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DETERMINATION OF POTENTIAL BIOACTIVE COMPOUNDS AND THEIR ANTIOXIDANT ACTIVITIES OF SELECTED UNDERUTILISED LEGUMES

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ABSTRACT

Indigenous underutilised legumes are important sources of bioactive compounds with potential to improve food and nutrition security, yet data on their phytochemical constituents remain limited. This study evaluated the bioactive profile, fibre fractions, and antioxidant properties of selected legumes, namely *Treculia africana*, *Vigna subterranea*, *Phaseolus vulgaris*, *Cajanus cajan*, *Vigna unguiculata*, *Sphenostylis stenocarpa*, *Pentaclethra macrophylla*, and *Arachis hypogaea*. Standard analytical methods were used to determine fibre components, antioxidant activity, and bioactive compounds. The results showed significant ($p < 0.05$) differences in fibre fractions among the samples. Hemicellulose, raffinose, cellulose, lignin, and stachyose varied across the legumes, with *Pentaclethra macrophylla* recording the highest hemicellulose content (17.18%), while *Vigna subterranea* had the lowest (12.15%). Higher levels of raffinose and related oligosaccharides were also observed in *Pentaclethra macrophylla* and *Arachis hypogaea*. Gas chromatography–mass spectrometry analysis identified a wide range of bioactive compounds associated with antioxidant and antimicrobial activities. Germination for three days significantly ($p < 0.05$) increased antioxidant capacity in all samples, with improvements ranging from 14.56% to 53.58%. The highest increase was recorded in *Vigna unguiculata* (53.5`8%), while *Arachis hypogaea* showed the least change (14.56%). However, in reducing power, *Phaseolus vulgaris* (57.14%) and *Vigna unguiculata* (57.25%) had the highest values at the end of 3-day germination. The findings indicate that these legumes are rich in functional compounds and respond positively to short-term germination, supporting their potential use in functional food development.

Keywords: Indigenous Legumes, Fibre Fractions, Bioactive Compounds, Antioxidant Capacity

INTRODUCTION

Legumes constitute a fundamental component of human diets globally, serving as affordable and sustainable sources of plant-based proteins, dietary fibre, essential minerals, and a wide spectrum of bioactive compounds that support human health and nutrition (James *et al*., 2020abc; Singh *et al*., 2022; Popoola *et al*., 2023; Samal *et al*., 2023). Their consumption has been associated with improved diet quality, enhanced satiety, and reduced risk of chronic non-communicable diseases, particularly in populations with limited access to animal protein sources (FAO, 2023; Aremu *et al*., 2021). Conventional legumes such as soybean, lentil, chickpea etc. have received much attention by scientists; however, indigenous and underutilized legumes remain insufficiently explored, even though they possess considerable nutritional density, bioactive reservoir, functional attributes that could contribute to food system applications. These underutilized legumes, including African yam bean (*Sphenostylis stenocarpa*), Bambara groundnut (*Vigna subterranea*), and pigeon pea (*Cajanus cajan*), offer potential health benefits due to their abundance of bioactive compounds.

Bioactive compounds in legumes constitute a diverse array of naturally occurring phytochemicals with significant physiological functions, including antioxidant, anti-inflammatory, antimicrobial, and cardioprotective effects (James *et al*., 2020ab; Singh *et al*., 2022). These activities are largely attributed to polyphenolic constituents such as flavonoids, phenolic acids, tannins among others which contribute to antioxidant potential through mechanisms involving free radical scavenging, metal ion chelation, and inhibition of lipid peroxidation (Popoola *et al*., 2023). Dietary antioxidants from legumes are increasingly implicated in the reduction of oxidative stress, a central factor in the pathogenesis of chronic non-communicable diseases such as cardiovascular disorders, diabetes mellitus, obesity, and certain cancers (Singh *et al*., 2022; Popoola *et al*., 2023). Also, the concentration and composition of these bioactive compounds vary across legume species, influencing their functional and nutritional quality as well as their suitability for different food applications.

Underutilized legumes constitute a strategically important yet underexploited class of food resources that can significantly strengthen global food and nutrition security, particularly in low- and middle-income regions where dietary diversification remains a challenge (Popoola *et al*., 2023; Samal *et al*., 2023; Oyeyinka *et al*., 2023). These ‘food reserve’ legumes are rich in dietary fibre fractions, essential amino acids, and micronutrients, while also serving as dense reservoirs of bioactive compounds such as polyphenols, flavonoids, tannins, and phytosterols, which collectively underpin their functional and health-promoting attributes (González-Montoya *et al*., 2018; James *et al*., 202abc; Singh *et al*., 2022; Popoola *et al*., 2023). Their fibre components, both soluble and insoluble fractions, play critical roles in improving gastrointestinal health, regulating postprandial glycaemia, reducing serum cholesterol, and supporting beneficial modulation of gut microbiota, thereby contributing to the prevention of metabolic disorders (Jenkins *et al*., 2019; Slavin, 2021). Furthermore, these lesser-utilized legumes function as nutritional buffers in food-insecure settings, offering resilient, climate-adapted sources of plant protein and micronutrients that can complement staple cereals and improve dietary quality (Aremu *et al*., 2021; FAO, 2023; Oyeyinka *et al*., 2023).

Consequently, characterization of their fibre composition, bioactive profile, and antioxidant capacity is essential for unlocking their full potential as functional food ingredients and for supporting their integration into nutraceutical formulations, food fortification strategies, and sustainable dietary systems aimed at addressing hidden hunger and chronic disease burdens.

MATERIALS AND METHODS

Materials and Preparations

Indigenous and underutilised legumes for this study included African breadfruit (*Treculia africana*) seeds, Bambaranut (*Vigna subterranean* L.), red bean (*Phaseolus vulgaris*), pigeon pea (*Cajanus cajan*), cowpea (*Vigna unguiculata* L.), African yam bean (*Sphenostylis stenocarpa*) seed, African oil bean (*Pentaclethra mycrophylla* Benth.) seed and groundnut (*Arachis hypogea* L.). The samples were procured in December 2017 from Umuahia Local Market and were botanically identified at the Department of Crop Production, Federal University of Technology, Minna, Nigeria. Each sample (200 g) was sorted, dry-cleaned, milled into flour using a disc attrition mill (Model ED-5, China), and sieved to a particle size of 1 mm. Owing to the unique nature of African breadfruit, the method of James and Nwabueze (2013a) was employed for seed coat removal. The seeds were washed with potable water and drained using a perforated basket. They were then partially cooked in boiling water for 15 min to facilitate separation of the seed coat from the endosperm. The partially cooked seeds were drained and allowed to stand for 20 min to soften the seed coat and permit cooling. The softened seeds were subsequently dehulled using an adjustable disc attrition mill, and the cotyledons (*dhal*) were manually separated from the seed coats. The dehulled seeds were oven-dried at 60°C for 17 h and milled to a particle size of 1 mm. The resulting eight flour samples were packaged in high-density polyethylene bags and stored under refrigeration (4 ± 2°C) until required for further laboratory analyses.

Determination of Dietary Fibre Fractions

The method described by AOAC (2015) was used for fibre determination. One gram of the sample was weighed and dissolved in ethanol in a 1.0 mL vial. The prepared solution was injected into a high-performance liquid chromatography (HPLC) system (Buck Scientific BLC 10/11, USA) equipped with a fluorescence detector set at an excitation wavelength of 295 nm and an emission wavelength of 325 nm. Separation was achieved using an analytical column (C18 silica-packed, 25 cm × 4.6 mm, 3 µm particle size; SWASTIK Industries, Vadodara, India) operating under reverse-phase conditions with a non-polar stationary phase and a polar mobile phase.

The mobile phase consisted of a methanol–acetonitrile mixture delivered at a flow rate of 1.0 mL/min. Standard solutions were prepared using the same procedure. The concentrations of dietary fibre fractions in the samples were determined by calibration against the standards. The percentage composition of each fraction was then calculated using equaation 1.

$$\mathrm{DF} = \frac{\text{A Sample x STD (ppm) x V Met. (mL)}}{\text{A STD x Wt. Sample (g)}} \qquad (1)$$

Where: DF = Concentration of dietary fibre in ppm; STD = Concentration of standard; A Sample = Area of sample; A STD = Area of standard; V Met. = Volume of methanol and Wt. Sample = Weight of sample.

Chemical Reagents

The extraction solvents used in this study included acetone (Lobal Chemie Pvt. Ltd., India; CAS No. 64-17-5), ethanol (Guangdong Guanghua Sci-Tech Co. Ltd., India; CAS No. 67-64-1), and methanol (Lobal Chemie Pvt. Ltd., India; CAS No. 67-56-1). All solvents were obtained from Finlab, Abuja, Nigeria.

Extraction of Total Phenolic Compounds in Legume Samples

Total phenolic content was determined using the Folin–Ciocalteu method as described by Rajha *et al*. (2014). An aliquot of 10 µL of the sample solution was mixed with 100 µL of commercial Folin–Ciocalteu reagent and 1580 µL of distilled water. After standing at room temperature for 5 min, 300 µL of saturated sodium carbonate solution was added. The mixture was allowed to develop colour for 2 h at room temperature, after which absorbance was measured at 760 nm using a UV–Vis spectrophotometer (UV-9200, UK). A calibration curve was prepared using gallic acid standards, and phenolic content was expressed as milligrams of gallic acid equivalents per litre (mg GAE/L). The values were subsequently converted to grams of gallic acid equivalents per 100 g dry matter (g GAE/100 g DM) to obtain total phenolic content (TPC).

Extraction of phenolic compounds from the raw legume samples was carried out by varying four factors: solvent type (methanol, ethanol, and acetone), solvent concentration (20–100%, v/v), extraction time (30–50 min), and temperature (40–60°C). Each factor was investigated independently while maintaining the others constant. Fixed conditions included a particle size of 0.50 mm and a solvent-to-solid ratio of 10:1 (v/v) (Tan *et al*., 2013; James *et al.*, 2020b).

Based on the evaluation of extraction yield under different conditions, the optimum extraction parameters for each legume were established as follows: African breadfruit yielded best in 40% acetone (v/v) for 30 min at 50°C; Bambara nut in 60% ethanol (v/v) for 40 min at 50°C; red bean in 100% methanol for 40 min at 50°C; pigeon pea in 100% methanol (v/v) for 40 min at 50°C; cowpea in 90% ethanol (v/v) for 50 min at 50°C; African yam bean in 100% acetone (v/v) for 30 min at 50°C; African oil bean seed in 60% acetone (v/v) for 50 min at 50°C; and groundnut in 40% acetone (v/v) for 30 min at 50°C.

Characterization of Bioactives

GC–MS analysis was performed using a GC Clarus 500 Perkin Elmer system equipped with an AOC-20i autosampler and a gas chromatograph coupled to a mass spectrometer. Separation was achieved on an Elite-1 fused silica capillary column (30 m × 0.25 mm internal diameter × 1 µm film thickness), composed of 100% dimethyl polysiloxane. The instrument operated in electron impact mode at 70 eV. Helium (99.999%) served as the carrier gas at a constant flow rate of 1 mL/min, and an injection volume of 0.5 µL was used with a split ratio of 10:1. The injector and ion source temperatures were maintained at 250°C and 280°C, respectively. The oven temperature programme commenced at 110°C (held for 2 min), increased at a rate of 10°C/min to 200°C, then at 5°C/min to 280°C, and finally held isothermally at 280°C for 9 min. Mass spectra were recorded at 70 eV with a scan interval of 0.5 s over a mass range of 40–550 Da.

Identification of Components

Interpretation of the GC–MS mass spectra was carried out using the National Institute of Standards and Technology (NIST) database, which contains over 62,000 spectral patterns. The spectra of unknown compounds were matched with those of known compounds in the NIST library to facilitate identification. The identities and molecular weights of the components present in the samples were thereby determined.

In Vitro Antioxidant Evaluation of the Extracts

Reducing Power Assay

Reducing power was determined according to the method of Singh *et al*. (2011). Briefly, 1 mL of the filtrate was mixed with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$], followed by incubation at 50°C for 20 min. The reaction was terminated by the addition of 2.5 mL of 10% trichloroacetic acid, after which the mixture was centrifuged at 3000 rpm (1000 × g) for 10 min. An aliquot (2.5 mL) of the supernatant was then combined with 2.5 mL of distilled water and 0.5 mL of 1% ferric chloride ($FeCl_3$) solution. The absorbance was measured at 700 nm. An increase in absorbance indicates higher reducing power. The percentage reducing power was calculated using equation 2.

$$\text{Reducing power} = [(Abs_{extract} - Abs_{blank})/Abs_{blank}] \text{ X } 100\%, \quad (2)$$

where $Abs_{extract}$ is absorbance of extracts, and Abs_{blank} is absorbance of water.

1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Assay:

The radical scavenging activity of the samples was evaluated using the method described by Siddiqua *et al.* (2010). Briefly, the reaction mixture consisted of 1 mL of the extract, 3 mL of methanol, and 150 µL of 0.1% DPPH solution. The mixture was allowed to stand for 30 min, after which absorbance was measured at 517 nm.

Radical scavenging activity was calculated using equation3:

%DPPH scavenging = [(Abs_control − Abs_sample) / Abs_control] × 100 (3)

where Abs_sample represents the absorbance of the extract solution and Abs_control represents the absorbance of the methanol–DPPH solution.

RESULTS AND DISCUSSION

Fibre Profile of Legume Samples

The interaction between dietary fibre and other bioactive compounds, such as polyphenols, plays an important role in determining their physiological effects. In addition, fermentation of dietary fibre in the large intestine leads to the production of short-chain fatty acids, which serve as an energy source for gut microflora (Singh *et al.*, 2016).

The fibre profile of the legume samples is presented in Table 1. The results indicate significant variations ($p < 0.05$) among all samples evaluated. Hemicellulose, raffinose, cellulose, lignin, and stachyose contents ranged from 12.15–17.18%, 14.26–18.67%, 38.19–44.09%, 4.03–8.17%, and 3.45–6.54% in African breadfruit, Bambara nut, red bean, pigeon pea, cowpea, African yam bean, African oil bean and groundnut, respectively.

African oil bean recorded the highest hemicellulose content (17.18%), followed by groundnut (16.43%) and African breadfruit (15.22%), while Bambara nut had the lowest value (12.15%). A similar pattern was observed for raffinose and hemicellulose, where African oil bean and groundnut exhibited significantly higher ($p < 0.05$) values. For lignin and stachyose, groundnut showed the highest contents (8.17% and 6.54%, respectively), whereas red bean (4.16%) and Bambara nut (3.45%) recorded comparatively lower values. Dietary fibre remains a key bioactive component in pulses.

Table 1: Fibre Profile of Legume Samples

Fibre (%)	ABF	BBN	RBS	PGP	CPB	AYB	AOB	GGN
Hemicell.	15.22^{c} ± 0.00	12.15^{g} ± 0.01	13.27^{f} ± 0.01	13.32^{e} ± 0.01	14.26^{d} ± 0.01	14.26^{d} ± 0.01	17.18^{a} ± 0.01	16.34^{b} ± 0.01
Raffinose	14.26^{h} ± 0.01	17.87^{c} ± 0.02	17.64^{d} ± 0.03	17.26^{e} ± 0.01	16.44^{f} ± 0.02	15.65^{g} ± 0.00	18.67^{a} ± 0.01	18.35^{b} ± 0.00
Cellulose	42.14^{c} ± 0.01	38.06^{h} ± 0.00	38.19^{g} ± 0.01	40.21^{f} ± 0.00	41.67^{e} ± 0.01	41.18^{d} ± 0.00	44.09^{a} ± 0.00	43.25^{b} ± 0.01
Lignin	7.08^{b} ± 0.00	4.03^{h} ± 0.01	4.16^{g} ± 0.01	4.19^{f} ± 0.01	5.14^{e} ± 0.01	6.12^{d} ± 0.00	7.04^{c} ± 0.00	8.17^{a} ± 0.01
Starchyose	5.26^{c} ± 0.01	3.45^{g} ± 0.00	3.74^{f} ± 0.02	3.87^{e} ± 0.01	3.95^{d} ± 0.00	3.98^{d} ± 0.01	6.00^{b} ± 0.00	6.54^{a} ± 0.02

According to Singh *et al*. (2016), both the seed coat and cotyledons of legumes are rich in soluble and insoluble dietary fibre. The values obtained in this study are comparable to the reported range of 14–32% for beans, chickpea, lentils, and peas. However, they are higher than the values reported for cereals such as wheat (3.00–15.02%), rice (0.86–4.33%), and barley (2.14–10.79%) (Dueñas *et al*., 2004; Singh *et al.*, 2016; Rebello *et al*., 2014).

Bioactive Potentials of Legume Samples

GC-MS Profiling of African Breadfruit Seed, Bambaranut, Red Beans and Pigeon Pea

The gas chromatography-mass spectrometry (GC-MS) study has identified a diverse array of bioactive compounds with significant pharmacological and therapeutic potentials in African bread fruit, Bambaranut, red bean and pigeon pea (Table 2). These bioactive compounds exhibit multifaceted biological activities, including antioxidant, antimicrobial, enzyme inhibitory, and sickle cell protective properties, underscoring their potentials as functional food ingredients.

African breadfruit contains a diverse array of bioactive compounds with significant physiological and pharmacological relevance, underscoring its potential as a functional food with therapeutic applications. Among the identified constituents are pyrazole derivatives, which are widely reported for their broad biological activities, including antimicrobial, anti-inflammatory, and anticancer effects (Patel *et al*., 2020); while, pyrazole-5-carboxylic acid functions as a bioactive modulator capable of interacting with biological receptors and enzymatic systems, with structural properties that support enzyme inhibition relevant to drug discovery.

Table 2: Bioactive Profiling of African Breadfruit, Bambaranut, Red Beans and Pigeon Pea

P/No.	Ret. time (sec)	Conc. Area (%)	Compound	Mol. weight (g/mol)	Bioactivity
			African breadfruit		
6	6.495	4.53	Pyrazole-5-carboxylic acid, 3-methyl-	126.115	Bio activator /receptor
11	6.702	0.82	5-Hydroxymethylfurfural	126.111	Inhibits the formation of sickle cells
16	7.117	3.24	1,2-Diethoxybenzene	166.22	Bio inhibitor
21	7.458	4.74	Ethanethioic acid, S-phenyl ester	152.21	Antioxidant
25	7.658	1.56	Phenyl-1-thio-.alpha glucopyranoside	272.32	Antioxidant
27	7.732	2.73	Hydroquinone	110.11	Treatment of hyper pigmented skin
			Bambaranut		
4	6.317	0.33	Phenol, p-[3-(methylamino)propyl]-	165.23	Antioxidant
26	7.843	0.82	10-Undecenoic acid, propyl ester	184.279	Antifungal
41	9.139	0.37	Phenol, o-(2-butenylthio)-	182.28	Antioxidant
51	10.769	0.09	2-Methyl-3-nitrophenol	153.14	Antioxidant
			Red bean		
2	6.161	4.38	Cumidine	135.21	Serine-type endopeptidase activity
10	6.628	1	2-Mercaptophenol	126.173	Inhibition of carbonic anhydrase
15	6.843	1.18	3-Thiopheneethanol	128.19	Antioxidant
17	7.391	2.13	Dichloroacetic acid, 2-isopropoxyphenyl ester	263.11	Antioxidant
49	11.835	0.08	Morpholine, 3-(4,5-dihydroxy) phenyl-	163.22	Antioxidant
			Pigeonpea		
4	6.028	0.1	Mexiletine	179.26	Ant arrhythmias
10	6.561	0.46	2-Dimethylamino-2'-methoxyacetophenone	193.24	Antioxidant
23	7.561	0.78	3,4-Dimethyl-2-prop-2-enyl-2,5-dihydrothiophene 1,1-dioxide	186.27	Antioxidant
25	7.717	0.83	Tetracaine	264.363	Aesthesia
27	7.784	0.32	4-(4-Methyl-[1,3,2]dioxaborinan-2-yloxy)-phenol	208.019	Antioxidant
29	8.183	3	Resorcinol monoacetate	152.149	Enzyme inhibitor
44	9.813	2.41	Acetamide, 2-amino-N-phenyl-2-thioxo-	194.252	Enzyme inhibitor
87	12.768	1.24	5-Cyclohexadecen-1-one	236.399	Enzyme inhibitors

In addition, 5-hydroxymethylfurfural (HMF), a compound formed during thermal processing, has been associated with inhibition of sickle hemoglobin polymerization, thereby reducing erythrocyte sickling, and it also exhibits antioxidant and anti-inflammatory activities relevant to sickle cell disease management (Antwi-Baffour *et al*., 2018; Zheng *et al*., 2021). Furthermore, African breadfruit expressed the presence of aromatic derivatives such as 1,2-diethoxybenzene which display antimicrobial and antifungal activity through disruption of microbial cell membrane integrity, alongside reported antioxidant and anti-inflammatory effects that enhance their pharmacological relevance (Chen *et al*., 2022; Madaan *et al*., 2020). Also, African breadfruit showed the presence of S-phenyl esters of ethanethioic acid which exhibit demonstrate strong free radical scavenging activity, thereby preventing oxidative damage linked to cardiovascular and neurodegenerative disorders (Gupta & Pandey, 2020; Rahman *et al*., 2021); while, glucopyranoside derivatives contribute to oxidative stress reduction through reactive oxygen species scavenging and exhibit anti-inflammatory and immunomodulatory properties relevant to chronic diseases such as diabetes and cardiovascular disorders (Lee *et al.*, 2019; Kumar *et al*., 2022). Hydroquinone, also detected in African breadfruit, is widely recognized for its dermatological application in the management of hyperpigmentation disorders such as melasma through inhibition of tyrosinase, the key enzyme in melanin biosynthesis (Grimes *et al*., 2020), although prolonged use requires caution due to potential adverse effects including exogenous ochronosis and skin irritation (Jung *et al*., 2021). Collectively, these compounds reflect the biochemical diversity of African breadfruit and reinforce its relevance in both nutritional and pharmacological research applications.

Bambara groundnut contains a diverse array of phenolic and related bioactive compounds that contribute to its antioxidant, antimicrobial, and health-promoting properties, thereby supporting its value as a functional food. In Bambara groundnut, these phenolic constituents play a central role in antioxidant activity by scavenging free radicals and suppressing oxidative chain reactions, which helps protect biological systems from oxidative stress (Huang *et al*., 2020). Phenol, p-[3-(methylamino) propyl]-, a substituted phenolic compound identified in Bambara groundnut, would contribute to the inhibition of lipid peroxidation and neutralization of reactive radicals, thereby reducing oxidative damage associated with cardiovascular diseases, neurodegeneration, and cancer development (Dai & Mumper, 2010; Shahidi & Ambigaipalan, 2015). Bambara groundnut also contains fatty acid derivatives such as 10-undecenoic acid and its esters, which are associated with antifungal activity through disruption of fungal cell membranes, increased permeability, and eventual cell death; while, ester forms such as propyl esters enhance solubility and antimicrobial effectiveness against spoilage and pathogenic organisms (Ghannoum & Rice, 1999; Kabara *et al.*, 1972; Kumar *et al.*, 2021a). In addition, sulfur-containing phenolic compounds present in Bambara groundnut, such as phenol, o-(2-butenylthio)-, exhibit enhanced antioxidant activity due to thio-functional groups that improve radical scavenging efficiency and confer protection against oxidative stress-related disorders including diabetes and cardiovascular diseases (Jacob *et al.*, 2011; Zhou *et al*., 2016). Similarly, nitrophenolic compounds such as 2-methyl-3-nitrophenol in Bambara groundnut demonstrate strong antioxidant and anti-inflammatory properties, where methyl and nitro substituents stabilize phenoxyl radicals and enhance free radical scavenging capacity (Aldini *et al*., 2018; Santos *et al*., 2019). Aside their antioxidant effects, these nitrophenol derivatives also modulate inflammatory pathways, further contributing to their potential role in disease prevention and health maintenance (Gülçin, 2020). Collectively, the presence of these compounds highlights the biochemical diversity of Bambara groundnut and reinforces its importance as a reservoir of functional bioactive molecules with applications in nutrition and health.

Red bean contains a variety of bioactive compounds that collectively contribute to its functional and health-promoting properties. Among these compounds, cumidine, an aromatic amine identified in red bean, is associated with serine-type endopeptidase activity, enzymes that are central to protein digestion, immune regulation, and blood coagulation processes, suggesting that its presence may support proteolytic balance and metabolic regulation in the body (Poreba *et al.*, 2014; Rawlings & Salvesen, 2012). In addition, 2-mercaptophenol, a sulfur-containing phenolic compound present in red bean, exhibits inhibitory activity against carbonic anhydrase enzymes, which are key regulators of pH balance, respiration, and ion transport; this inhibitory potential indicates possible relevance in the management of conditions such as glaucoma, epilepsy, and certain cancers where carbonic anhydrase modulation is therapeutically important (Supuran, 2008; Supuran *et al*., 2003; Carta *et al.*, 2020). Red bean also contains 3-thiopheneethanol, a sulfur-containing heterocyclic compound with notable antioxidant properties that contribute to the neutralization of reactive oxygen species, thereby reducing oxidative stress linked to neurodegenerative, cardiovascular, and metabolic disorders (Lobo *et al*., 2010; Lai *et al*., 2017). Furthermore, dichloroacetic acid derivatives identified in red bean, particularly the 2-isopropoxyphenyl ester form, have been linked to metabolic regulation and mitochondrial function, suggesting potential roles in reducing oxidative stress and supporting cellular energy metabolism in conditions such as neurodegenerative and metabolic diseases (Stacpoole, 2011; Liang *et al*., 2019). In addition, morpholine derivatives such as 3-(4,5-dihydroxy) phenyl-morpholine contribute to antioxidant defense through hydrogen-donating activity typical of phenolic structures, thereby enhancing the ability of red bean to counteract oxidative stress and inflammation-related damage (Rice-Evans *et al*., 1997; Pietta, 2000). Therefore, the presence of these compounds highlights the biochemical diversity of red bean and its relevance as a source of bioactive molecules with potential physiological and therapeutic benefits.

Pigeon pea contains a diverse range of bioactive compounds suggesting its potential as a functional food with notable therapeutic relevance. The presence of mexiletine, a class IB sodium channel blocker, is particularly significant as it stabilizes cardiac membranes by reducing sodium ion influx and suppressing abnormal myocardial electrical activity, with established clinical use in the management of ventricular arrhythmias and neuropathic pain, thereby suggesting possible cardioprotective implications of pigeon pea bioactive (Catterall *et al.*, 2010; Johannesen *et al.*, 2016ab). In addition, acetophenone derivatives contribute to the antioxidant potential of pigeon pea through phenolic and methoxy functional groups that enhance free radical scavenging, supporting their role in mitigating oxidative stress associated with neurodegenerative, cardiovascular, and cancer-related diseases (Pietta, 2000). Likewise, thiophene-based compounds identified in pigeon pea reported to demonstrate strong antioxidant activity, where methyl and alkenyl substituents enhance electron-donating capacity and improve reactive oxygen species neutralization, reinforcing their relevance in conditions such as diabetes, cancer, and cardiovascular disorders (Pérez *et al*., 2016; Gülçin, 2020). Pigeon pea also contains tetracaine, an ester-type local anesthetic that acts by inhibiting voltage-gated sodium channels, thereby reducing nerve impulse transmission and explaining its established use in ophthalmic, spinal, and surface anesthesia, while also indicating potential analgesic and neuroactive properties within the crop’s phytochemical profile (Covino, 1985; Rosenberg *et al.*, 2004). Furthermore, resorcinol derivatives present in pigeon pea have been linked to inhibition of key metabolic enzymes such as tyrosinase and lipoxygenase, suggesting potential applications in managing hyperpigmentation, inflammatory conditions, and microbial infections (Kumar & Pandey, 2013; Ali *et al.*, 2018); while, resorcinol monoacetate may contribute to similar enzyme-regulatory effects in metabolic pathways. Additionally, thioamide derivatives exhibit inhibitory activity against proteases and oxidoreductases, which are essential regulators of metabolic processes, thereby indicating their potential in therapeutic development targeting cancer, infections, and inflammatory diseases (Santos *et al.*, 2019). Macrocyclic ketones such as 5-cyclohexadecen-1-one further contribute by inhibiting enzymes involved in lipid metabolism and microbial defense, suggesting possible applications in obesity management, diabetes control, and antimicrobial interventions (Carbone *et al*., 2019ab). These findings showed the biochemical complexity of pigeon pea and reinforce its value as a reservoir of pharmacologically active compounds.

Bioactive Potentials in Cowpea, African Yam Bean Seed, African Oil Bean Seed and Groundnut

Table 3: Bioactive Profiling of Cowpea, African Yam Bean Seed, African Oil Bean Seed and Groundnut

P/No.	Ret. time (sec)	Conc. Area (%)	Compound	Mol. weight (g/mol)	Bioactivity
			Cowpea bean		
10	10.635	1.85	Acetamide, 2-amino-N-phenyl-2-thioxo-	194.252	Bio inhibitor
17	11.687	0.38	6-Octadecenoic acid, (Z)-	282.468	Enzyme inhibitor
23	12.102	2.94	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxy phenyl]-	207.273	Enzyme inhibitor
24	12.183	2.21	Pyrido[2,3-d]pyrimidine, 4-phenyl-	207.23	Antioxidant
31	12.628	6.76	2-Methyl-7-phenylindole	207.27	Antioxidant
34	12.909	3.58	Silane, trimethyl[5-methyl-2-(1-methylethyl)phenoxy]-	222.40	Antioxidant
36	12.998	2.22	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	282.29	Antioxidant
			African yam bean seed		
1	11.827	1.5	2-Methyl-5,5-diphenyl-4-(methylthio)imidazole	234.302	Enzyme inhibitor
3	12.072	0.99	1H-Indole, 1-methyl-2-phenyl-	207.276	Enzyme inhibitor
8	12.331	1.87	6-Octadecenoic acid	282.468	Bio modulator
12	12.59	4.34	2-Methyl-7-phenylindole	207.276	Bio receptor
15	12.746	3.01	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-	207.273	Inhibitor, reducing agent
17	12.827	4.11	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	298.298	Antimutagene
22	13.094	6.74	2(1H)-Quinolinone, 1,4-dihydroxy-	177.159	Tumor inhibitor
24	17.064	1.02	Thiocarbamic acid, N,N-dimethyl, S-1,3-diphenyl-2-butenyl ester	311.4411	Modulators
28	18.997	0.78	Bicyclo[6.1.0]non-4-ene-9-carboxamide, N-(4-acetylphenyl)-	283.3648	Helps in biosynthesis
30	19.701	1.58	1,2-Bis(trimethylsilyl)benzene	222.478	Enzyme Inhibitor
32	19.997	1	Benzo[h]quinoline, 2,4-dimethyl-	207.276	Antimicrobial
			African oil bean seed		
20	7.295	1.1	Phenol, 4-butoxy-	166.22	antitumor
22	7.406	0.8	Thiodiglycol	122.182	Enzyme inhibitors
27	7.746	2.3	Dimethyl dopamine, N,N-	181.235	Enzyme inhibitor
34	8.724	1.4	N'-Benzyl-N,N-dimethyl ethylene diamine	178.279	Antitumor
39	9.05	0.63	3-Methoxy-4-methyl-(2-aminobutyl)benzene	167.208	Bio stimulator
40	9.094	0.41	N-methyl-1-[4-(methyl sulfanyl) phenyl]propan-2-amine	195.324	Bio inhibitor
43	9.331	1.16	Venlafaxine	277.402	Antidepressant
44	9.383	1.24	Psilocin	204.273	Hallucinogen
50	9.783	0.67	2,2'-Bis[(3-dimethylaminopropyl) amino carbonyl] diphenyl disulfide	248.362	Bacterial/enzyme inhibitor
52	9.894	1.65	Mexiletine	179.259	Anti-arrhythmia
57	10.628	1.16	2,2'-Bis[(3-dimethylaminopropyl) amino carbonyl] diphenyl disulfide	248.362	Bacterial/enzyme inhibitor

P/No.	Ret. time (sec)	Conc. Area (%)	Compound	Mol. weight (g/mol)	Bioactivity
67	11.331	0.79	Amitriptyline	277.403	Antidepressant
			Groundnut		
1	5.887	18.4	Benzofuran, 2,3-dihydro-	120.15	Antioxidant
5	6.199	2.88	Benzaldehyde, 2-methyl-	120.151	Antioxidant
9	10.057	1.05	3-Methyl-2-nitrophenol	153.14	Antioxidant
10	10.309	2.29	Paromomycin	615.634	Antibacterial
21	12.131	0.88	3,4-Dimethylbenzoic acid	150.17	Antioxidant
32	17.449	1.15	N-(3-Chlorophenyl)maleimide	242.055	Antimutagene
35	18.619	0.99	1H-Indole, 5-methyl-2-phenyl-	207.27	Antioxidant

Compounds identified in cowpea, African yam bean seed, African oil bean seed and ground have been reported to exhibit enzyme inhibition, serve as antioxidants, and possess bio-inhibitory properties, revealing the functional and therapeutic potential of these legumes (Table 3).

Cowpea contains a range of bioactive compounds that contribute to its functional and health-promoting properties through enzyme modulation, antioxidant activity, and antimicrobial effects. Among these, acetamide derivatives identified in cowpea are associated with enzyme inhibition and antimicrobial activity, suggesting potential roles in metabolic regulation, microbial control, and disease prevention (Santos *et al*., 2019). In addition, cowpea contains 6-octadecenoic acid (oleic acid), a monounsaturated fatty acid known to influence lipid metabolism by inhibiting key enzymes involved in fatty acid synthesis, while also exhibiting anti-inflammatory and antimicrobial properties that support its relevance in functional food and nutraceutical applications (Huang & Froehlich, 2013; Sales-Campos *et al*., 2013). Cowpea also contains hexahydropyridine derivatives, nitrogen-containing heterocyclic compounds that demonstrate enzyme-inhibitory activity against oxidoreductases and proteases, with the presence of dihydroxyphenyl groups further enhancing antioxidant potential and pharmacological significance (Patel & Purohit, 2018). Furthermore, pyridopyrimidine compounds in cowpea contribute to antioxidant and neuroprotective functions by scavenging reactive oxygen species and limiting oxidative stress-induced cellular damage, thereby indicating potential roles in the prevention of neurodegenerative disorders, cardiovascular diseases, and cancer (Rashid *et al*., 2019). Indole derivatives such as 2-methyl-7-phenylindole also occur in cowpea and are recognized for their strong antioxidant properties, where phenyl substitution enhances free radical scavenging and supports cellular defense mechanisms against oxidative damage linked to chronic diseases (Kumar *et al*., 2021b). In addition, organosilicon compounds, including silane derivatives present in cowpea, contribute to cellular protection by stabilizing membranes and reducing oxidative damage, with phenoxy groups further enhancing antioxidant efficiency (Yang *et al*., 2014ab). Biphenyl derivatives identified in cowpea further strengthen its antioxidant profile through free radical neutralization and reduction of oxidative stress, with functional groups such as acetoxymethyl and methoxycarbonyl improving compound stability and bioavailability (Shahidi & Ambigaipalan, 2015). Hence, these compounds demonstrate the biochemical diversity of cowpea and its importance as a source of bioactive molecules with potential applications in nutrition, disease prevention, and health promotion.

African yam bean seed contains a wide range of bioactive compounds that show its potential as a functional food with important physiological and therapeutic benefits, particularly as enzyme inhibitor, antioxidant activity, and antimicrobial action. The presence of imidazole derivatives in African yam bean seed suggests its ability to inhibiting oxidoreductase and protease enzymes (Hearn & Aguilar, 2021). In a similar manner, indole-based compounds identified in African yam bean seed are known to modulate key enzymes such as tyrosine kinases, proteases, and oxidoreductases, and their structural similarity to tryptophan allows them to interact with biological pathways linked to neuroprotection, antimicrobial defense, and metabolic balance (Kumar *et al*., 2021b). The seed also contains 6-octadecenoic acid (oleic acid), a bioactive lipid associated with anti-inflammatory, cardioprotective, and lipid-regulating functions, including cholesterol modulation, improved insulin sensitivity, and immune response regulation (Sales-Campos *et al*., 2013). However, indole derivatives such as 2-methyl-7-phenylindole contribute to antioxidative defense and receptor-mediated biological activities relevant to neurological health (Kumar *et al*., 2021a). In addition, hexahydropyridine derivatives present in African yam bean seed exhibit antioxidant and enzyme-inhibitory properties, with dihydroxyphenyl groups enhancing radical-scavenging capacity and supporting protection against oxidative stress-related disorders such as cardiovascular and neurodegenerative diseases (Patel & Purohit, 2018). Tyramine-based compounds in the seed demonstrate antimutagenic potential by reducing oxidative DNA damage, with methylenedioxy functional groups strengthening their biological activity and suggesting relevance in cancer prevention (Ali *et al*., 2018). Quinolinone derivatives further extend this therapeutic potential through their ability to inhibit cell proliferation and induce apoptosis in tumor cells, with hydroxyl substitutions improving their effectiveness in modulating cancer-related pathways (Rashid *et al*., 2019). Thiocarbamic acid derivatives contribute to enzyme regulation and redox signaling, indicating possible roles in metabolic control and disease intervention (Santos *et al*., 2019); while, bicyclic carboxamides are associated with enhanced biosynthetic and lipid metabolic pathways, supporting cellular energy production (Carbone *et al*., 2019a). Moreover, trimethylsilyl benzene derivatives identified in African yam bean seed exhibit inhibitory effects on lipid-metabolizing enzymes, suggesting potential applications in managing obesity and cardiovascular disorders (Yang *et al*., 2014a); whereas, benzoquinoline derivatives demonstrate antimicrobial activity through disruption of microbial enzyme systems, reinforcing their value in food preservation and pharmaceutical applications (Kumar *et al*., 2021ab). In conclusion, these compounds highlight the biochemical complexity of African yam bean seed and its significance as a reservoir of bioactive constituents with broad nutritional and therapeutic potential.

Gas chromatography–mass spectrometry profiling of African oil bean seed reveals a wide spectrum of bioactive compounds that indicate its potential as a functional food with diverse therapeutic applications, including antitumor, enzyme-inhibitory, antimicrobial, antidepressant, and anti-arrhythmic effects. The presence of phenolic compounds with butoxy functional groups in African oil bean seed is shown to enhance lipophilicity and cellular permeability, which may support anticancer activity through inhibition of tumor cell proliferation and induction of apoptosis (Shahidi & Ambigaipalan, 2015). Sulfur-containing compounds such as thiodiglycol identified in African oil bean seed exhibit enzyme-inhibitory activity, particularly through interactions with thiol groups in enzyme active sites, thereby influencing metabolic and detoxification pathways (Kumar & Pandey, 2013). Dopamine-related derivatives present in the seed are also implicated in neurotransmitter metabolism and cellular signaling, with potential inhibition of oxidative enzymes such as monoamine oxidase, suggesting relevance in neurodegenerative conditions and mood regulation (Zhou *et al*., 2020). African oil bean seed further contains ethylenediamine derivatives with reported antitumor activity, where benzyl functional groups enhance lipophilicity and support interactions with DNA and enzymes involved in cancer progression (Ali *et al*., 2018); while, benzene derivatives bearing methoxy and methyl substituents contribute to neuroprotective and anti-inflammatory effects through modulation of cellular signaling pathways and oxidative stress responses (Sales-Campos *et al*., 2013). Sulfur-containing amines in the seed also participate in enzyme modulation and neurotransmitter-related pathways, contributing to antimicrobial and neuroprotective functions (Santos *et al*., 2019). Important compounds such as venlafaxine and amitriptyline detected in African oil bean seed are associated with antidepressant activity through modulation of neurotransmitter reuptake, suggesting possible neuropharmacological benefits (Katzman, 2009; Rosenberg *et al*., 2004); while, psilocin, a serotonin receptor agonist, indicates potential effects on cognitive and neurological processes that warrant further investigation (Nichols, 2016). In addition, disulfide-containing compounds contribute to antimicrobial activity by interfering with bacterial thiol metabolism, reinforcing the antibacterial potential of African oil bean seed (Carbone *et al*., 2019ab). The detection of mexiletine, a class IB sodium channel blocker, further suggests possible cardiovascular benefits through stabilization of cardiac electrical activity and management of arrhythmias (Johannesen *et al*., 2016ab). In summary, these compounds highlight the biochemical diversity of African oil bean seed and its significance as a source of bioactive constituents with applications in nutrition, disease prevention, and therapeutic development.

Groundnut contains a wide range of bioactive constituents among these, benzofuran derivatives which contribute to oxidative stress control through effective free radical neutralization and have been associated with anti-inflammatory and neuroprotective effects (Ahmed *et al*., 2021). In a similar manner, benzaldehyde derivatives present in groundnut exhibit both antioxidant and antimicrobial functions, where structural substitutions such as methyl groups enhance their reactivity and ability to inhibit lipid peroxidation and cellular oxidative damage (Prakash *et al*., 2018; Singh & Sharma, 2020). Nitrophenolic compounds further support this antioxidative capacity by scavenging reactive species and reducing inflammation, thereby contributing to protection against chronic conditions including cardiovascular and neurodegenerative diseases (Kumar *et al*., 2017). The detection of paromomycin-like compounds suggests an additional antimicrobial dimension, as such aminoglycoside structures are known to inhibit microbial protein synthesis, indicating possible roles in microbial control and food safety (Grollman, 2018; Wang *et al*., 2021). Benzoic acid derivatives, including substituted forms such as 3,4-dimethylbenzoic acid, enhance this protective profile by limiting oxidative degradation and suppressing microbial growth, which is relevant for both physiological and preservation functions (Huang *et al*., 2020). In addition, maleimide derivatives identified in groundnut have been linked to antimutagenic activity through their ability to protect DNA integrity against mutational damage (Wang *et al*., 2016). Indole derivatives further complement this biochemical profile by providing antioxidant and anti-inflammatory effects, supporting cellular defense mechanisms and contributing to neuroprotective and anticancer potential (Gómez-Verduzco *et al*., 2022). Therefore, these compounds highlight the biochemical complexity of groundnut and underscore its relevance as a functional food with multiple roles in health maintenance and disease prevention.

Antioxidant Capacity and Reducing Power of the Raw and 3-Day Germinated Legumes

The antioxidant activities of the raw and three-day germinated legume samples were determined using the DPPH• radical scavenging assay and the reducing power method. The results (Table 4) showed that all samples exhibited appreciable antioxidant capacities, with significant variations ($p<0.05$) among them. In the raw state, African oil bean and groundnut exhibited the highest antioxidant activities 52.16% and 52.17%, respectively. These were followed by Bambara groundnut (45.13%) and African breadfruit (35.80%), while African yam bean seed recorded the lowest value (21.75%). A similar pattern was observed for reducing power, where groundnut, Bambara groundnut, and African breadfruit demonstrated significantly ($p<0.05$) higher values of 35.17%, 32.27%, and 29.86%, respectively, whereas African yam bean seed had the lowest reducing capacity (21.63%).

Germination for three days resulted in a significant ($p<0.05$) enhancement of antioxidant activity across all legumes, with percentage increases of 17.24%, 18.42%, 52.84%, 43.03%, 53.58%, 53.18%, 14.65%, and 14.56% for African breadfruit, Bambara groundnut, red bean, pigeon pea, cowpea, African yam bean seed, African oil bean, and groundnut, respectively. Among these, cowpea exhibited the greatest improvement (53.24%), whereas groundnut showed the least increase (14.56%). At the end of the three-day germination period, groundnut and African oil bean retained significantly ($p<0.05$) higher antioxidant activities 61.06% and 61.11%, respectively, followed by red bean (60.12%) and pigeon pea (57.84%).

Table 4: Antioxidant Capacity and Reducing Power of the Raw and 3-day Germinated Samples

Sample	DPPH of Raw (%)	DPPH of 3-day germinated (%)	Reducing power of Raw (%)	Reducing power of 3-day germinated (%)
ABF	$35.80^{c}\pm0.00$	$43.26^{f}\pm0.26$ (+17.24)	$29.86^{c}\pm0.01$	$32.92^{g}\pm0.07$ (+9.30)
BBN	$45.13^{b}\pm0.69$	$55.32^{d}\pm0.11$ (+18.42)	$32.27^{b}\pm0.02$	$34.00^{f}\pm0.00$ (+5.09)
RBS	$28.35^{e}\pm0.01$	$60.12^{b}\pm0.02$ (+52.84)	$25.49^{e}\pm0.01$	$59.47^{b}\pm0.20$ (+57.14)
PGP	$32.95^{d}\pm0.00$	$57.84^{c}\pm0.06$ (+43.03)	$26.10^{d}\pm0.00$	$49.11^{d}\pm0.01$ (+46.85)
CPB	$25.64^{f}\pm0.02$	$55.24^{d}\pm0.03$ (+53.58)	$23.54^{f}\pm0.02$	$55.06^{c}\pm0.08$ (+57.25)
AYB	$21.75^{g}\pm0.00$	$46.45^{e}\pm0.32$ (+53.18)	$21.63^{h}\pm0.01$	$35.74^{e}\pm0.04$ (+39.48)
AOB	$52.16^{a}\pm0.00$	$61.11^{a}\pm0.13$ (+14.65)	$22.63^{g}\pm0.01$	$24.38^{h}\pm0.40$ (+7.18)
GGN	$52.17^{a}\pm0.02$	$61.06^{a}\pm0.08$ (+14.56)	$35.17^{a}\pm0.01$	$60.06^{a}\pm0.08$ (+41.44)

Values are means and standard deviations of three determinations. Values not followed by the same superscript in the same column are significantly different ($p < 0.05$).
Key: ABF = African breadfruit, BBN = Bambaranut, RBS = Red bean, PGP = Pigeon pea, CPB = Cowpea, AYB = African yam bean seed, AOB = African oil bean, GGN = Groundnut and (-/+ = % decrease/increase)

The observed enhancement in antioxidant activity following germination may be attributed to biochemical changes such as the activation of hydrolytic enzymes and the synthesis or release of bound phenolic compounds. Antioxidant activity in legumes has been widely associated with their phenolic constituents, which act as effective free radical scavengers and reducing agents (Gujral *et al*., 2012; James *et al*., 2020abc). More recent studies have further confirmed that germination improves the bioavailability of phenolics and other antioxidant compounds through the breakdown of complex macromolecules and the formation of low-molecular-weight metabolites with higher antioxidant potential (Gan *et al*., 2022). In the present study, the relatively high antioxidant capacity of groundnut may be linked to its higher total phenolic content compared to the other legumes.

Similarly, three-day germination significantly ($p<0.05$) improved the reducing power of the legumes by 9.30%, 5.09%, 57.14%, 46.85%, 57.25%, 39.48%, 7.18%, and 41.44% for African breadfruit, Bambara groundnut, red bean, pigeon pea, cowpea, African yam bean seed, African oil bean, and groundnut, respectively. Cowpea (57.25%) and red bean (57.14%) showed the highest increases, while Bambara groundnut recorded the least improvement (5.09%). At the end of germination, groundnut exhibited a significantly ($p<0.05$) higher reducing power (60.06%), whereas African breadfruit had the lowest value (32.92%).

The results indicate that short-term germination enhances both the radical scavenging activity and electron-donating capacity of legumes, thereby improving their functional and nutraceutical potential. These findings are consistent with recent reports that germination is an effective, low-cost bioprocessing technique for improving the antioxidant profile and health-promoting properties of legumes and other plant-based foods (Gan *et al*., 2022).

CONCLUSION

This study demonstrates that selected indigenous and underutilized legumes are valuable sources of dietary fibre and bioactive compounds with significant antioxidant potential. Variations in fibre fractions, particularly the higher levels of hemicellulose and raffinose in *Pentaclethra macrophylla* and *Arachis hypogaea*, indicate strong prospects for physiological benefits, including improved gut function and metabolic health. GC–MS results further revealed a wide range of phytochemicals associated with antioxidant, antimicrobial, antifungal, and enzyme inhibitory activities, confirming their functional relevance beyond basic nutrition. Short-term germination enhanced antioxidant capacity across all samples, likely through enzymatic activation and increased availability of phenolic compounds, with *Vigna unguiculata* showing the greatest response, while *Arachis hypogaea* and *Pentaclethra macrophylla* retained superior activity. Therefore, these legumes show considerable promise as functional food ingredients, and furthermore *in vivo* studies are needed to validate their health benefits and support their broader utilization in sustainable food systems.

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