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In-vitro Comparative Antioxidant Effects and Proximate Composition of Peeled and Unpeeled Pigeon-pea (*Cajanus cajan*) Seed

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Background: Pigeon pea is an economical source of natural antioxidants and its use could be a means of attaining prospect in ensuring food safety. This *in vitro* study intends to compare the primary constituents and oxidation inhibition potentials of peeled pigeon pea (PPP) and unpeeled pigeon pea (UPP) seed.
Methods: PPP and UPP samples were milled into flour and their corresponding soluble free phenolic extracts were prepared. Its flour was used to determine the proximate composition. The soluble-free phenolic extracts were used to assay for total phenols, total flavonoids, reducing property, DPPH radical scavenging ability iron-chelating ability and lipid peroxidation inhibition potential.

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Results: Proximate composition of peeled pigeon pea (PPP) had higher fat and carbohydrate content while the protein, fibre, ash and moisture content were higher in unpeeled pigeon pea (UPP). The phenolic constituents and reducing property of the Peeled pigeon pea was significantly ($p < 0.05$) higher than that of unpeeled pigeon pea with the value (12.80 mg/g >11.85 mg/g), (6.07 mg/g > 5.46 mg/g) and (7.40 > 5.62) respectively. The results showed that both PPP and UPP extracts have antioxidant potentials. However, PPP had higher nitrogen monoxide (NO) radical scavenging ability and iron (Fe) chelating ability than UPP. Contrary to this trend, UPP exhibited greater DPPH radical scavenging activity and more effective inhibition of lipid malondialdehyde (MDA), the final byproduct of lipid peroxidation.

Conclusion: These findings provide additional evidence supporting the potential of pigeon pea as a natural source of antioxidants. The primary constituents, such as phenolics, flavonoids, and other bioactive compounds, are likely responsible for its remarkable antioxidant properties. This highlights its value as a functional ingredient in the food industry, promoting its application in developing health-focused food products.

Keywords: Antioxidants; proximate; Cajanus cajan; lipid peroxidation; flavonoids; phenol; carbohydrate; fats.

1. INTRODUCTION

Generally, internally produced and naturally occurring reactive oxygen species (ROS) are mainly produced by living cells as byproducts in the oxidative reaction process of the mitochondrial chain during metabolism (Nathan and Cunningham-Bussel, 2013). The impact of ROS on cell physiology is far-reaching. Moderate levels of ROS have positive effects, such as eliminating invading pathogens, promoting wound healing, and supporting repair processes (Bhattacharyya et al., 2014). Nevertheless, oxidative stress has been associated with the onset of chronic conditions, including cancer, atherosclerosis, coronary artery disease, stroke, hypertension, diabetes, neurological disorders, and a compromised immune system (Zhu et al., 2008). Natural antioxidants are therefore considered as an alternative to retard the oxidation process with minimal side effect unlike synthetic antioxidants butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) which have the tendency to promote carcinogenesis and are therefore under strict regulations due to their potential health hazards (Odubanjo et al., 2017). The majority of researchers have discovered natural antioxidants in various parts of plants (Lai et al., 2012; Atanassova et al., 2011). Phenolic and flavonoid compounds are recognized as safe, natural antioxidants. Additionally, various in vivo studies have demonstrated a strong correlation between high dietary intake of these natural antioxidants and increased life expectancy (Lai et al., 2012). Natural antioxidants are beneficial in lowering the risk of numerous chronic diseases, various cancers, diabetes, obesity, and hypertension, while also enhancing endothelial function (Lai et al., 2012; Atanassova et al., 2011).

Pigeon-pea (*Cajanus cajan* L. Millsp) is a medicinal herbaceous leguminous plant which belongs to the Fabaceae family (Florence et al., 2014). The pigeon pea plant grows to a height of approximately 1 to 4 meters, featuring a deep taproot that extends up to 2 meters. Its stem is ribbed, angled, and covered with fine hairs (Hossain et al., 2013). The leaves are typically green and have long hairs, arranged in a spiral pattern around the stem and positioned alternately (Datta and Lal, 2012). The flowers are yellow with a red exterior color (Hossain et al., 2013). There is still few scientific information on pigeon pea compared to other major legumes such as cowpea, soya beans etc. due to its underutilization, low exploitation and cultivation. Currently, the demand for sustainable development and economic recovery in developing regions is crucial, particularly in terms of ensuring access to high-quality protein-rich foods and improving food security. On comparing plant protein to that of animal's, it is generally accepted that plant protein is inferior due to some large polysaccharides that are not easily digested by the animal. Conversely, the high cost of while animal protein has therefore been rendered the kind of food that cannot be easily purchased by the common man (Chinedu and Nwinyi, 2012). Vegetable protein is a great source of dietary protein and they are more economical than animal products such as meat, fish, and poultry (Hossain and Awad, 2018). Pigeon pea seeds contain protein levels ranging from 21.0% to 29.0%, with approximately 50% carbohydrates, primarily starch (Aggarwal et al.,

2015). They are also abundant in minerals like potassium, phosphorus, magnesium, calcium, iron, and zinc, but have low levels of sodium and copper (Adepoju et al., 2019). Given these nutritional benefits, this study aimed to further assess the proximate composition, total phenolic content, total flavonoid content, reducing capacity, iron-chelating ability, DPPH radical scavenging activity, nitric oxide radical scavenging potential, and lipid peroxidation inhibitory effects of pigeon pea.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh pigeon pea (C. cajan) samples used in this study were sourced from Osele market, located in Ikare Akoko, Ondo State, Nigeria. (7.5248 ºN, 5.7669 ºE). The bean seed was identified and authenticated with designated Voucher Number-264 as that of (*Cajanus cajan* (L.) Millsp.) in the herbarium of Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba Akoko, Ondo State.

2.2 Preparation of Samples

The dry pigeon pea seed that were purchased were cleaned and sorted to eliminate any extraneous materials and dirt. Approximately, 2 kg of the seed were split into two portions. One portion was soaked in 2 liters of water for around 24 hours to separate the hulls from the cotyledons, then sundried and further dried in an electric oven. Both the dried dehulled peas and the hulled peas were milled into a fine flour using an attrition mill; they were labeled PPP and UPP, respectively, and stored in an airtight container for further analysis.

2.3 Chemicals and Equipment

The following substances were sourced from Sigma Aldrich: 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS), iron (II) sulfate, H2O2, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX). Gallic acid, quercetin, butylated hydroxytoluene (BHT), trichloroacetic acid (TCA), Folin-Ciocalteu's phenol reagent, acetone, methanol, and ethanol were sourced from Sigma Chemical Co., St. Louis, MO, USA. Additional reagents were supplied by the Biochemistry Department at Obafemi Awolowo University, Ile-Ife, Osun State.

2.4 Extraction of Soluble Free Phenolic Compounds

Each powdered sample (100 g) was blended with 80% acetone in a 1:4 ratio (w/v) using a chilled blender for 10 minutes. To ensure complete homogenization, the blending was continued for an additional 3 minutes. The resulting mixtures were filtered through Whatman No. 2 filter paper using a Buchner funnel under vacuum. The solid residues were retained for the extraction of bound phenolics. The filtrates were then concentrated using a rotary evaporator under vacuum at 45 ºC. The extracts were frozen at −4 ºC (Chu et al., 2002) and stored in airtight containers until further analysis.

2.5 Primary Composition

According to the AOAC (1995) methods, the PPP and UPP flours were evaluated for moisture level, ash, crude protein, fat, and crude fiber. Carbohydrate content was determined by difference. The reported values are the means of three replicate samples accompanied by their standard deviations.

2.6 Total Phenol Determination

The total phenol content was subsequently expressed as Gallic Acid Equivalent (GAE). The total phenolic content of each extract was determined following the procedure described by Singleton et al. (1999). A mixture was prepared by combining 0.1 mL of the sample with 0.9 mL of water, followed by the addition of 0.2 mL of Folin-Ciocalteu's phenol reagent. This mixture was vortexed and left to stand for 5 minutes. Next, 1.0 mL of 7% (w/w) sodium carbonate (Na2CO3) solution was added, and the volume was adjusted to a final 2.5 mL. The solution was then incubated at room temperature for 90 minutes. Absorbance was recorded at 750 nm against a blank, where water replaced the sample. The phenolic content was expressed as Gallic Acid Equivalent (GAE).

2.7 Determination of Total Flavonoid Content

A slightly modified method based on Meda et al. (2005) was used to assess the total flavonoid content of each extract. To 0.1 mL of the extract or standard, 0.1 mL of distilled water was added, followed by the addition of 0.1 mL of 5% sodium nitrite. After 5 minutes, 0.1 mL of 10% aluminum

chloride and 0.2 mL of sodium hydroxide were introduced, and the volume was adjusted to 2.5 mL with distilled water. The absorbance was then measured at 510 nm against a blank. The total flavonoid content of the beans was expressed using quercetin as a standard.

2.8 Reducing Property

The reducing activity of each extract was evaluated by measuring its potential to decrease FeCl3 solution, following the method outlined by Benzie and Strain (1999). A FRAP reagent was formulated by combining 300 mmol/L acetate buffer at pH 3.6, 10 mmol/L of 2,4,6-tri-(2-pyridyl)-1,3,5-triazine, and 20 mmol/L of FeCl3•6H2O in a ratio of 10:1:1. To this reagent, 50 µL of each extract (at a concentration of 0.1 mg/mL) and 50 µL of standard ascorbic acid solutions (at concentrations of 20, 40, 80, 120, 160, and 200 µg/mL) were added. The mixture was then incubated for 10 minutes, and the absorbance was recorded at 630 nm against a blank containing 50 µL of distilled water. The reducing power of the plant was determined using quercetin as a reference standard.

2.9 DPPH Free Radical Scavenging Assay

A method described by Braca et al. (2001) was used to assess the free radical-scavenging activity of each extract against DPPH free radicals. Various volumes of each extract sample (ranging from 0 to 100 µL) were added to 1 mL of 0.3 mM DPPH solution in methanol. The mixture was thoroughly mixed and left to incubate in the dark for 30 minutes. Following incubation, the absorbance was recorded at 517 nm, using a DPPH control that contained 1 mL of methanol without the extract.

The per cent of inhibition was calculated using the formula:

% Inhibition = ((Standard - sample) / Standard) x 100%)

2.10 Fe^{2+} Chelation Assay

The ferrous ion chelating (FIC) assay was performed according to the method described by Singh and Rajini (2004), with slight modifications. Solutions of 2 mM FeCl2•4H2O and 15 mM ferrozine were diluted to one-twentieth of their initial concentrations. Each extract sample (ranging from 0 to 100 µL) was mixed with 1 mL of FeCl2•4H2O. Following a 5-minute incubation, the reaction was triggered by adding 1 mL of ferrozine. The mixture was then vigorously agitated, followed by an additional 10-minute incubation. The absorbance of the resulting solution was recorded using a spectrophotometer at 562 nm. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was determined using the formula:

Iron Chelating Effect (%) = ((A control - A sample) / A control) × 100

where A control represents the absorbance of the control sample (containing FeCl2 and ferrozine for complex formation), and A sample denotes the absorbance of the tested sample.

2.11 Nitric Oxide Radical Scavenging Assay

The ability of each extract to scavenge nitric oxide was assessed spectrophotometrically using Griess reagent, as outlined by Govindarajan et al. (2003). In brief, 0.3 mL of sodium nitroprusside (5 mM) was mixed with 1 mL of each extract. The test tube mixtures were incubated under light conditions at 25 ºC for 150 minutes. After incubation, 0.5 mL of Griess reagent (composed of equal parts of 1% sulfanilamide in 5% ortho-phosphoric acid and 0.01% naphthyl-ethylenediamine in distilled water, prepared 12 hours earlier) was added. The absorbance was then recorded at 546 nm using a spectrophotometer, and the percentage inhibition of nitric oxide radical generation was determined.

2.12 Bioassay

2.12.1 Preparation of liver homogenate

Using mild diethyl ether anesthesia, the rats were euthanized followed by the quick removal of the liver, it was then placed on ice and weighed. The liver tissue was subsequently homogenized in cold saline (1:10 w/v) using roughly 10 vertical strokes at about 1200 rpm in a Teflon glass homogenizer (DuPont, Wilmington, DE). The homogenate was centrifugally separated for 10 minutes at 3,000 g, resulting in a pellet that was disposed of, while the low-speed supernatant (S1) was collected and reserved for the analysis of lipid peroxidation (Belle et al., 2004).

2.12.2 Lipid peroxidation assay

The analysis of lipid peroxidation was performed using a refined technique based on Ohakawa et al. (1979). In brief, 30 µL of 0.1 mol/L Tris-HCl buffer (pH 7.4), of the reaction mixture was combined with the homogenate fraction

containing 100 µL, varying volumes of each sample extract (0-100 µL), and 30 µL of the pro-oxidant solution (250 µM FeSO4).The total volume was brought to 300 µL with water and incubated at 37 °C for 1 hour. To develop the color reaction, 300 µL of 8.1% sodium dodecyl sulfate was added to the mixture, followed by 600 µL of acetic acid/HCl (pH 3.4) and 600 µL of 0.8% thiobarbituric acid (TBA). This mixture was then incubated at 100 °C for 1 hour. The thiobarbituric acid reactive species produced were quantified at 532 nm using a spectrophotometer. Malondialdehyde (MDA) served as the standard, and the thiobarbituric acid reactive substances (TBARS) generated were expressed as equivalents of MDA.

2.13 Data Analysis

The results from the three replicate measurements were aggregated and reported as mean ± standard deviation. All data were subjected to analysis of variance (ANOVA), followed by Newman–Keuls multiple range post hoc tests, with a significance level established at $p < 0.05$. Statistical evaluations were performed using GraphPad Prism version 5.00 for Windows. The IC50 (the extract concentration needed to attain 50% reducing activity) was determined using linear regression analysis.

3. RESULTS AND DISCUSSION

The Primary composition of the PPP and UPP flours was analyzed and is shown in Table 1. The UPP sample had a higher moisture content (8.44%) compared to the PPP sample (8.06%). Pigeon pea is one of the less consumed and underutilized plants. However, the reported values in this study are lesser than those earlier researched in some other common beans and cowpea (Nwadike et al., 2018). This is an indication that it can easily be stored for a longer period.

The protein content of the PPP and UPP flours ranged from 12.19% to 15.23%. The UPP flour (15.23%) had a higher value compared to the PPP flour (12.19%). The protein levels are greater than those found in fluted pumpkin seeds (Telferia occidentalis), making pigeon pea a viable alternative source of plant-based protein (Agunbiade and Ojezele, 2010; Gu et al., 2023). The consumption of this type of beans points to the fact that it would also benefit in the area of mental growth and development (Gichohi-Wainaina et al., 2022).

The fat content values of PPP and UPP flours were 2.33% and 2.90% respectively. Fat obtained from plants are of great importance because of its wide distribution in foods and vital roles in cell maintenance (Williams and Lenkat, 2018). The incorporation of pigeon pea would be of great economic value and health benefit as it is readily available in our environment.

Ash forms part of proximate components that represents the residue remaining after the organic matter in foodstuff has been completely ignited and it's a source of mineral content in food. Ash content of the UPP (4.63%) was significantly higher than that of PPP (2.72%). These results align with the research carried out by Ibeabuchi et al., 2017 as the undehulled beans showed higher ash content value than the dehulled.

The presence of crude fibre in food has some relevant effects in gastrointestinal tract among which is constituting the faecal bulk, removal of bile acids and stimulation of neutral steroids that lower the body cholesterol level. The crude fibre content observed for both PPP (0.02%) and UPP (3.25%) samples is lower than the 5.44% value that obtained in the earlier researched soya bean (Ogbemudia et al., 2017).

The values for the carbohydrate content of the PPP and UPP were 74.11% and 66.10% respectively. The values are higher compared to the carbohydrate content (31.25%) in *Telfairia occidentalis* seeds (Agunbiade and Ojezele, 2010). Carbohydrate in beans unlike sweet bars is an essential component that has been reported to contain some compounds such as stachyose and raffinose that serve as substrates for an increase in bifidobacteria. This condition favours bacterial fermentation in the human gut to positively impact gut metabolism (Řezáčová, 2010).

The total phenolic concentration of the PPP and UPP extracts was analyzed and is shown in Table 2. The PPP extract displayed higher content of total phenol than the UPP extract at 12.80 mg/g of GAE and 11.85 mg/g of GAE respectively. Similarly, a pattern was observed with the total flavonoid content as well. (Table 2) as PPP extract (6.07 mg/g QRT) showed higher value than that of UPP extract (5.46 mg/g QRT). Earlier and recent investigations reported phenolics as one of the most prominent and thoroughly researched category of phytochemicals and have consistently

highlighted their remarkable antioxidants and medicinal properties. The amount of total phenol in a plant indicates the total amount of phenolics and flavonoids present. Because of their recognized antioxidant and therapeutic properties, flavonoids are highly valued for their various natures (Cartea et al., 2011). Furthermore, flavonoids have been identified as the primary components of nutraceuticals, playing a role in reducing cellular oxidative stress (Lobo et al., 2010). As a result of these facts, the function of antioxidants in human body viz a viz the scavenging of reactive oxygen species (ROS) has necessitated the awareness about the consumption of plants. The supply of dietary antioxidant is therefore considered the most practical approach to reducing various chronic degenerative diseases (Shodehinde and Oboh, 2012; Muscolo et al., 2024).

One of the analyses of plant extract property is according to the ability to reduce iron (III) to iron (II). The PPP and UPP extracts were assessed and their results are presented in Table 2 which shows PPP to have higher ($p<0.05$) reducing power (7.40 mg/g GAE) than the UPP extract (5.62 mg/g GAE). The findings regarding the reducing property align with the total flavonoid and total phenol content. The reducing capacity of a plant sample may indicate a substantial potential for exhibiting antioxidant activity. Nevertheless, the activity of many antioxidants is associated with various mechanisms, including the breakdown of peroxides, metal chelation, prevention of chain initiation, and radical scavenging, among others (Chaudhary et al., 2023).

The mechanism by which antioxidants scavenge various reactive oxygen species (ROS) in vitro is considered a means of preventing the chain initiation step. The current results, which demonstrate the inhibition of free radicals by PPP and UPP phenolic extracts in a dose-dependent manner, are illustrated in Fig. 1, with their respective half-maximal inhibitory concentration (IC50) values shown in Table 3. Importantly, the UPP extract demonstrated a considerably greater free radical scavenging capacity (IC50 = 5.65 mg/mL) in comparison to the PPP extract ($p < 0.05$). However, a difference was observed between the levels of phenolic content and the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity in both the PPP and UPP extracts. The discordance between these parameters in this present study may be attributed to the presence of some non-phenolic substances in the extracts such as amino acids, phytate and peptides which could have contributed to the higher DPPH free radical scavenging ability of UPP extract. This supports the findings of Djordjevic et al. 2011, in which he studied red kidney bean and soya bean of higher total phenol content did not generally display higher DPPH radical scavenging ability.

Table 1. Proximate composition of PPP and UPP flours (%)

Nutrient	Moisture	Protein	Fat	Fibre	Ash	CHO
PPP (%)	8.06±0.31^{b}	12.19±0.00^{b}	2.90±0.18^{a}	0.02±0.00^{b}	2.72±0.11^{b}	74.11±0.60^{a}
UPP (%)	8.44±0.21^{a}	15.25±0.86^{a}	2.33 0.38^{b}	3.25±0.00^{a}	4.63±0.07^{a}	66.10±0.77^{b}

Mean (n= 3) in the same column followed by different letters are significantly different at p < .05.
CHO: Carbohydrate

Table 2. Total phenol content, total flavonoid content, total antioxidant capacity and reducing property of PPP and UPP extracts

Parameter	Total phenol content (mg/g GAE)	Total flavonoid content (mg/g QRT)	Reducing property (mg/g GAE)
PPP	12.80 ± 0.72^{a}	6.07 ± 0.33^{a}	7.40 ± 0.20^{a}
UPP	11.85 ± 0.25^{b}	5.46 ± 0.25^{a}	5.62 ± 0.33^{b}

Mean (n= 3) in the same column followed by different letters are significantly different at p < .05

Table 3. IC_{50} values of DPPH radical scavenging ability, NO radical scavenging ability and iron chelating ability of PPP and UPP extracts

Samples	IC_{50} (mg/mL)		
	DPPH	NO	Fe
PPP	5.65	3.67	9.48
UPP	6.18	3.83	9.48

Mean (n= 3) in the same column followed by different letters are significantly different at p < .05

The study of reactive oxygen species continues to attract the attention of researchers as they are considered one of the major risk factors that could later progress into pathogenesis. Neurodegenerative diseases as one of the considered pathological conditions are generated through the interrelation between free radical-induced mitochondrial dysfunction and cytotoxicity (Bhat et al., 2015). In the current study, we assessed nitric oxide (NO) levels in a dose-dependent manner within the range of 0.06 - 0.26 mg/mL, with results illustrated in Fig. 2. The findings indicated no significant difference ($p < 0.05$) in scavenging activity at lower concentrations; however, both PPP and UPP extracts demonstrated higher inhibitory activity at elevated concentrations. Notably, the PPP extract exhibited a greater inhibitory capacity than the UPP extract. The IC50 value for the PPP extract was lower (3.67 mg/mL), indicating greater activity compared to the UPP extract (3.83 mg/mL). NO serves as a universal neuronal messenger that can cause neuronal damage in conjunction with other reactive oxygen species. Additional research into the role of NO in promoting synaptic plasticity (Pitsikas, 2015) and its potential to impair learning may be mitigated by reducing its donor levels (Paul and Ekambaram, 2011).

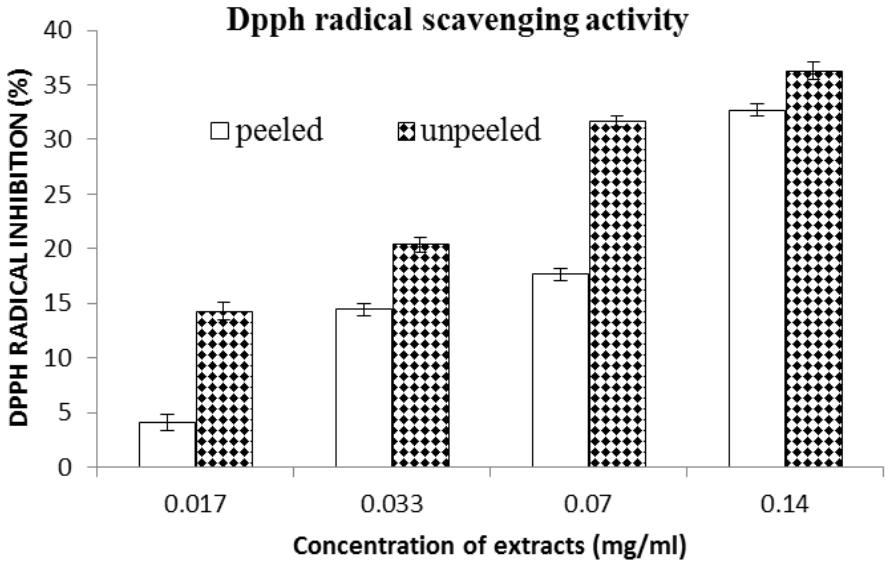

Fig. 1. DPPH radical-scavenging ability of free phenolic extracts of PPP and UPP

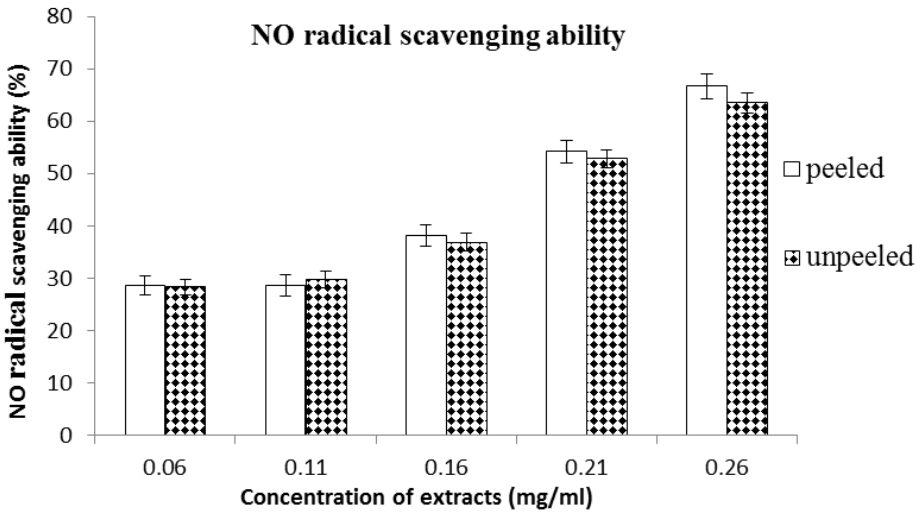

Fig. 2. Nitric oxide radical-scavenging ability of free phenolic extracts of PPP and UPP

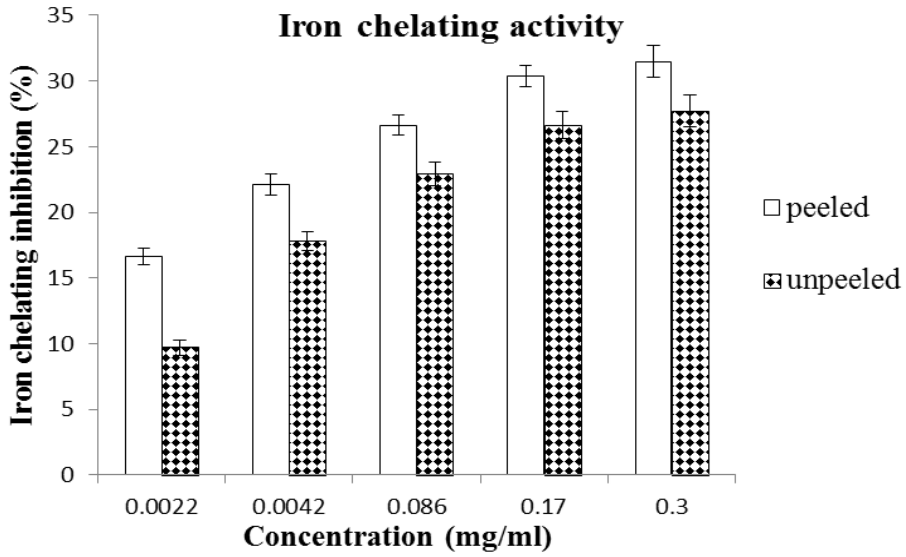

Fig. 3. Iron chelating ability of free phenolic extracts of PPP and UPP

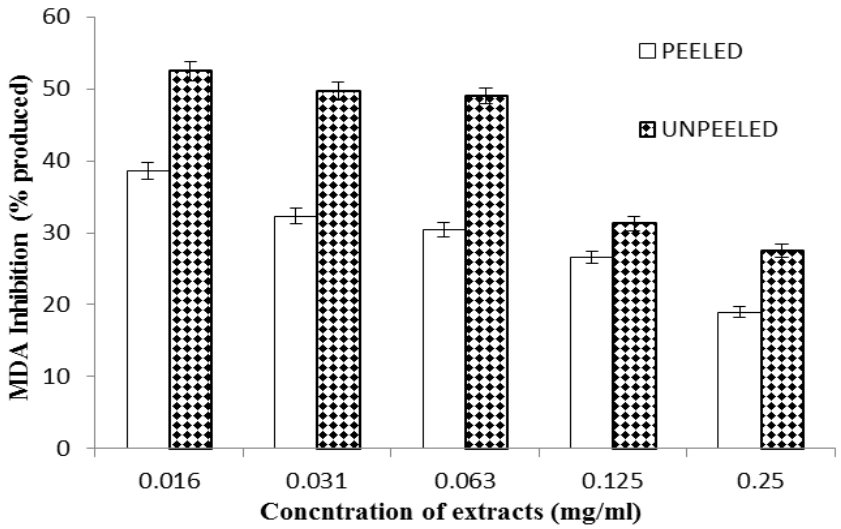

Fig. 4. Inhibition of lipid peroxidation assay of free phenolic extracts of PPP and UPP

The present *in vitro* study agrees with the earlier researched work of Pitsikas, 2015 on dose-dependent functioning of NO on neuronal actions. The low concentrations elicited neuroprotective properties while the higher concentrations promoted inflammatory actions and were considered neurotoxic. Also, the ability of PPP and UPP extracts could be suggested to possess remarkable antioxidant and medicinal properties that could be exploited to improve neuronal functions.

There are indications from earlier researched work that phenolics carry out their mode of action as antioxidants to chelate and/or scavenge iron (Fe). Excessive Fe production can lead to harmful effects by reacting with hydrogen peroxide (H2O2) to generate hydroxyl radicals through the Fenton reaction (Moreira da Silva et al., 2010). In this in vitro study, the results regarding the iron-chelating ability of PPP and UPP extracts are illustrated in Fig. 3. Both extracts demonstrated the capacity to chelate iron (Fe) in a dose-dependent manner (0 - 0.3 mg/mL). However, the PPP extract showed a greater inhibitory effect compared to the UPP extract. Notably, both extracts exhibited the same IC50 value (9.48 mg/mL), indicating no significant difference in their chelating ability. The exact reason for the identical IC50 value cannot be definitively determined, but it is important to mention. Nonetheless, the iron chelating ability

mirrored the trends observed in total phenolic content, total flavonoid content, and reducing properties.

Likewise, an increase in the concentration of ROS has been associated with a direct assault on the oxidative damage to biomolecules like lipids, proteins, and DNA, contributing to pathological conditions and hastening the aging process (Volinsky et al., 2013). The dose-dependent protective effects (0 – 0.25 mg/mL) of PPP and UPP extracts against iron-induced lipid peroxidation in cultured rat liver are illustrated in Fig. 4. The result revealed a significant inhibitory effect on $FeSO_4$. However, UPP extract displayed higher inhibitory activity than PPP extract which follows the trend observed in DPPH radical scavenging ability (Fig. 1). Malondialdehyde (MDA), a secondary product of the lipid peroxidation chain reaction, has been widely utilized as a dependable biomarker for assessing oxidative stress in the field of biomedical research (Giera et al., 2012). The result of the present research on MDA showed lack of agreement between phenolic contents and reducing property values (Table 2). According to Djordjevic et al. 2011, an observed discordance between total phenol concentration and the ability of lipid peroxidation inhibition in plants should not be established that plants with higher phenolic values are necessarily more proficient in reducing lipid peroxidation. The justification for this is based on the complex nature of lipid peroxidation inhibition as uncompounded phenols, polyphenols with high-molecular weight and other non-phenolics antioxidants partake in its mechanism of action.
More and more studies have continued to substantiate the findings that the accumulation of excess iron leads to pathophysiological settings. It of the general opinion that hydroxyl radical formation through the redox cycling of Fenton reaction in which free Fe^{2+} interact with hydrogen peroxide (as an oxidant) to form Fe^{3+}. Fe^{2+} and Fe^{3+} are precursors for the initiation of lipid oxidation chain reaction. However, the formation of a complex with the prooxidants could prevent the onset of lipid peroxidation in a biological system (Shodehinde and Oboh, 2012). Based on this, it could be suggested that the PPP and the UPP extract under study possess the ability to bond with Fe (II) to consequently prevent the initiation of lipid peroxidation.

4. CONCLUSION

The findings of this study emphasized the proximate composition, phenolic content, and antioxidant potential of PPP and UPP in vitro. Notably, there was a difference in antioxidant capacity between the two samples. The inhibitory effects observed in both PPP and UPP can be linked to their quantified phenolic contents. Thus, it can be proposed that including pigeon pea in the diet may enhance overall health by reducing the accumulation of free radicals and lowering the risk of developing degenerative diseases.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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