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Comparative analysis of bioactives and protein characteristics in six genotypes of yam bean tubers

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Yam bean (*Pachyrrhizus* spp.) is a diverse genus of high-moisture tuber crops with useful agricultural properties but limited nutritional and health benefits due to its low nutrient density. Only a few species with limited genotypic variability are produced globally, such as *P. erosus* (jicama), *P. tuberosus* (goiteño), and *P. ahipa* (ahipa). However, there is a deficit of compositional information on indigenous landraces, which could assist in the future selection and breeding of yam bean tubers with improved nutritional and health properties. The present study evaluated the proximate composition, protein characteristics, and some phytochemical properties of two indigenous landraces, one of *P. tuberosus* and the other of *P. erosus*. Individual tuber weight and moisture content of whole tuber were significantly higher ($p \geq 0.05$) in *P. erosus* than in *P. tuberosus* samples. While *P. erosus* showed greater protein digestibility, *P. tuberosus* exhibited superior biochemical properties with respect to total phenolic content, antioxidant capacity, and ascorbic acid levels, with significant variation observed among genotypes and species. HPLC analysis further identified diverse phenolic compounds in the yam bean tuber, including gallic acid, coumaric acid, and ferulic acid. These findings provide detailed compositional data on under-studied indigenous yam bean landraces, supporting their future use in food and nutrition research.

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Sustainability spotlight

This study characterised underutilised indigenous yam bean genotypes in an attempt to support crop diversification and nutritional security in alignment with the UN Sustainable Development Goal 2 (Zero Hunger). Identifying genotypes with higher bioactive compounds and protein quality contributes to improved nutrition and public health outcomes, contributing to SDG 3 (Good Health and Well-being). The findings from the study could support the development of value-added functional foods, promoting sustainable agro-industrial innovation (SDG 9: Industry, Innovation and Infrastructure).

1. Introduction

Yam bean (*Pachyrrhizus* spp.) is a neotropical legume genus with edible tuberous roots native to Central and South America. It is now also grown widely as a commercial crop in Asia and parts of Africa.[1] The genus consists of five species, *P. tuberosus*, *P. ahipa*, *P. erosus*, *P. ferrugineus*, and *P. panamensis*, of which the first three species are cultivated to obtain edible tuberous roots, while the remaining two have only been reported to be found in the wild.[2] Yam bean has attracted significant attention because of the high tuber-yielding capacity of the genus,[3] which makes this crop economical to develop as a staple food. Additionally, it has a mildly sweet taste that is appealing to consumers.[4] Its nitrogen fixation ability, which minimises nitrogen fertiliser use, makes it sustainable for use as an annual crop and in crop rotation.[5] In addition, the ability of the yam bean plant to grow in the tropics and dry areas with poor soil conditions makes its breeding, introduction and promotion worthwhile considering as an economical field crop.

Although yam bean has been studied for its agronomic performance, it remains relatively underexplored compared to more established root crops, such as potato, cassava, and sweet potato. When comparing the consumption, utilisation, and research emphasis on the two economic products of yam bean, the tuber and the seed, the former has received significantly less attention.[6] Previous reports indicate that the tuber is high in moisture and thus not as nutrient-dense as sweet potato and potato. Upon drying, however, the resulting yam bean tuber flour contains useful levels of carbohydrates, protein, including

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essential amino acids, dietary fibre, minerals, and vitamins.[7] Importantly, yam bean tuber flour has been highlighted for its low fat content, which reduces susceptibility to lipid oxidation and rancidity. Buckman *et al.*[8] reported that this feature makes the flour advantageous as a substitute in health-oriented foods, particularly for overweight individuals. In addition, the high protein content in the flour also makes it suitable for weaning foods for children. The yam bean tuber has thus been classified as a "healthy" food with ongoing potential as a raw material for large-scale food production.[2] Recent studies have also demonstrated substantial variability in elemental composition across yam bean genotypes.[9] Upon investigating thirty genotypes of yam bean tubers in eastern India, a significant difference occurred in major nutrients such as potassium, calcium, manganese, and zinc, as well as several trace elements, with some lines identified as particularly nutrient-rich. These findings highlight the influence of genotype and environment on yam bean composition, thus reinforcing the importance of characterising its nutritional and functional potential in greater depth.

Despite these promising studies, a comprehensive evaluation of the protein characteristics, bioactive compounds, and genotype-specific variations in yam bean tubers remains scarce. Addressing this gap, the present work investigates the proximate composition, protein characteristics, phenolic content, and antioxidant activity of selected genotypes of *P. erosus* and *P. tuberosus*.

This research may identify variations in these compositional attributes that could be considered in breeding future commercial varieties with improved nutritional and health properties.

2. Materials and methods

2.1. Raw materials

Six different genotypes of yam bean tuber species *P. tuberosus* and *P. erosus*, originating from Peru, Guatemala, Costa Rica and Mexico, were grown in experimental fields of the University of Western Australia located in Perth, Western Australia during February and August 2019. For this, seeds from different yam bean genotypes (Table 1) were soaked in warm water (30 °C) for 2 h. The seeds were then placed in a Petri-dish on sterilised filter paper, covered with moist filter paper, and incubated at room temperature until the radicle appeared. All seeds germinated within 3–5 days, and the germinated seeds were carefully planted in 5 L pots filled with steam-sterilised potting soil. The pots were then placed in a phytotron, where the temperature was maintained between 18 and 22 °C, and were watered daily by hand. Four weeks after sowing, the young plants were moved to Greenhouse A3. Twelve pots were placed on each bench, and each pot was provided with a dripper for irrigation. The plants were watered twice daily for 30 s. A total of 125 mL of fertiliser with microminerals was provided by hand every two weeks. Also, each plant was provided with a wire cage trellis to allow the runners to grow up against it (Fig. 1).

Fig. 1 Yam bean plants at 7 weeks after planting in experimental fields of the University of Western Australia, Perth.

Plants were monitored daily and treated preventively for trips and aphids. All plants were harvested on the 23rd week upon reaching the size of a large apple. Harvested tubers (Fig. 2), free from mechanical damage or decay, were washed after removing extra roots and dried at room temperature overnight before analysis.

2.2. Weight and colour measurement

Each tuber was weighed using calibrated scales, and the colour was measured using a colorimeter (BYK-Gardner) with a D65 illuminator and a 10° observer. The obtained values of L^*, a^*, and b^* were used to calculate the chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and the hue value (artg (b^*/a^*)) as indicated by Moreno *et al.*[10]

2.3. Experimental design and sample preparation

Three biological replicates of six accessions were chosen for the analysis, and three analytical replicates were conducted for each

Table 1 Origin and accession number of yam bean cultivars and varieties planted at the University of Western Australia, Perth

No.	Species	Accession	Collected from	Origin
13	*P. tuberosus*	CIP 209013 (A13)	CIP, Peru	Peru
14	*P. tuberosus*	CIP 209014 (A14)	CIP, Peru	Peru
15	*P. tuberosus*	CIP 209015 (A15)	CIP, Peru	Peru
16	*P. erosus*	CIP 209016 (A16)	CIP, Peru	Guatemala
46	*P. erosus*	CIP 209046 (A46)	CIP, Peru	Cartago Costa Rica
51	*P. erosus*	CIP 209051 (A51)	CIP, Peru	Mexico

Fig. 2 Harvested yam bean tubers belonging to the species *P. tuberosus* at accessions (a) CIP 209013, (b) CIP 209014, and (c) CIP 209015 and *P. erosus* at accessions (d) CIP 209016, (e) CIP 209046, and (f) CIP 209051.

specific analysis. Tuber pieces weighing 50 g were freeze-dried at ChemCenter, Perth, Western Australia. The samples were kept at −18 °C until further analysis. The moisture content of freeze-dried samples was determined according to the hot-air oven method using a representative 1 g sample.[11]

2.4. Total protein content and *in vitro* protein digestibility

The total protein content was determined using the Kjeldahl method following AOAC.[11] Briefly, 1 g of the sample was digested and then neutralised to release ammonia, which was distilled and captured in a boric acid solution. The nitrogen content was then quantified by titrating against 0.1 N HCl, and the protein content was calculated by applying a conversion factor. The results were expressed on a percentage dry basis. The *in vitro* protein digestibility (IVPD) of the ground freeze-dried yam bean samples was determined following a pepsin digestion method as per Licata *et al.*[12] and Villarino *et al.*[13] and using wheat flour as the internal standard (IS). A sample weighing 250 mg was taken into 50 mL centrifuge tubes and mixed with 0.75 mg of pepsin (1 : 2500 units per mg activity; Chem-Supply, Gillman, SA-5013) and 7.5 mL 0.1 N HCl. The mixture was then incubated at 37 °C for 3 h in a shaking water bath with 126 rpm. Post incubation, the solution was neutralised using 0.2 N NaOH. The undigested protein was then precipitated by adding 25 mL 10% TCA and centrifuging at 1000*g* for 30 min. The digested protein in the supernatant was determined using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, USA). Different concentrations of bovine serum albumin (catalog number P0914) were used as the protein standard. The working reagent was prepared using Reagent A (bicinchoninic acid solution) and Reagent B (copper(II) sulfate pentahydrate 4% solution) in a 50 : 1 ratio. For the assay, a 200 μL working reagent was mixed with a 25 μL sample and incubated at 37 °C for 30 min, followed by measuring absorbance at 562 nm. The digestibility was calculated as the protein hydrolysed relative to the total protein in the sample using the following equation.

$$\text{IVPD}(\%) = \frac{\text{protein in supernatant (mg)}}{\text{total protein in sample (mg)}} \times 100$$

2.5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out under both reducing and non-reducing conditions following Wong *et al.*[14] using NuPAGE 12% bis-tris gels (Invitrogen, Carlsbad, CA, USA). Concisely, 85 mg of samples were prepared in NuPAGE running buffer (Invitrogen, Carlsbad, CA, USA). Under reducing conditions, 0.5 mL of mercaptoethanol was used. Electrophoresis was performed in NuPAGE MES SDS running buffer at 200 V until the electrophoretic front reached approximately 1 cm from the bottom of the gel. The gel was carefully dismounted by separating the two plates of the cassette and pushing it down into the staining solution containing Coomassie blue stain (Bio-Rad Laboratories, California, USA). Destaining was performed by soaking the gel in deionised water three times, and the gel was then compared to the Novex Sharp pre-stained protein standard for determining the molecular weight of protein bands.

2.6. Extraction of bioactives

Free phenolic compounds were extracted following the method described by Tomsone *et al.*[15] with slight modifications. Briefly, 5 g of finely diced fresh tuber sample was homogenised (Ultra-Turrax) with 20 mL of 80% (v/v) ethanol for 1 min followed by centrifugation (Eppendorf Centrifuge 5810 R) at 3000*g* for 15 min and 4 °C. The supernatant was then filtered using Whatman filter paper No. 541, and the collected filtrate was stored at −18 °C until further analysis.

2.6.1. Total phenolic content. The total phenolic content was measured by the Folin–Ciocalteu method using gallic acid as the standard (0–360 mg L^{-1}), and the results were expressed as mg gallic acid equivalent per 100 g dry matter and g of gallic acid equivalent per kg of fresh tuber.[16] For this, 10 μL aliquot of the extract was mixed with 10 μL of Folin–Ciocalteu Reagent in a 96-well clear flat bottom microplate, followed by 5 min incubation at room temperature. Later, 80 μL of 7% sodium carbonate and 100 μL of distilled water were added to the mixture. The plate was then covered with aluminium foil and shaken at 150 rpm for 2 min using a microplate shaker, followed by incubation for 1 h. The absorbance was then measured at 750 nm using a multi-plate reader (BioTek Synergy HT).

2.6.2. Phenolic profile analysis. The phenolic profile of tuber extract of Accession 14 was analysed using a high-performance liquid chromatography (HPLC) system attached to a diode array detector (DAD) (Agilent Technologies, Polo Alto, CA, USA) modified from the method described by Wu *et al.*[17] Accession 14 was selected for this analysis due to its darker tuber colour, which is generally associated with higher phenolic content, making it a suitable representative sample for preliminary identification of major bioactive compounds. A solvent system containing 0.1% formic acid in LC-MS grade water was used as Solvent A, and LC-MS grade acetonitrile was used as Solvent B to generate a linear gradient. The system was run at a flow rate of 0.5 mL min^{-1} using different concentrations of acetonitrile and flow time as follows: 0–15% for 10 min, 15–50% for 40 min, 50–70% for 2 min, 70–100% for 1 min, 100% for 5 min, 100–0% for 1 min and 0% for 6 min. Phenolic compounds in the samples were identified and quantified using the external standard method under the specified HPLC-DAD conditions and expressed as μg/100 g of sample. The different standards used were gallic acid, caffeic acid, *p*-coumaric acid, protocatechuic acid, ferulic acid, luteolin, apigenin, and catechin.

2.7. Antioxidant capacity

2.7.1. DPPH radical scavenging assay. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was carried out using the method of Fukumoto and Mazza[18] and Mohdaly *et al.*[19] with some modifications. Briefly, 20 μL aliquots of the extract were mixed with 20 μL of distilled water in a 96-well plate flat-bottom microplate on ice. Later, 200 μL of methanolic DPPH radical solution (118.3 mg L^{-1}) was added to each well and mixed on a microplate shaker (2 min and 150 rpm). The absorbance was measured at 515 nm after a 30-minute incubation in the dark on ice using a multi-plate reader. A control without extract and using 20 μL methanol was also analysed in each plate. The DPPH radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right] \times 100$$

where A is the absorbance at 515 nm.

2.7.2. ORAC assay. The oxygen radical absorbance capacity (ORAC) assay was performed in a 96-well black-walled, clear-bottom polypropylene microplate as described by Huang *et al.*[20] Briefly, 25 μL of the extract, diluted 50 times, was mixed with 150 μL of fluorescein (0.084 μmol L^{-1}) and incubated at 37 °C for 30 min. This was followed by adding 25 μL of 153 mmol L^{-1} 2,2-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and shaking for 10 s to initiate the reaction. The fluorescence intensity at 37 °C was monitored kinetically at an excitation wavelength of 485 nm and an emission wavelength of 528 nm every minute for a total of 120 min using a multi-detection microplate reader (Synergy HT, BioTek Instruments, Inc., Winooski, VT). The areas under the curve (AUC) were calculated for both the blank (control) and sample readings from a fluorescence–reaction time graph, and the antioxidant activity was determined using Trolox as a standard (0–50 μmol L^{-1}) and expressed as milligrams of Trolox equivalent (mg TE)/100 g.

2.8. Ascorbic acid content

Ascorbic acid content was analysed using the commercially available Megazyme kit (Megazyme Inc., Bray, Ireland). Precisely, 1 g sample was homogenised in 3% (w/v) metaphosphoric acid and 10 mM EDTA, followed by filtration using Whatman filter paper No. 1. In a cuvette, 0.1 mL sample was mixed with 1.52 mL of distilled water (37 °C) and 0.5 mL buffer solution. For the blank, 1.5 mL distilled water was used, and 0.02 ascorbic acid

oxidase was added along with 0.1 mL sample. The mixture was then incubated for 3 min at 37 °C, mixing the content every 1 min for 5 seconds. Later, 0.2 mL MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] buffer was added, and incubation was continued for 3 min at 37 °C. The absorbance of the sample (A1) and the blank was recorded at 578 nm, followed by the addition of 0.2 mL PMS (5-methylphenazinium methyl sulfate). The sample absorbance was again recorded at 578 nm (A2), and the concentration and content of L-ascorbic acid were calculated using the following formula.

$$\text{Concentration of L-ascorbic acid } (C, \text{ g L}^{-1}) = \frac{V \times \text{MW} \times (A_2 - A_1)}{\varepsilon \times d \times v}$$

where V = final volume (2.52 mL), MW = molecular weight of L-ascorbic acid (176.13 g mol^{-1}), ε = extinction coefficient of MTT-formazan at 578 nm = 16 900 L mol^{-1} cm^{-1}, d = light path (1 cm), and v = sample volume (0.1 mL)

$$\text{Content of L-ascorbic acid (g/100g)} = \frac{C_{\text{L-ascorbic acid}} \text{ (g/L sample solution)}}{\text{sample weight(g/L sample solution)}} \times 100$$

2.9. Statistical analysis

Statistical analysis was conducted using IMB SPSS statistics 26 (IBM Corp., Armonk, New York). One-way analysis of variance (ANOVA) was carried out to determine the significant difference in traits among the genotypes for all the responses studied. The Tukey test was used as a post hoc test to compare the mean value differences. A *T*-test was carried out to determine the significant difference between the two species, *P. tuberosus* and *P. erosus*, and the statistical differences were tested at $p < 0.05$. Additionally, a correlation analysis of the different responses was performed using R Studio.

3. Results and discussion

3.1. Tuber characteristics and colour analysis

A summary of the differences in tuber characteristics and colour of six yam bean tuber genotypes is presented in Table 2. The average weight of the yam bean tubers varied significantly among the six genotypes, ranging from 241 g to 537 g, the lowest for A13 (*P. tuberosus*) and the highest for A46 (*P. erosus*). No significant differences were observed among the *P. tuberosus* genotypes among A13, A14, and A15. However, in *P. erosus,* A16 displayed an intermediate weight, showing significant differences from A46 and A13. This trend highlights the potential of *P. erosus* genotypes for greater tuber biomass production than *P. tuberosus* genotypes. Moisture content exhibited significant variation across genotypes, ranging from 60.66% in A14 (*P. tuberosus*) to 85.98% in A46 (*P. erosus*). Besides, there was a higher variation in the moisture content of *P. tuberosus* (CV 6.19%) compared to that of *P. erosus* (CV 2.67%). The *P. erosus* genotypes consistently displayed higher moisture content than the *P. tuberosus* genotypes, which also explains their higher

Table 2 Changes in weight, moisture, and colour characteristics of the yam bean tuber genotypes[a]

	P. tuberosus			*P. erosus*		
Species	Accesion13	Accesion14	Accesion15	Accesion16	Accesion46	Accesion51
Origin	Peru	Peru	Peru	Guatemala	Cartago Costa Rica	Mexico
Weight	241 ± 60.83^{a}	312 ± 78.59^{a}	294 ± 32.42^{a}	375 ± 64.87^{ab}	537 ± 16.23^{b}	416 ± 144.22^{ab}
Moisture	67.15 ± 2.52^{b}	60.66 ± 1.24^{a}	67.58 ± 2.37^{b}	84.94 ± 0.79^{c}	85.98 ± 2.58^{c}	85.63 ± 6.33^{c}
Whole tuber						
L^*	48.59 ± 5.05^{b}	44.70 ± 4.09^{ab}	41.91 ± 2.06^{a}	69.31 ± 3.22^{c}	66.74 ± 5.52^{c}	67.65 ± 3.71^{c}
a^*	8.28 ± 1.34^{d}	1.74 ± 2.45^{a}	1.50 ± 0.91^{a}	5.30 ± 0.92^{bc}	6.12 ± 1.05^{c}	4.42 ± 1.35^{b}
b^*	12.93 ± 2.87^{b}	7.46 ± 3.06^{a}	8.44 ± 2.65^{a}	26.99 ± 1.16^{c}	26.70 ± 1.50^{c}	26.55 ± 2.19^{c}
Chroma	15.44 ± 2.61^{b}	7.69 ± 3.69^{a}	8.58 ± 2.77^{a}	27.51 ± 1.23^{c}	27.41 ± 1.48^{c}	26.94 ± 2.19^{c}
Hue angle	56.83 ± 6.62^{a}	78.18 ± 8.30^{b}	80.45 ± 2.72^{b}	78.91 ± 1.70^{b}	77.07 ± 2.23^{b}	80.49 ± 2.81^{b}
Tuber flesh						
L^*	84.09 ± 0.78^{a}	83.23 ± 1.91^{a}	79.27 ± 19.60^{a}	84.04 ± 2.36^{a}	83.77 ± 1.72^{a}	82.28 ± 4.46^{a}
a^*	4.89 ± 1.14^{c}	0.37 ± 1.51^{a}	0.52 ± 0.35^{a}	−0.58 ± 0.33^{b}	−0.87 ± 0.23^{b}	−1.00 ± 0.21^{b}
b^*	30.40 ± 3.04^{c}	10.20 ± 6.05^{a}	11.31 ± 1.23^{ab}	11.91 ± 1.11^{b}	12.29 ± 0.79^{b}	12.36 ± 1.02^{b}
Chroma	30.80 ± 3.18^{c}	10.21 ± 6.19^{a}	11.33 ± 1.22 ^{ab}	11.93 ± 1.11^{ab}	12.33 ± 0.78^{b}	12.40 ± 1.02^{b}
Hue angle	80.97 ± 1.31^{b}	87.91 ± 2.44^{b}	87.20 ± 2.19^{b}	−75.17 ± 45.68^{a}	−85.89 ± 1.27^{a}	−85.35 ± 0.90^{a}
Freeze-dried tuber						
L^*	90.14 ± 2.98^{c}	86.38 ± 1.84^{ab}	88.42 ± 3.42^{abc}	87.23 ± 4.49^{abc}	88.68 ± 1.88^{bc}	85.43 ± 3.97^{a}
a^*	2.09 ± 0.55^{a}	0.71 ± 0.21^{b}	0.42 ± 0.29^{b}	−0.76 ± 0.54^{c}	−1.00 ± 0.54^{c}	−1.11 ± 0.68^{c}
b^*	12.53 ± 1.23^{c}	3.31 ± 1.20^{a}	5.22 ± 1.19^{b}	6.33 ± 1.15^{b}	11.20 ± 1.82^{c}	11.19 ± 2.19^{c}
Chroma	12.72 ± 1.21^{d}	3.40 ± 1.18^{a}	5.25 ± 1.15^{b}	6.70 ± 1.11^{b}	11.25 ± 2.19^{c}	11.27 ± 11.27^{cd}
Hue angle	80.43 ± 2.73^{bc}	76.48 ± 6.65^{b}	84.58 ± 5.20^{c}	−83.01 ± 5.42^{a}	−84.83 ± 3.06^{a}	−84.35 ± 3.45^{a}

[a] Different superscripts along the rows indicate significant differences in the quality parameter studies among the genotypes ($p < 0.05$).

biomass weight. Moisture content values were ranked in decreasing order: Accession46 ≈ Accession51 ≈ Accession16 > Accession15 ≈ Accession13 > Accession14. This variation also suggests potential differences in water-binding capacity and structural composition between species, which could influence their post-harvest processing and storage characteristics.[21]

Upon virtual examination, the whole tuber displayed yellowish-white, red, and purple colours, whereas the flesh colours were white, yellow and white with a purple tinge. The tuber samples were evaluated for their colour characteristics in terms of the following parameters: lightness (L^*), redness/greenness (a^*), blueness/yellowness (b^*), chroma, and hue. A higher chroma value indicates more vivid and saturated colours, reflecting greater colour intensity. On the other hand, higher hue angles correspond to a shift toward yellow or green tones, signifying lighter or more neutral colouration.[22] For the whole tuber, lower L^*, chroma, and hue values obtained for the *P. tuberosus* genotypes indicate darker and less saturated surface colours compared to *P. erosus*, which were consistently lighter and more vibrant, especially in yellow pigmentation. However, both species displayed a lighter colour in the tuber flesh, with *P. erosus* genotypes exhibiting a more neutral tone with less red pigmentation, whereas *P. tuberosus* retained noticeable yellow and red hues. Freeze-dried tubers exhibited reduced differences in colour parameters compared to fresh forms, with all genotypes retaining high lightness and subdued chromaticity. However, *P. erosus* genotypes maintained a slightly brighter and yellowish appearance than *P. tuberosus*, suggesting better pigment stability during drying. These findings indicate that the yam cultivars tested in this study cover various colour and tuber characteristics.

3.2. Total protein and *in vitro* protein digestibility

The protein content and *in vitro* protein digestibility (IVPD) of the six yam bean genotypes are presented in Fig. 3. The total protein content showed significant differences ($p < 0.05$) among the genotypes. Similar to moisture content, considerable variation in total protein content was observed in *P. tuberosus* (CV 17.99%) compared to *P. erosus* (CV 8.45%). For *P. tuberosus*, A15 exhibited the highest protein content (9.92 ± 0.64%, db), while A14 had the lowest (6.87 ± 0.96%, db). However, among *P. erosus* genotypes, no significant differences ($P > 0.05$) were observed, but the content was relatively higher, with A51 achieving the highest value (9.09 ± 1.08%, db). These results highlight the variability in protein content between genotypes within the same species and across species. Higher protein levels in *P. erosus* genotypes could be associated with their adaptation to different agro-climatic conditions, as previously reported in tropical root and tuber crops.[23] Such variations might also result from genetic differences and biosynthetic pathways influencing nitrogen assimilation and protein deposition in tubers.[5] Zhang *et al.*,[24] in their study of changes in nutrients and medicinal composition of Chinese yam (*Dioscorea opposita*) tubers during storage, obtained protein content ranging from 13.02 to 15.13% under ambient temperature conditions. However, Forsyth and Shewry[3] reported a total protein content of 2.7% on a dry basis and 0.35% on an as-is basis in *Catalina* variety of *P. erosus*. These values are significantly lower than the total protein content obtained in other genotypes of *P. erosus* analysed in the present study. In a different study, Nursandi *et al.*[25] reported the effect of time of harvesting on the nutritional content of yam bean and found a higher protein content for the tuber harvested at the 16th week (6.51%) compared to the 22nd week (4.79%). Notably, the contents were lower compared to the results obtained in the present study. Similarly, Buckman *et al.*[8] reported a comparatively lower protein content in *P. erosus*, contrasting with the higher values obtained in the current analysis. These variations could be attributed to differences in genotype, environmental conditions, location, soil fertility, and cultivation practices.[25,26]

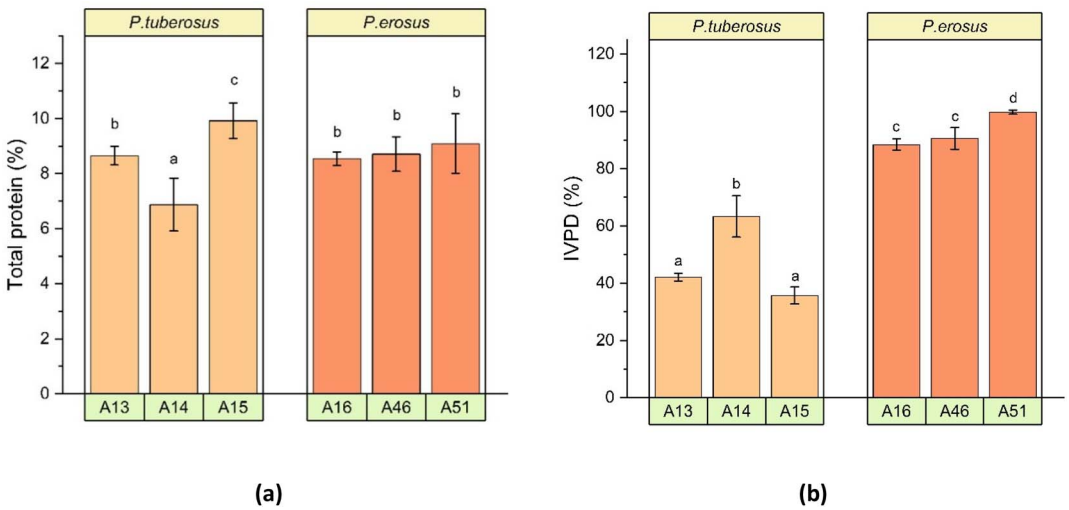

Fig. 3 Change in (a) total protein content and (b) *in vitro* protein digestibility of different yam bean tuber genotypes. Values represent mean ± standard deviation of three analytical replicates and three biological replicates of each genotype. Different lowercase letters indicate significant differences ($p < 0.05$).

Heider *et al.*[27] obtained minimum and maximum protein contents of 4.7% and 8%, respectively, for *P. tuberosus* when eight different accessions were studied. Compared with other tuber species, Leonel *et al.*[28] found that the protein content of five potato tubers ranged between 1.45 and 2.35 g/100 g, with lower protein levels obtained in soil with high phosphorus availability. In a different study, Choi *et al.*[29] obtained a higher crude protein content for whole potato samples, ranging from 7.56 to 10.33 g/100 g dry weight (DW). However, for sweet potato, a crude protein content ranging from 1.2–3.3% on a dry basis was reported by Senanayake *et al.*[30] in 5 different varieties from Sri Lanka.

The *in vitro* protein digestibility (IVPD) also varied significantly among genotypes ($p < 0.05$). Among *P. tuberosus* genotypes, A14 showed the highest digestibility (63.26%), while A15 had the lowest value (35.74%). Conversely, all *P. erosus* genotypes exhibited superior digestibility, with A51 achieving the highest IVPD (99.77%). This marked difference in digestibility between species highlights *P. erosus*'s potential as a highly digestible protein source. The high digestibility observed in *P. erosus* genotypes could potentially be associated with lower levels of anti-nutritional factors such as tannins and phytates, which are known to interfere with protein digestibility; however, no references are available to substantiate this in regard to yam bean tuber, and as this was not measured in the current study, this should be considered a hypothesis for future investigation.

Interestingly, Ekwere *et al.*,[31] in their attempt to develop an infant food formulation from African yam bean (AYB) (*Sphenostylis stenocarpa*) seeds and groundnuts, obtained a negative correlation between the anti-nutrient content and IVPD. Anti-nutrients are chemical substances in foods that cause adverse physiological responses by decreasing the overall nutritional quality and impairing protein digestibility and mineral availability.[32] In a recent study to develop protein concentrate from yam tubers (*Dioscorea cayennensis*), do Nascimento *et al.*[33] performed simulated gastrointestinal digestion free amino acid analysis to evaluate the digestibility. They observed that the proteins were fragmented into low molecular weight molecules, suggesting the gastrointestinal enzymes easily digest yam proteins. Additionally, a considerable increase in essential amino acid concentration was observed at the end of digestion, which indicates their *in vitro* bioaccessibility. Therefore, an increased IVPD in *P. erosus* could be attributed to the protein structure, making it much more susceptible to enzymatic hydrolysis when compared with *P. tuberosus*. Hence, it indicates that the higher digestibility of *P. erosus* may be explained by its lower molecular weight protein, and the lower digestibility of *P. tuberosus* might be due to the higher molecular weight of protein components. Despite the moderate protein content, the relatively low IVPD of *P. tuberosus* genotypes underscores the need for processing interventions such as fermentation or heat treatment to mitigate anti-nutritional factors and improve digestibility.[34] However, no study has been conducted on the digestibility properties of Yam bean protein, yet it is difficult to compare it with existing literature. However, IVPD values obtained for other tuber proteins are 73–90% for potato,[35] 46.71% for cassava,[36] 45.1 ± 0.7% for Chinese sweet potato,[37] and 4.31–6.74% for wild yam (*Dioscorea* spp.).[38]

3.3. Electrophoretic profile

Fig. 4 and 5 show the protein components of different *P. tuberosus* and *P. erosus* varieties under reducing (with mercaptoethanol) and non-reducing (without mercaptoethanol) conditions, respectively. Twelve protein components were detected in *P. tuberosus* under reducing and non-reducing conditions. Molecular weight values detected under both conditions were similar, except for the 61.2 kDa value (Table 3) observed under non-reducing conditions. On the other hand, in *P. erosus*, fewer bands were detected under the non-reducing condition (9 bands) compared to the reducing condition (12 bands). In addition, only six molecular weights detected under reducing and non-reducing conditions were close to each other. However, when comparing the two species, the molecular weights of protein components were found to be identical, except for 68 kDa detected in *P. erosus*. Although yam bean accessions did not show any difference in the electrophoretic

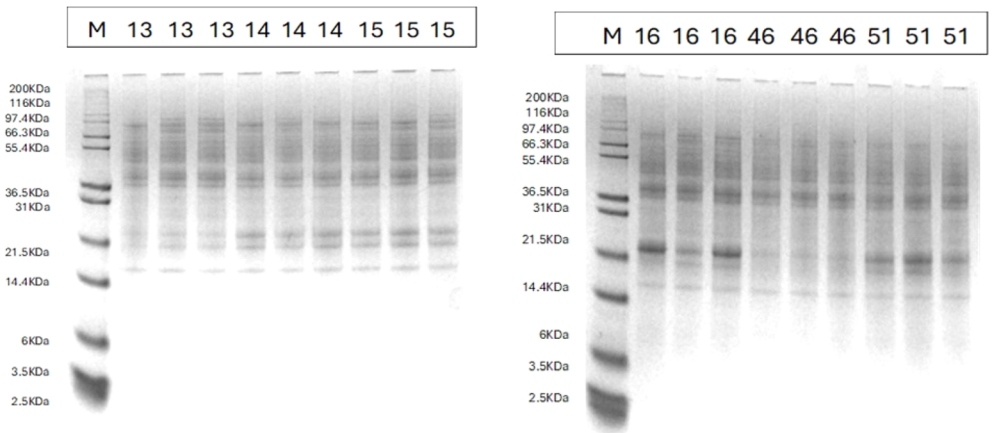

Fig. 4 Electrophoretic profile of *P. tuberosus* and *P. erosus* species under non-reducing conditions. Samples: (M) protein marker, (13, 14, and 15) accessions of *P. tuberosus*, and (16, 46, and 41) accessions of *P. erosus*.

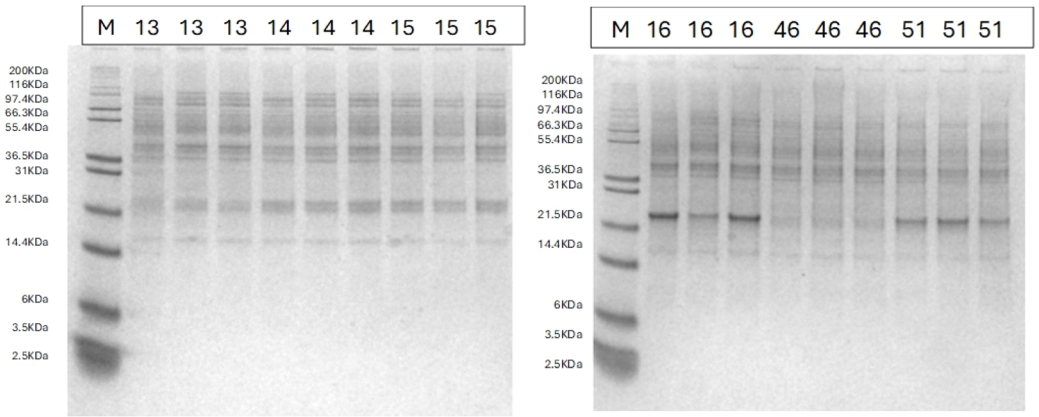

Fig. 5 Electrophoretic profile of *P. tuberosus* and *P. erosus* species under reducing conditions. Samples: (M) protein marker, (13, 14, and 15) accessions of *P. tuberosus*, and (16, 46, and 41) accessions of *P. erosus*.

pattern for *P. tuberosus*, accessions 16 and 51 (*P. erosus*) showed intense bands of 21.0 kDa and 20.4 kDa under both reducing and non-reducing conditions, respectively. These results suggest the plausible presence of disulphide-linked high molecular-weight protein aggregates cleaved into smaller bands.[39] Interestingly, the protein components of *P. erosus* under non-reducing conditions had more mobility than those under reducing conditions (Table 3). This could be again due to an intermolecular disulphide bond in the protein of *P. erosus.*[40] A similar trend was observed by Arogundade *et al.*[41] when they studied the stabilisation properties of ultrafiltered AYB (*Sphenostylis stenocarpa*) protein isolate made from seeds.

In contrast, no such trend was observed in *P. tuberosus*. Forsyth and Shewry[3] reported five major bands with molecular weights of 44 kDa, 30 kDa, 27 kDa, 22 kDa, and 17 kDa in *Pachyrhizus ahipa*, similar to what was observed in the present study for *P. tuberosus* and *P. erosus.* Zhang *et al.*[42] reported dioscorin as the storage protein in yams, accounting for over 80% of water-soluble proteins in many yam species, and its protein band had a molecular weight of 31 kDa.[43] To compare with other tubers, the major proteins present in potato tubers (*Solanum tuberosus*) are patatin, with a molecular weight between 40 and 42 kDa and protease inhibitors, with a molecular weight of 20–23 kDa.[44] Like *P. erosus*, mercaptoethanol did not alter the major proteins in potatoes, indicating the absence of intermolecular disulphide bonds.[45]

Table 3 Molecular weights of major components of protein present in *P. tuberosus* and *P. erosus*

P. tuberosus		*P. erosus*	
Reducing	Non-reducing	Reducing	Non-reducing
99.8 kDa	105.8 kDa	93 kDa	84.9 kDa
90.9 kDa	88.7 kDa	85.5 kDa	77.2 kDa
82.5 kDa	80.6 kDa	81.9 kDa	45.0 kDa
49.9 kDa	61.2 kDa	68.1 kDa	42.0 kDa
47.6 kDa	49.1 kDa	58.8 kDa	38.2 kDa
44.9 kDa	45.1 kDa	47.1 kDa	31.0 kDa
40.0 kDa	39.1 kDa	43.9 kDa	20.4 kDa
39.8 kDa	38.4 kDa	40.1 kDa	18.8 kDa
34.5 kDa	35.9 kDa	38.9 kDa	16.2 kDa
20.9 kDa	20.8 kDa	37.0 kDa	
20.1 kDa	19.1 kDa	21.0 kDa	
15.8 kDa	16.1 kDa	16.3 kDa	
15.8 kDa	16.1 kDa	16.3 kDa	

3.4. Total phenolic content

Phenolic compounds play a crucial role in plant defence mechanisms and human health due to their potent antioxidant properties, which are associated with the prevention of oxidative stress-related diseases such as diabetes, cancer, and cardiovascular disorders.[46] There exists significant variation in the total phenolic content (TPC) among the analysed genotypes, and the *P. tuberosus* genotypes (A13, A14, and A15) showed remarkably higher TPC values compared to the *P. erosus* genotypes (A16, A46, and A51), as illustrated in Fig. 6(a). Specifically, the TPC of *P. tuberosus* ranged from 50.23 to 55.94 mg/100 g, with the highest value recorded for A14. On the other hand, the TPC values of *P. erosus* genotypes were significantly low and ranged from 30.2 to 35.63 mg/100 g, with the highest value for A46 and the lowest for A51. Values for total phenolic content are ranked in decreasing order: accession 14 > accession 13 ≈ accession 15 > accession 46 ≈ accession 16 > accession 51. The higher phenolic content observed in *P. tuberosus* genotypes suggests that they would be more appropriate for use in functional foods or nutraceuticals intended to enhance human health by means of dietary antioxidants.[47]

Previous studies have also documented significant variability in phenolic composition among different yam bean species, emphasising the role of genotype and environmental interactions. A good number of literature studies exist on the phenolic composition of yam bean seed extracts;[48,49] however, limited information is available on that of the tuber. Baiyeri and Samuel-Baiyeri[50] studied the bioavailability of micronutrients and the anti-nutrient composition of AYB tubers from three accessions, obtaining a total phenolic content ranging from 61.05 to 92.03 mg/100 g. They also observed a significant proportion of anti-nutrients, with oxalates and phytates being the major ones. In a different study, Konyeme *et al.*[51] studied the

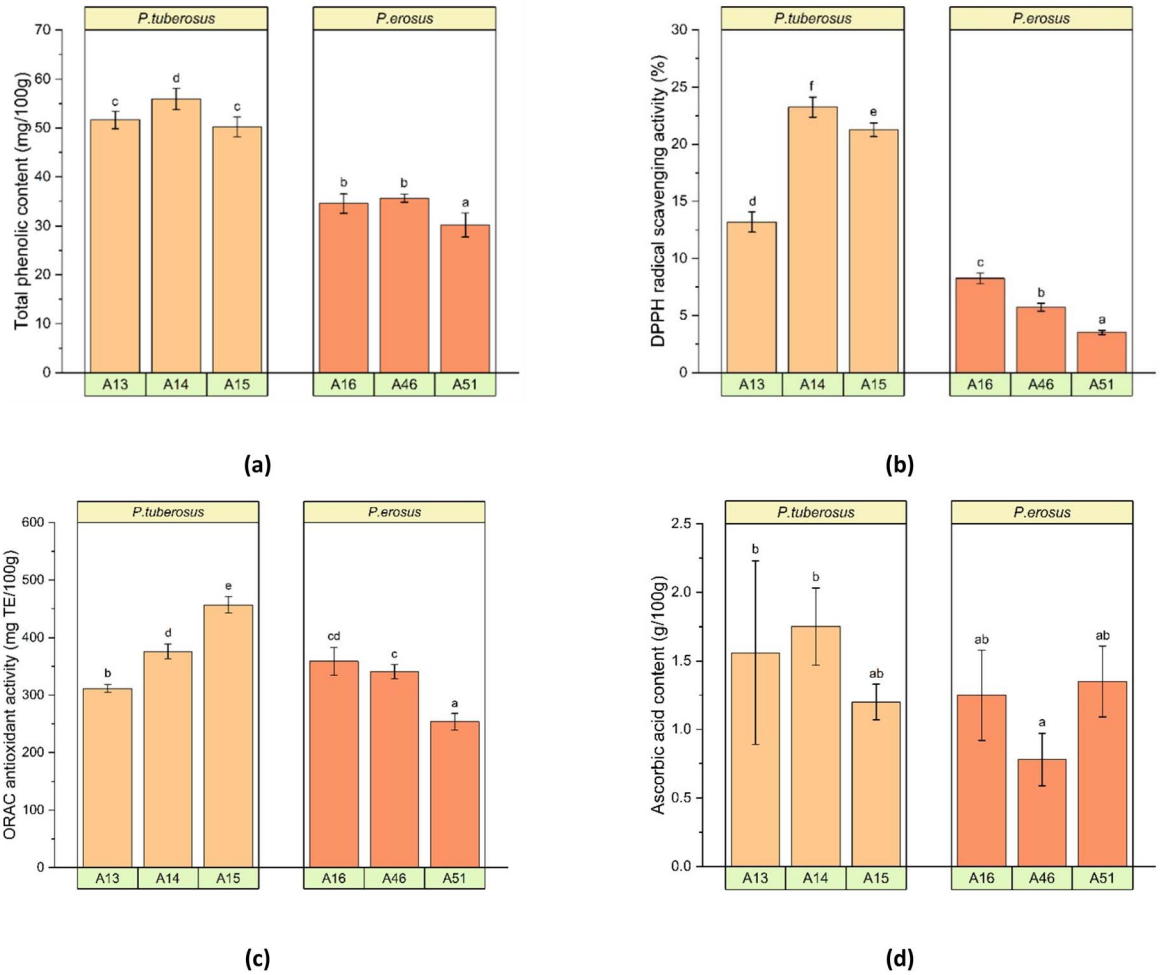

Fig. 6 Change in bioactive composition of different yam bean tuber genotypes: (a) total polyphenolic content, (b) DPPH radical scavenging activity, (c) ORAC antioxidant activity, and (d) ascorbic acid content. Values represent mean ± standard deviation of three analytical replicates and three biological replicates of each genotype. Different lowercase letters indicate significant differences ($p < 0.05$).

phytochemical composition in tubers of seventeen accessions of the AYB and found a phenolic content ranging from 16.45 to 32.29 g/100 g, along with a significant presence of other phytochemicals, including flavonoids, saponins, glycosides, organic acids, phytates, hydrogen cyanide, alkaloids, tannins, and trypsin inhibitor. A substantial difference in TPC between the two tuber species in the present study could be attributed to genetic factors and environmental conditions during cultivation, which are known to impact phenolic accumulation.[52]

Remarkably, the higher phenolic levels observed in *P. tuberosus* also correspond to its deeper pigmentation as described in the colour analysis (Section 3.1). Phenolic compounds, particularly anthocyanins and flavonoids, are major contributors to red and purple hues, whereas carotenoids are associated with yellow tones.[53] Thus, the darker and more saturated surface colours in *P. tuberosus* align with its higher TPC values, while the lighter and more neutral flesh tones of *P. erosus* are consistent with its comparatively lower phenolic content.

An interesting observation was made when comparing the results obtained with those of other tuber species. For instance, Perla *et al.*[54] compared the mature tuber of 5 cultivars and 9 advanced selections of potato for its total phenolic content and found that red-fleshed advanced selection had the highest total phenolic content of 4.4 mg GAE/g of DW and white fleshed variant of Russet nugget had the lowest phenolic content of 0.9 mg GAE/g DW. Similarly, Rytel *et al.*[55] studied the phenolic composition of two variants of raw and cooked coloured (red and purple) fleshed potatoes and reported that purple-fleshed tuber showed the highest phenolic content (292 mg GAE/100 g DW) as compared to the red-fleshed tuber (279.7 mg GAE/100 g DW). For sweet potato (*Ipomoea batatas*), Kourouma *et al.*[56] obtained a phenolic content of 2.59 mg GAE/g DW for the orange-fleshed variety, whereas Nevara *et al.*[57] found a value of 4.43 mg GAE/g DW for the purple-fleshed variant. These values are comparable to the findings of the present study, where the TPC ranged from 30.2 to 55.94 mg GAE/100 g fresh weight and 1.30 to 3.15 mg GAE/g DW. These findings also indicate a higher number of phenolic compounds in purple and red tuber cultivars than in white-fleshed cultivars. As phenolic compounds are responsible for pigmentation, highly pigmented samples have a higher phenolic content.

3.5. Phenolic profiling

The phenolic profiling of yam bean tuber was conducted using High-Performance Liquid Chromatography (HPLC) with detection at 280 nm. The chromatographic analysis demonstrates the richness and diversity of phenolic compounds in yam bean tuber. The obtained chromatogram is presented in Fig. 7. Gallic acid was identified at a retention time of 7.351 min, coumaric acid at 21.866 min, and ferulic acid at 23.298 min. Gallic acid is widely recognised for its potent antioxidant properties and is often found in plant-based foods.[58] Similarly, both coumaric acid and ferulic acid belong to the hydroxycinnamic acid family, which is known for its strong antioxidant and anti-inflammatory activities.[59]

In addition to these identified compounds, several other peaks (at retention times of 5.598, 11.101, 12.309, 13.194, 13.418, and 14.944 min) remained unidentified, despite their characteristic absorbance at 280 nm. These peaks likely represent other phenolic acids, flavonoids, or phenolic derivatives. Further studies employing advanced analytical techniques, such as mass spectrometry, are necessary to accurately identify these compounds.

3.6. Antioxidant activity

The antioxidant activity of yam bean genotypes was evaluated using DPPH radical scavenging activity (Fig. 6(b)) and ORAC assay (Fig. 6(c)). The DPPH radical scavenging activity followed a trend similar to TPC, with *P. tuberosus* genotypes exhibiting significantly higher activity compared to *P. erosus* genotypes. The scavenging activity in *P. tuberosus* genotypes ranged between 13.21 and 23.26%, with the highest activity observed in A14, while the lowest activity was recorded in A13. In contrast, *P. erosus* genotypes demonstrated a range of 3.51–8.26%, with A16 showing the highest value and A51 the lowest. The radical scavenging activity was ranked in decreasing order: accession 14 > accession 15 > accession 13 > accession 16 > accession 46 > accession 51. These trends closely mirror the total phenolic content observed in the respective genotypes, indicating a positive correlation between TPC and antioxidant activity. Genotypes with higher TPC values, particularly A14, also exhibited stronger DPPH radical scavenging capacity, supporting the role of phenolic compounds as primary contributors to free radical neutralisation. The correlation between phenolic content and antioxidant activity is well-documented in the literature, since phenolic compounds are considered to be one of the major contributors to the neutralisation of free radicals through the donation of hydrogen atoms or electrons.[60] The lower scavenging activity in *P. erosus* genotypes might also reflect the presence of other bioactive compounds, such as flavonoids, in lower quantities compared to *P. tuberosus*. These differences highlight the significance of phenolic content as a key determinant of antioxidant efficacy, indicating that *P. tuberosus* genotypes may provide greater protective benefits against oxidative damage. A considerable amount of literature is available on the antioxidant studies of *Dioscorea* genus yam tubers.[61] The literature states that the storage protein, dioscorin, steroidal sapogenin, and diosgenin constitute the major bioactive compounds in yams.

The oxygen radical absorbance capacity (ORAC) assay provided further insights into the antioxidant potential of the genotypes, showing significantly higher activity in *P. tuberosus* genotypes (311.64–456.81 mg TE/100 g) compared to *P. erosus* genotypes (253.59–358.98 mg TE/100 g). Among *P. tuberosus*, A15 exhibited the highest ORAC value, indicating its superior ability to quench reactive oxygen species, whereas A13 recorded the lowest value. In *P. erosus*, the highest ORAC activity was observed in A16, with A51 exhibiting the lowest value. The antioxidant capacity was ranked in decreasing order: accession

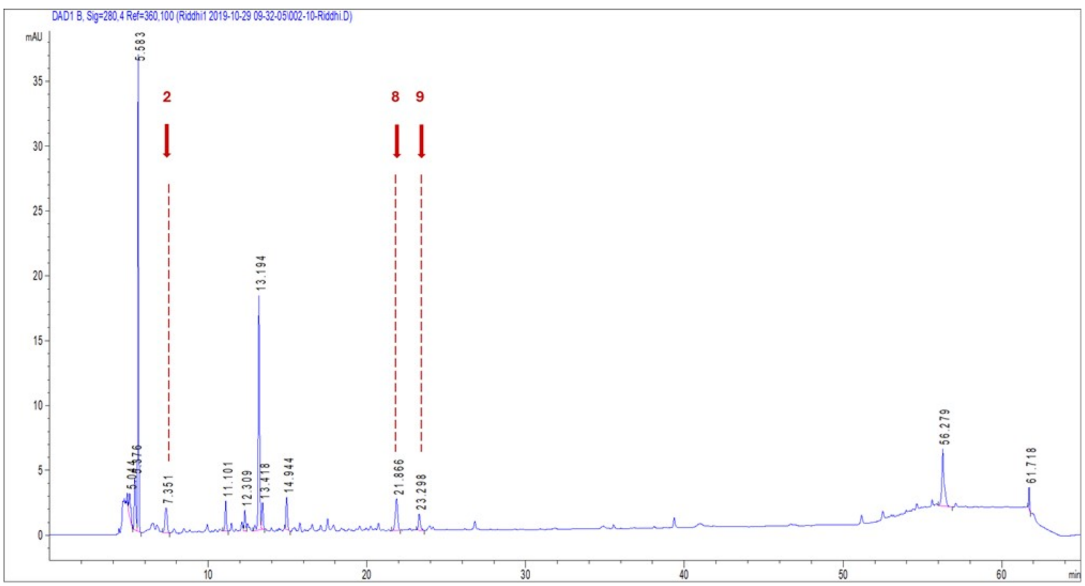

Fig. 7 Chromatographic profile of phenolic acids observed in *P. tuberosus* (Accession 14) species. Peak names: 2, gallic acid; 8, coumaric acid; 9, ferulic acid.

15 > accession 14 ≈ accession 16 ≈ accession 46 > accession 13 > accession 51. The ORAC assay is considered a comprehensive measure of antioxidant activity as it accounts for both hydrophilic and lipophilic compounds.[62] The higher ORAC values in *P. tuberosus* could be attributed to its diverse array of antioxidants, including phenolic acids and flavonoids, which act synergistically to enhance free radical scavenging. The results indicated the presence of biochemically active phenolics that possess antioxidant capacity, as well as the coloured variant of yam bean genotypes and species, which had significantly higher amounts of antioxidant capacity in terms of % radical scavenging activity and amount equivalent to Trolox.

The literature available on the antioxidant capacity of yam bean tuber is very scant. Therefore, results are compared with those of other tubers such as potato (*Solanum Tuberosum*) and sweet potato (*Ipomoea batatas*). These two tubers are also available in different skin colours, and the colour of the skin is positively correlated with antioxidant activity. In the case of potatoes, Perla *et al.*[54] found that the purple-fleshed variant had the highest DPPH radical scavenging ability of 93–94%, and the white-fleshed variant demonstrated the lowest DPPH scavenging activity of 33%. Similar results were reported on sweet potatoes, where the purple genotype exhibited higher antioxidant activity than the orange genotype.[56,57]

3.7. Ascorbic acid content

Ascorbic acid, commonly known as vitamin C, is a key water-soluble antioxidant that contributes to the overall antioxidant capacity of plants. Thus, although not their primary nutritional trait, the presence of ascorbic acid can influence antioxidant capacity and the overall functional potential of yam bean tuber. Fig. 6(d) shows the ascorbic acid composition of the different yam bean tuber genotypes. The ascorbic acid content in *P. tuberosus* genotypes ranged from 1.20 to 1.75 g/100 g, with A13 and A14 showing relatively higher levels compared to A15. In contrast, *P. erosus* genotypes exhibited lower ascorbic acid levels, ranging from 0.78 to 1.35 g/100 g, with A51 showing the highest value. The ascorbic acid content of all genotypes is ranked in decreasing order: accession 14 > accession 13 > accession 51 > accession 16 > accession 15 > accession 46. The variability in ascorbic acid content among genotypes may be attributed to differences in metabolic pathways that regulate ascorbic acid biosynthesis and degradation.[63] Ascorbic acid plays a crucial role in scavenging reactive oxygen species and regenerating other antioxidants, such as vitamin E, further enhancing the plant's defence mechanism against oxidative stress. The relatively higher ascorbic acid content in *P. tuberosus* genotypes reinforces their superior antioxidant profile compared to *P. erosus.* At this stage, no information is available indicating the ascorbic acid content of the yam bean tuber. Hence, ascorbic acid values for other tubers, such as potato and sweet potato, are considered for comparison. Jayanty *et al.*[64] reported that depending on post-harvest and pre-harvest conditions, the ascorbic acid content of potato tuber could be as high as 460 mg/100 g fresh weight. Additionally, a lower amount of ascorbic acid (10–40 mg/100 g fresh weight) has been reported for potato cultivars.[65] This reflects that the amount of ascorbic acid is higher in yam bean tuber (770–1750 mg/100 g fresh weight) as compared to other tubers.

4. Conclusion

A selective evaluation of yam bean tuber genotypes showed their great potential as a functional food ingredient due to their favourable physicochemical, nutritional, and bioactive properties. The study focused primarily on proximate composition, protein digestibility, and antioxidant properties. The protein analysis showed that the yam bean tuber is a valuable source of plant protein, though variable among genotypes. Protein digestibility assessments underlined its nutritional quality, hence its suitability as a protein source in food applications. The electrophoretic profile provided insights into the diverse protein fractions present in the tuber, reflecting the complex structural composition with possibly significant potential impact on functionality. The *P. tuberosus* species showed superior phenolic content, antioxidant activity, and ascorbic acid levels compared to *P. erosus*, indicating that the former holds greater potential as a source of dietary antioxidants and functional food components. Additionally, HPLC phenolic profiling indicated the presence of gallic acid, coumaric acid, and ferulic acid, among many unidentified peaks that all contribute to the antioxidant capacity of the tuber.

Further research should be directed toward the characterisation of the unidentified phenolic compounds and their health benefits, as well as the impact of different processing conditions, such as thermal and non-thermal treatments, on the bioactive and nutritional properties of the tuber. Furthermore, investigating genotype–environment interactions and processing techniques that optimise its functionality and shelf life could open up new perspectives for this tuber in various food applications. In addition, research is required on amino acid profiling, carbohydrate fractionation, and functional properties, as these investigations would provide a more detailed understanding of the nutritional and techno-functional potential of yam bean tubers, enhancing their relevance for industrial and dietary applications.

Author contributions

RRB: visualization, drafting of the original manuscript, reviewing, and editing. GS and RGM: investigation, methodology, data curation, formal analysis, and drafting of the original manuscript. ASE and KP: resources, manuscript review and editing. NUS: data analysis, visualization, and drafting of the original manuscript. AS: review and editing. SJ: conceptualization, methodology, project administration, supervision, and manuscript review and editing.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included within the article.

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