

AN APPRAISAL OF PROTEIN FRACTION AND NUTRACEUTICAL BENEFITS OF SOME COMMONLY CONSUMED LEGUMES IN EASTERN NIGERIA

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ABSTRACT: To document for the populace the nutritional values of the five most consumed legumes in Eastern Nigeria, the protein fractions (albumin, globulin, prolamin, and glutelin fractions) of *Voandzeia geocarpa* (Okpa), *Phaseolus coccineus* (Akidi), *Cajanus cajan* (Fiofio/agbugbu), *Phaseolus multifarioris* (Ozaki/azam) and *Vigna unguiculata* (Beans) are assayed. The Biuret reagent spectrophotometric method was followed using bovine serum albumin (30%) as a standard. The result reveals that *C. cajan*, ranked the best of all the legumes appraised followed by *V. geocarpa*, *P. coccineus*, *P. multifarioris*, and *V. unguiculata*. *V. unguiculata*, *P. multifarioris* and *V. geocarpa* are richer in albumin while *C. cajan* and *V. geocarpa* are rich in globulin. Based on the nutritional and medicinal richness of these legumes, there is need to encourage the people to increase their consumption to compensate for animal protein deficiency in diet menu of average Eastern Nigerian family.

Keywords: Legumes, protein fraction, nutraceutical benefits, commonly consumed.

Introduction

Plants that produce seeds within the pods belong to the family called Fabaceae/Leguminosae and they include peas, lentils, beans, peanuts, and other podded plants that are consumed as food (Singh Sandhu & Kumar Chaturvedi, 2025; Savita et al., 2021; Grygier et al., 2022).

Legumes are rich in protein (composed of about 20–45%), amino acids, healthy carbohydrates with low effect on blood sugar (6–62%), dietary fiber (3–31%), essential minerals, mono and polyunsaturated healthier fats (1.6–49.7%), inorganic constituents after burning (ash 2–4.7%) and vitamins for the human diet. Legumes possess hypocholesterolemia, antiatherogenic, anticarcinogenic and hypoglycemic

properties among other health benefits and verities enjoyed by different societies of the world for humans and animals' food (Singh et al., 2022; Guo et al., 2024; Ndovie et al., 2025; Maphosa & Jideani, 2017; Polak et al., 2015).

The four most studied major constituents of legume seed protein fraction that varies among species are albumin and globulin fractions while prolamin and glutelin fractions are very low (Vasconcelos et al., 2010; Teka et al., 2020; Adewole et al., 2023; Sánchez-Chino et al., 2015; Zhang et al., 2024).

Albumin is the water-soluble fraction that helps maintain the colloid osmotic pressure (COP), that transport water soluble such as bilirubin, hormones, metals, vitamins, and drugs, that regulates pH as a buffer, acts as an antioxidants, coagulator, and aids wound healing in the body (Moman ET AL., 2022; Iizaka et al., 2011; Belinskaia et al., 2021; Evans, 2002; Miller, A. & Jedrzejczak, 2001; Kouris-Blazos & Belski, 2016). Albumin is essential in maintaining growth and repairing tissues. It also plays a major role in fighting off infections, provides a source of energy to the body, and helps keep a balance among body fluids (Messina, 2016).

Globulin is the fraction of protein that dissolves and can be extracted with sodium chloride solution. It plays an important role in immune defense, liver function, blood clotting, and infection control. It consists of numerous serum proteins responsible for the transport of nutrients, and metabolic reactions and the complement systems as well as functioning as antibodies. Compared to albumin, Globulin proteins has a higher molecular weight and can function as enzymes in many metabolisms, carrier proteins, or antibodies (Messina, 2016; Villasante et al., 2025; Busher, 1990; Sánchez-Chino et al., 2015; Ndidi et al., 2014).

Prolamin, a protein fraction from legumes is soluble in 70% ethanol, largely due to its abundance of hydrophobic amino acids such as alanine, proline, leucine, and glutamine. It demonstrates superior water holding and antioxidant capacity in addition to reported antihyperglycemic and antihypertensive potential that may contribute to improved health and prevention of chronic disease. However prolamin is characterized by low levels of essential amino acids such as lysine and tryptophan showing that prolamin have very low nutritional value. Foods rich in prolamins (eg gluten) act as binders holding foods together with high thermal stability. Despite these properties, they exhibit low digestibility in the small intestine and are associated with celiac disease (Gammoh et al., 2023; Xing et al., 2023; Day, 2013; Shewry 2019; Chirido & Arranz, 2015; Ortiz-Sánchez et al., 2013; Daly et al., 2020; Cebolla et al., 2018; Shah et al., 2024; Holding, 2014).

Glutelin is a water-insoluble protein fraction that becomes soluble in very dilute alkaline or acidic solutions, as well as in detergents. It is primarily responsible for the elasticity and viscous characteristics of bakery products, owing to its high molecular weight and its ability to form complexes with other proteins such as gliadin. (Desikan, 2024; Ye et al., 2023; Zhang et al., 2022; Marchetti et al., 2012; Li et al., 2024). Glutelin's are rich in proline and glutamine amino acids, with varying amount of phenylalanine, valine, and leucine which are responsible for their low aqueous solubility. Other essential amino acids such lysine, methionine, and threonine are present as a nutrient store for plant embryos, animals and humans (Takahashi et al., 2019; Ergün et al., 2018). The major

function is to supply nitrogen and amino acids during the germination of seeds (Guo et al., 2021; Duvnjak et al., 2021; Limami et al., 2002; Onomo et al., 2010).

A diet rich in plant proteins like legumin reduced the levels of triglycerides, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) (bad) cholesterol and thereby reducing the incidence of cancer, atherosclerosis, diabetes, high blood pressure, and other chronic metabolic diseases because it acts as an antioxidant, anti-inflammatory and alternative chemo preventive therapy against various chronic metabolic diseases. While on the other hand, it raises the level of high-density lipoprotein (HDL) (good) cholesterol and support healthy albumin, globulin levels, which if low may cause health challenges such as liver disease, kidney disease, or malnutrition (Islam & Siddiqua, 2020; Papaodyssea et al., 2025; Najjar et al., 2018; Angeles et al., 2021; Elliott et al., 2022; Shahnaz et al., 2024; Rao & Poonia, 2023; El-Sehrawy et al., 2025).

This study assays protein fractions (namely - albumins, globulins, glutelin, and prolamins) of the five most commonly consumed legumes in South Eastern Nigeria (*Vigna geocarpa*, *Phaseolus coccineus*, *Cajanus cajan*, *Phaseolus multiflorus*, and *Vigna unguiculata*) in order to evaluate the nutraceutical benefits associated with each legume.

Materials and methods

Materials

The choice seeds of legumes were bought from the Eke Market Agbani of Enugu State Nigeria. Bovine serum albumin, sodium chloride, sodium hydroxide, ethanol, copper sulphate, sodium potassium tartrate and potassium iodide were all of analytical grade and obtained from Conraws Scientific LTD Enugu Nigeria.

Sample preparation

Twenty grams of clean, dried and destoned legume seeds (*V. geocarpa*, *P. coccineus*, *C. cajan*, *P. multiflorus* and *V. unguiculata*) were ground and sieved repeatedly to obtain fine powder set aside for subsequent analysis respectively.

Extraction of albumin, globulin, prolamin and glutelin fractions

To obtain albumin fraction 0.5 g of each ground sample was ground with 0.5 g acid washed sand to release the intracellular protein present in the sample and homogenized with 100 ml distilled water and filtered. The filtrate was used for spectrophotometric analysis (Nugraha. & Fitriani, 2023; Birghila et al., 2015). The process was repeated but homogenized separately with 150 ml of 1M sodium hydroxide to obtain globulin fraction (Soto-Madrid et al., 2022; Tan et al., 2011; Uttam et al., 2023; Akasha, 2014), with 100 ml 70% ethanol to obtain prolamin (Tapia-Hernández et al., 2019; Min-Xuan et al., 2010; Yang et al., 2021), and with 50 ml of 0.1 M sodium chloride solution to obtain glutelin fraction (Idowu et al., 2021; Fashakin et al., 2023; Makeri et al., 2017; Deb et al., 2023).

Spectroscopic analysis

The protein assay was spectroscopically studied by adding 4 ml of biuret reagent to 1 ml of 0.0, 1, 2, 2.5, 5 and 10 % bovine serum albumin (used in constructing the standard curve Table 1 and Fig.1) alongside each extracted sample fraction of albumin, globulin, prolamin and glutelin respectively. The mixture was vortexed three times to mix well and left undisturbed at room temperature for 20 minutes. The Peak

pick method was employed using UV 250D PC series. The spectrophotometer was zeroed with distilled water and the reagents. Then the optical density of each was determined at 540 nm and recorded (Mahesha, 2012; Birghila et al., 2015; Sari et al., 2024).

Table 1: Standard curve preparation

Bovine serum albumin (30 %)	3.3	2.7	2	1.3	0.7
Distilled water (ml)	6.7	7.3	8.0	8.7	9.3
Biuret reagent (ml)	4.0	4.0	4.0	4.0	4.0
Absorbance	0.83	0.67	0.51	0.34	0.18
Protein concentration (%)	10	8	6	4	2

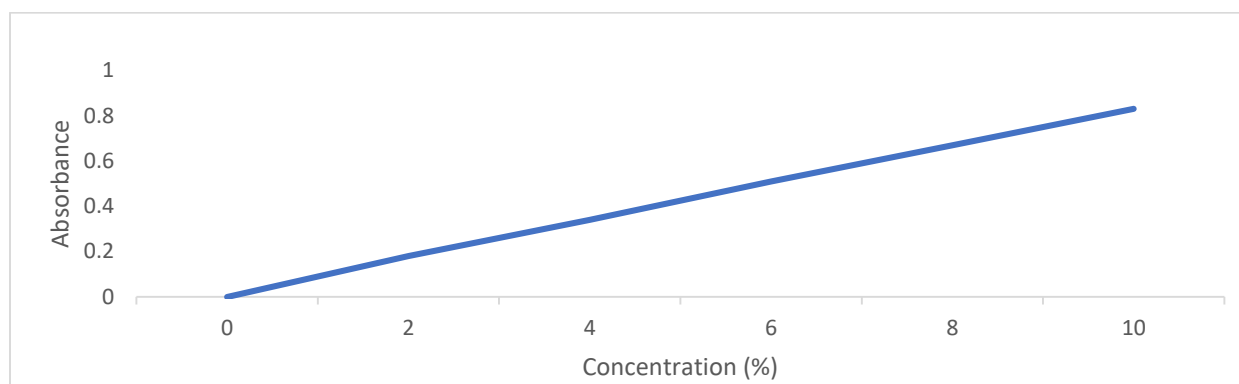


Figure 1: Standard curve graph using bovine serum albumin (30 %)

4.0 Results and discussion

Plant protein are known for decreased mortality risk especially cardiovascular, and cancer mortality among other numerous biological activities including chemopreventive activities. The result of this study showed that the legumes assayed are all an excellent source of protein and increased consumption will significantly improve nutrition of poor third world nations (Langyan et al., 2022; Yanni et al., 2024; Perraud et al., 2022; Rodríguez et al., 2022; Torheim & Fadnes, 2024; Lisciani et al., 2024).

These protein fractions can be classified according to a study as: albumin (water soluble), globulin (saline soluble); prolamin (alcohol soluble) and glutelin (alkaline soluble) (Oluwajuyitan & Aluko, 2025). The composition of the protein fractions in the five legumes studied are summarized in Table 2 & Figure 2. Among the five legumes assayed for protein fractions, *V. geocarpa*, *P. coccineus* were found to be richer in the four protein fractions (albumin, globulin, prolamin and glutelin) followed by *C. cajan* and *V. unguiculate* while *P. multifarioris* was the least.

The results showed that the albumin protein fraction ranked second among other fractions in all the five legumes assayed. In comparing the five legumes, the *V. geocarpa* and *V. unguiculate* were found to be richer in albumin (4.8 and 4.6 %) followed by *P. coccineus* and *P.s multifarioris* (3.9 %) while *C. cajan* had a much smaller proportion of albumin (2.7 %). The percent fraction range of albumin in the total proteins content is 10.3 -19.2 % (Table 2 & Fig. 2). Lu et al. 2022 reported a similar result (Lu et al., 2022).

Table 2: Protein fractions of *V. geocarpa*, *P. coccineus*, *C. cajan*, *P. multifaricus* and *V. unguiculate*

Protein fractions (%)	Samples				
	<i>V. geocarpa</i>	<i>P. coccineus</i>	<i>C. cajan</i>	<i>P. multifaricus</i>	<i>V. unguiculate</i>
Albumin	4.8 ± 0.03	3.9 ± 0.01	2.7 ± 0.04	3.9 ± 0.07	4.6 ± 0.05
Globulin	19.9 ± 0.08	16.4 ± 0.03	24.2 ± 0.02	15.0 ± 0.06	9.2 ± 0.01
Prolamin	0.8 ± 0.02	1.0 ± 0.05	0.3 ± 0.03	0.77 ± 0.07	0.77 ± 0.09
Glutelin	2.2 ± 0.03	2.6 ± 0.06	2.3 ± 0.01	2.2 ± 0.07	2.2 ± 0.04

The results revealed that the globulin is far higher than other fractions (66-71.7 %) of the five legumes appraised. This result agrees with previous studies that globulin is the main storage protein fraction of the leguminous seeds (Sánchez-Chino et al., 2015; Onyango, 2022; Ohanenye et al., 2022; Oluwajuyitan & Aluko, 2024; Sashikala et al., 2015; Koziol et al., 2012). *C. cajan* (24.2 ± 0.02) had a higher concentration of globulin than *V. geocarpa* (19.9 ± 0.08 %), *P. coccineus* (16.4 ± 0.03 %) while *P. multifaricus* had a slightly lower concentration of globulin (15.0 ± 0.06 %). The least globulin fraction was found on *V. unguiculate* (9.2 ± 0.01 %) (Table 2 & Fig. 2).

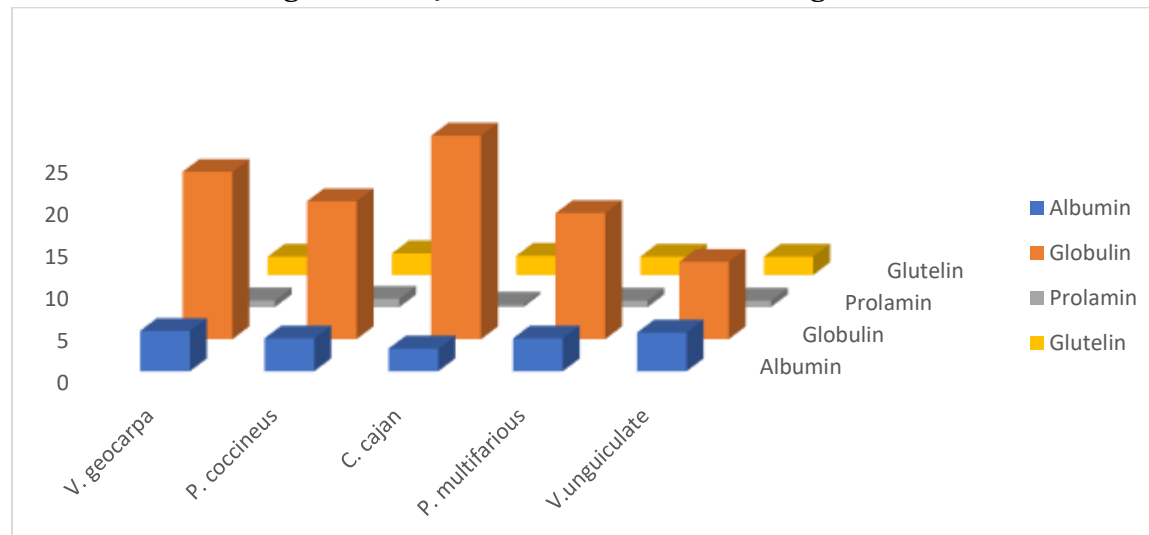


Figure 2: Protein fractions of *V. geocarpa*, *P. coccineus*, *C. cajan*, *P. multifaricus* and *V. unguiculate*. The prolamin content of the five legumes assayed for protein fraction differed from each other, but the results are in agreement with previous studies that prolamin constitute the least of total amount of proteins in legumes. Generally, prolamins fraction is not found in legumes or present in very low proportions. Results of the present study showed that in the five legumes, the concentration of prolamin was found to be the least protein fraction in all the five legumes. The observed low level of prolamin in the five tested legumes (*V. unguiculate* (4.6 ± 0.07%), *P. coccineus* (4.2 ± 0.03 %), *P. multifaricus* (3.5 ± 0.02 %), *V. geocarpa* (2.9 ± 0.08 %) and *C. cajan* (1.0 ± 0.04%) indicates that legumes are not rich in

prolamin protein fraction (Table 2 & Figure 2). This study agrees with some previous studies that prolamins fraction is not found in legumes or present in very low proportions (Carbonaro & Nucara, 2022; Yang et al., 2021; Holding, 2014; Vasconcelos et al., 2010; Akinhanmi et al., 2008; Oliveira et al., 2017; Sánchez-Chino et al., 2015; Rajnincová et al., 2019).

The concentration of glutelin in *V. geocarpa*, *P. coccineus*, *C. cajan*, *P. multifarioris* and *V. unguiculate* ranges from 2.2-2.6 %. It was found out from the studies that *P. coccineus* is richer in glutelin (2.6 %) followed by *C. cajan* (2.3 %) while *V. geocarpa*, *P. multifarioris*, and *V. unguiculate* have the same amount of glutelin (2.2 %). Legumes generally are not rich in glutelin's and cannot be employed in bread baking due to its inability to be elastic, and to trap gas during leavening (Neji et al., 2022; Nam et al., 2025; Rashidah et al., 2007).

The highest protein content (Table 3, Fig. 3) was observed in *C. cajan* (29.5 ± 0.03 %), followed by *V. geocarpa* (27.7 ± 0.01), *P. coccineus* (23.9 ± 0.07 %), *P. multifarioris* (21.9 ± 0.04 %), while *V. unguiculate* (16.8 ± 0.02 %) showed the least content.

Table 3: Percent total amount of proteins of *V. geocarpa*, *P. coccineus*, *C. cajan*, *P. multifarioris* and *V. unguiculate*

Sample	Percent total protein (%)
<i>V. geocarpa</i>	27.7 ± 0.01
<i>P. coccineus</i>	23.9 ± 0.07
<i>C. cajan</i>	29.5 ± 0.03
<i>P. multifarioris</i>	21.9 ± 0.04
<i>V. unguiculate</i>	16.8 ± 0.02

The total protein fraction (%) was calculated using the formula below

$$\text{Total protein fraction (\%)} = \frac{\text{Protein fractions (\%)}}{\text{Percent total protein (\%)}} \times 100 \quad \text{"Eq. 1"}$$

These five legumes that were studied contained a significant amount of protein (16.8-29.5 %) (Table 3 & Figure 3). The rich protein content of these five legumes may be due to the root's nitrogen-fixing bacteria which transforms the impractical nitrogen gas into ammonium ion used in protein syntheses (Maphosa & Jideani, 2017).

C. cajan (29.5 %) undoubtedly ranked first among the legumes assayed. *V. geocarpa* (27.7 ± 0.0 %), *P. coccineus* (23.9 ± 0.07 %), and *P. multifarioris* (21.9 ± 0.04 %) are the second, third, and fourth richer in protein content among the legumes widely consumed in Eastern Nigeria. While *V. unguiculate* (16.8 ± 0.02) has the least protein content.

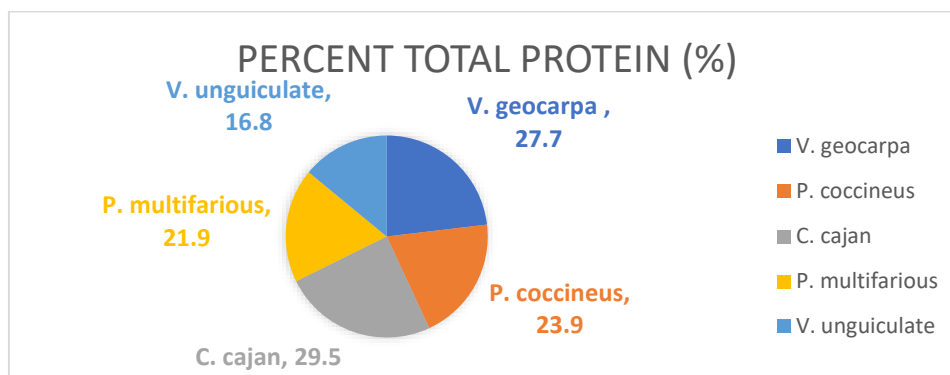


Figure 3: Percent total amount of proteins of V. geocarpa, P. coccineus, C. cajan, P. multifariorus and V. unguiculate

Foods rich in albumin protein fraction are known to contain all the nine essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) and hence can be called complete protein (Lopez & Mohiuddin, 2024). It was found from the studies that V. unguiculate (27.4 %), is richer in albumin followed by P. multifariorus (17.8 %), V. geocarpa (17.3 %), and P. coccineus (16.3 %), while C. cajan (9.2 %) was the least (Table 4 & Figure 4). These legumes screened are food rich in albumin that is vital for regulating body fluids such as blood pressure and serves as a carrier for nutrients and other body chemicals such as vitamins, enzymes and hormones and medications. Adequate intake can enhance repair and build tissue for optimal performance of vital metabolic function and overall well-being (Belinskaia et al., 2021; Spada et al., 2021; Ashraf et al., 2025;). Inadequate albumin called Hypoalbuminemia can be a very big health challenges in heart failure, malnutrition, severe burns, and kidney disease (Gounden et al., 2023; Grodin et al., 2016; Biancucci et al., 2025; Don & Kaysen, 2004).

Table 4: Percent fraction out of total amount of proteins of V. geocarpa, P. coccineus, C. cajan, P. multifariorus and V. unguiculate

Total protein fraction (%)	Samples				
	V. geocarpa	P. coccineus	C. cajan	P. multifariorus	V. unguiculate
Albumin	17.3 ± 0.02	16.3 ± 0.07	9.2 ± 0.01	17.8 ± 0.04	27.4 ± 0.06
Globulin	71.8 ± 0.03	68.6 ± 0.05	82.0 ± 0.02	68.5 ± 0.04	54.8 ± 0.01
Prolamin	2.9 ± 0.08	4.2 ± 0.03	1.0 ± 0.04	3.5 ± 0.02	4.6 ± 0.07
Glutelin	7.9 ± 0.04	10.9 ± 0.06	7.8 ± 0.03	10.1 ± 0.01	13.1 ± 0.05

Globulin mainly are composed of nine essential amino acid (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) Among them, C. cajan (82.0 ± 0.02 %) ranks highest followed by V. geocarpa (71.84 ± 0.03 %), then P. coccineus (68.6 ± 0.05 %) and P. multifariorus (68.5 ± 0.04 %) while V. unguiculate (54.8 ± 0.01%) had the least globulin (Table 4 & Figure 4)..The studies indicated that each of the five tested legumes are rich in globulin and will significantly

promote healthier liver and kidney function, blood clotting, and fight infection (Maphosa & Jideani, 2017; Carbonaro & Nucara, 2022; Çakir et al., 2019; Affrifah et al., 2023).

The observed low level of prolamin in the five tested legumes ranges between 1.0-4.6 % of the total protein content (V. unguiculate (4.6 ± 0.07%), P. coccineus (4.2 ± 0.03 %), P. multifarious (3.5 ± 0.02 %), V. geocarpa (2.9 ± 0.08 %) and C. cajan (1.0 ± 0.04%) (Table 4 & Figure 4) indicates that legumes that are not rich in prolamin protein fraction and are less likely to trigger risks of immunogenic reaction in individuals with celiac disease (Cebolla et al., 2018; Daly et al., 2020; Carbonaro & Nucara, 2022; Rajnincová et al., 2019; Herrera-Quintana et al., 2025; Mlyneková et al., 2014; Taylor, 2014; Balakireva & Zamyatnin, 2016).

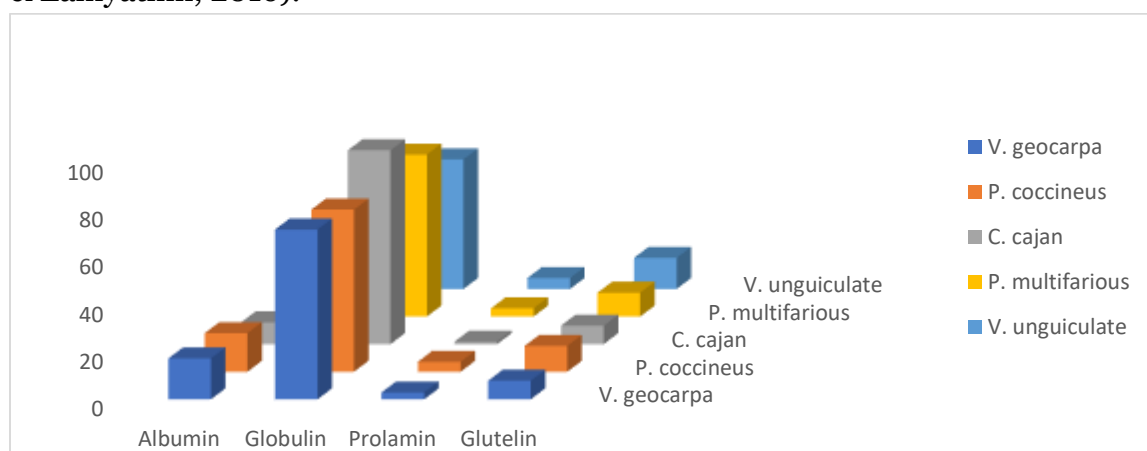


Figure 4: Percent fraction out of total amount of proteins of V. geocarpa, P. coccineus, C. cajan, P. multifarious and V. unguiculate

The concentration of legumes glutelin ranges from 10-20 % (Neji et al., 2022), interestingly in this study (Table 4 & Fig. 4), only V. unguiculate have a high concentration (13.1 %) of glutelin followed closely by P. coccineus (10.9 %) and P. multifarious (10.1 %) with exception of V. geocarpa (7.9 %) and C. cajan (7.8 %) having a low concentration of glutelin. The flour of V. unguiculate, P. coccineus, and P. multifarious can be employed in cake baking due to the high concentration of glutelin (10–15%) that qualifies them (Daniela et al., 2023; Khalid et al., 2023; Mesta-Corral et al., 2024) except for V. geocarpa and Cajanus cajan (7.9 and 7.8 % respectively).

Conclusion

C. cajan was found to have more protein content (29.5 %) and globulin protein fraction (82.0 %) than V. geocarpa with total protein content of 27.7 % and globulin protein fraction of 71.8 %, P. coccineus with total protein content of 23.9 % and globulin protein fraction of 68.6 %, and followed closely by P. multifarious with total protein content of 21.9 % and globulin protein fraction of 68.5 % while V. unguiculate have the least total protein content (16.8 %) and globulin protein fraction of 54.8 %. Albumin as the second richest protein fraction in the legumes screened was found to be highest in V. unguiculate (27.4 %), followed by P. multifarious (17.8 %), V. geocarpa (17.3 %), and P. coccineus (16.3 %), while C. cajan (9.2 %) was the least. The legumes screened were found not to be rich in prolamin protein fraction. Glutelin was found to be significant in V. unguiculate, P. coccineus, and P. multifarious.

To prevent disease and live a healthier life, sufficient amounts of these legumes must be included in our diet. People suffering from malnutrition, liver disease, kidney disease, diabetes, and other organ problems need to increase the consumption of these legume. The floor of *V.unguiculate*, *P.coccineus*, and *P. multifarions* can be employed in cake baking due to the high concentration of glutelin.

Author Contributions

Conception was by Ogbuanu, C.C., & Ike, O.C. Methodology was by Eze, C.N. & Anekwe, C.J. Investigation was by Okwesili, L.C., Akpata, I., & Ajah, N.D. Writing—Original Draft Preparation by Amobi, T.C, Writing—Review and Editing by Ugwoke, C. Funding Acquisition by all authors. All authors have read and agreed to the published version of the manuscript.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper, and it was financed collectively by authors.

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