
Vitamin C	1.60±0.45*	50.00±0.88	8.01±0.42
Peanut extract	1.18±0.61	42.12±0.52	3.04±0.11

*Values are expressed as mean ±SD (n=3)

Discussion

Proximate composition of *Arachis hypogaea* seed

The lipid composition of peanuts is previously shown to fall within the range of 33.6-54.95% (Asibuo *et al*., 2008, Aslam Shad *et al*., 2009, Campos-Mondragon *et al*., 2009, Musa *et al*., 2010, Shokunbi *et al*., 2012) [5, 6, 10, 27, 35]. These results are comparable with the values obtained in this study (Table 1).

Abbey *et al*., (2001) [2] in their study found that the carbohydrate content of peanuts is 10%. This is comparable our findings in this present study (Table 1). However, Asibuo *et al*., (2008) [5], Campos-Mondragon *et al*., (2009) [10] and Shokunbi *et al*., (2012) [35] reported a higher carbohydrate content of peanuts in the range of 17.03-27.16% which are above the values obtained in the present study. This disparity may be due to species differences.

The crude protein content (26.26%) obtained in this study is comparable with values reported by Onyeike and Oguike, (2003) [29], who reported that peanuts contain 26.4%. Asibuo *et al*., (2008) [5], Campos-Mondragon *et al*., (2009) [10], Musa *et al*., (2010) [27] and Shokunbi *et al*., (2012) [35] showed that the crude protein content of peanut ranges from17.1-31.0%. We believe like other investigators that the variation in the data could be attributed to the nutrient and soil texture of places and regions where the peanuts were planted coupled with species differences in the cultivars evaluated in each study (Shokunbi *et al*., 2012) [35].

Campos-Mondragon *et al*., (2009) [10], Aslam Shad *et al*., (2009) [6] and Shokunbi *et al*., (2012) [35] reported that the ash content of peanut seeds ranges between 2.0-3.45%. This is in accord with what was discovered in this study, 2.38% (Table 1). Results from this study also harmonises with the report by Abbey *et al*., (2001) [2] in terms of the moisture content of raw peanut seeds. They found it to be 6.0%, which is comparable to 6.72% obtained in the present study.

Phytochemical Analyses of *Arachis hypogaea* seeds

Several antioxidant phytochemicals are shown to be found in peanuts (Isanga and Zhang, 2007) [20]. Data from qualitative phytochemical analyses of the ethanolic extracts of *Arachis hypogaea* seeds revealed the presence of tannins, flavonoids, saponins, alkaloids, coumarins, quinones, steroids, cardiac glycosides and terpenoids (Table 2). Prabasheela *et al*. (2015) [31] also found as in the present study, that the ethanolic extract of *Arachis hypogaea* seeds contained flavonoids, tannins, terpenoids, saponins, alkaloids in addition to steroids. Shad *et al*., (2009) [6] also reported the occurrence of tannins and saponins. The occurrence of saponins in peanut seeds was shown by Kuang *et al*. (2017) [22]. Khaopha *et al*. (2012) [21] reported that *p*-coumaric acid was a major phenolic acid present in peanut seed extract. The occurrence of alkaloids, glycosides, tannins and saponins in peanut seed extracts was also reported by Marka *et al*., (2013) [24].

In order to establish the relative amount of the phytochemical constituents in *Arachis hypogaea* seeds, some phytochemicals were quantified – namely: tannin, total phenolics, proanthocyanidin and flavonoids (Table 3). The results gotten from the present study revelaed that the total phenolic content of the seeds was 28.37±0.69 mg GAE/g extract (Table 3). The relatively high total phenolic content may be as a result of numerous phenolic compounds present in peanuts such as proanthocyanidins which are known to be abudant in the peanut skin (Yvonne *et al*., 2007) [42]. A high level of total proanthocyanidin content (993.00±23.09mg AAE/g extract) (Table 3) was also observed. The total tannin content and the flavonoid content were also relatively high (Table 3).

Wang *et al*. (2007) [40] reported a total phenolic content comparable to that found in this present study. Khaopha *et al*. (2012) [21] in their evaluation of the total phenolic content of some phenolic acid in the testae and testa-free kernels of 15 Valencia-type peanut, found that the total phenolic contents of all peanut testae were much greater than those of the testa-free peanut kernels. In their investigation, the total phenolic content in the testae varied from 2.47±0.96 to 84.53±5.57 mg GAE/g dry weight. The result from this study (using peanut kernel with testa) falls within this range (Table 3). There are reports indicating that colour of peanut testa has a strong correlation with the total phenolic content of the peanut kernels (Chukwumah *et al*., 2009) [12]. Peanut testae with red colour showed a wide range of variation with respect to the total phenolic content (16.33±9.14 to 61.44±4.43 mg GAE/g dry weight (Khaopha *et al*., 2012) [21]. Result from Khaopha *et al*. (2012) [21] studies showed that the peanut genotype, 2019 (K vemena) which is red in colour like the peanut used in this study contained 23.56±0.31 mg GAE/g for testa and 0.11±0.01 mg GAE/g for testa-removed peanut kernel. The result of their study is comparable with the result obtained from our present study. Khaopha *et al*. (2012) [21] reported that peanuts contain an abundant amount of polyphenols such as coumarins and anthocyanidin. Yadav *et al*. (2014) [41] also reported that peanuts had total phenolic content ranging from 14.50±0.95 to 101.7±5.54 mg GAE/g dry skin.

In vitro antioxidant capacity of *Arachis hypogaea* seed extract

DPPH, FRAP and TBARS were employed to determinethe antioxidant potential of peanut seed extract in this present study. The DPPH free radical scavenging ability increased sharply with increase in the concentration of the ethanolic extract of the seeds (Table 5). Evident from the measured IC_{50} values, ethanolic extract of peanut seeds had a better DPPH radical quenching capacity compared to the standard vitamin C (Table 5). So the radical quenching capacity of peanut extract may be related to the abudance polyphenols found in peanuts as other investigators have reported (Roginsky and Lissi, 2005; Wang *et al.*, 2007; Yandav *et al*., 2014) [32, 40, 41]. The ability of plant phenolics to donate hydrogen or electron and to form stable radicals makes them powerful antioxidants (Scalbert *et al*., 2005) [34]. The antioxidant ability of these compounds is closely related to their redox characteristics that help to adsorb and neutralize reactive oxygen species (Hasan *et al*., 2008) [19].

Although, the ethanolic seed extract of peanut had a considerable high ferric antioxidant reducing power, it was however lower relative to the standard vitamin C (Table 5). This is not entirely surprising since justified the mechanism of action of polyphenols as antioxidant is predominantly by the transfer of H- atom (which explains their better DPPH radical quenching capacity) rather than electron transfer mechanism which is required in FRAP (Maru *et al*., 2014) [25].

The ethanolic extract of the seed of *Arachis hypogaea* was able to inhibit the peroxidation of lipid initiated by ferrous sulphate in egg-yolk homogenate in concentration dependent manner. Results obtained from this study shows that the ethanolic extract of the seed was able to inhibit TABRS production in a manner comparable to the standard, vitamin C suggesting that peanuts are a rich source of antioxidants (Table 5).

Conclusion

The general observation from this study was that antioxidant reaction of *A. hypogaea* seeds is concentration-dependent. As the concentration of the phenolic compounds increases, their ablility to scavenge free radicals also increased.

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