

Characterization of *Cissus quadrangularis* L. (Veldt Grape) aerial fractions: Integrating nutritional and biophysical properties for functional food potential

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Abstract

In this study, we investigated the nutritional and biophysical properties of underutilized L. (Veldt grape) aerial fractions, leaf (CQL), stem pulp (CQP) and stem pulp with peel (CQWP), to assess their functional food potential. Each fraction exhibited tissue-specific biochemical traits. CQL showed elevated protein (16.82%) with moderate protein digestibility (47.55%), enriched essential amino acids (6.22%), unsaturated fatty acids (3.38%) and a favourable nutritional index (6.88%). However, its limited biomass and high oxalate (9.62%) restrict direct applicability. CQWP, characterized by high fibre (18.31%) and minerals, yet elevated antinutrients, impede protein digestibility (19.33%). CQP presented a balanced nutritional profile, characterized by moderate protein (11.32%), elevated essential amino acid index (EAAI: 50.95%), lower antinutrient levels, and a favourable nutritional index (5.76%). Its predominant amorphous structure (~ 80%) enhanced palatability and protein digestibility (53.57%). All fractions exhibited a flavonoid-rich antioxidant profile, with GC-MS confirming the presence of shared and distinct phytochemicals. Biophysical analyses (FTIR, XRD, SEM), thermal studies (TGA, DSC) and colloidal behaviour (zeta potential, PDI) further demonstrated structural functionality, thermal stability and dispersibility relevant to food processing. Collectively, these findings provide the unique nutritional and structural profiles of veldt grape aerial fractions that can support specific food applications. This work establishes a scientific basis for positioning the veldt grape as a nutrient-dense, underexploited crop resource with promising value in future functional food innovations.

INTRODUCTION

Sustainable food systems are vital for ensuring food security and providing nutritious food, especially as the global population is expected to reach 10 billion by 2050 [1]. Current systems are under pressure due to rising food demand, environmental issues and resource depletion. Despite these global challenges, malnutrition affects over 42% of the population, who cannot afford a healthy diet, and more than 2.3 billion people suffer from micronutrient deficiencies, leading to significant health issues [2]. Globally, 193 million people experience severe food insecurity, with 720–811 million individuals facing hunger in 2020, and an estimated 660 million may still face hunger in 2030 [3, 4]. These challenges highlight the significance of integrating nutrition, sustainability, and biodiversity into food production. Furthermore, the UN Decade of Action on Nutrition emphasizes this by promoting the development of sustainable, resilient food systems that provide healthy and nutritious diets [1]. Enhancing dietary diversity through sustainable crop use is essential for achieving global nutrition goals and alleviating hidden hunger.

Underutilized and neglected plant species provide a significant opportunity to improve the dietary diversity, address the micronutrient problem and build sustainable food systems. Though more than 30,000 edible plant species have been identified, less than 150 are cultivated, with just 103 providing 90% of calories in the human diet. Presently, global food systems rely on four staple food crops (rice, wheat, maize and potato) that contribute 60% of the global energy supply [1]. This narrowing of the food basket had reduced the nutritional diversity and dietary intake. Underutilized plants are rich in protein, carbohydrates, essential amino acids, fatty acids, minerals, vitamins and dietary fibre [5]. Their

antioxidant, anti-inflammatory and therapeutic properties add functional values, making them a potential candidate for developing nutrient-rich foods that help combat non-communicable diseases. Reviving such nutrient-dense plants in modern diets may enhance biodiversity and sustainable strategies to reduce hidden hunger and improve global food security [1].

Among the array of underexploited plants, *Cissus quadrangularis* L. (Veldt Grape) from the Vitaceae family is well known for its medicinal properties. It is a large, succulent, four-winged, perennial plant native to West Africa, India, Malaysia, and Sri Lanka, locally called hadjod or veld grape [6]. Traditionally, plant constituents have been used to treat conditions such as asthma, indigestion, epilepsy, wounds, bone healing, and convulsions [7]. It is rich in phytochemicals, phenols, flavonoids, terpenoids, alkaloids, and phytosterols, along with essential vitamins (vitamin C) and minerals (calcium, iron, phosphorus), contributing to a wide range of biological activities, including antioxidant, anti-inflammatory, antimicrobial, anti-hemorrhoidal and anthelmintic effects [8]. These phytoconstituents are associated with diverse therapeutic benefits in managing chronic diseases such as diabetes, obesity, immune dysfunction, metabolic disorders, cancer, and arthritis.

Although its medicinal properties were well-documented, its nutritional relevance and application in modern food systems remain extremely underexplored. Existing studies report only a few investigations on incorporation into food matrices. For instance, the study by [7] developed an energy bite using this Veldt grape powder to enhance consumer awareness; The studies by [9, 11] incorporated this into bread formulations to improve nutritional value; The study by [10] demonstrated that adding cissus to Tteokgalbi, a Korean meat-based dish, enhanced antioxidant and anti-bacterial activity; and the study by [12] developed cookies containing veldt grape, though sensory limitations, such as throat irritation, were noted. In India, its culinary use is largely limited to traditional dishes such as chutneys, dosa batters, pickles, and powder blends [12]. In Korea, cissus extracts are recognized as functional ingredients with reported fat-reducing potential and are available as powder and tablet forms [8].

This limited and inconsistent usage underlines a critical gap in understanding the plant's complete nutritional and antinutritional, *in-vitro* protein digestibility, nutritional quality evaluation, physiochemical properties, thermal stability, techno-functional attributes, structural and functional characteristics, along with their technological suitability for broader food applications, a gap previously reported in literature [7]. Techniques used for characterization include: Fourier Transform Infrared Spectroscopy (FTIR) for functional and structural fingerprinting, X-Ray Diffractometer (XRD) for crystalline behaviour, Gas chromatography Flame Ionisation Detection (GC-FID) and High-performance Liquid Chromatography (HPLC) for fatty acid and amino acid profiling, Inductively Coupled Plasma Mass Spectrometer (ICPMS) for micronutrient analysis. Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) for thermal studies, Zeta sizer for their electrokinetic potential and particle size profile. Morphological features were analysed using High-Resolution Scanning Electron Microscopy (HR-SEM) and an optical microscope.

In this study, we aim to strategically valorize the aerial fractions of the veldt grape by comprehensively evaluating their nutritional, functional, structural and thermal attributes and interpreting these findings in line with functional food development and bridge the gap in understanding the full potential of plant's benefits. By examining the leaf (CQL), stem with peel (CQWP), and stem pulp (CQP) using advanced analytical techniques, we seek to understand how their unique profiles influence digestibility, thermal stability, technological suitability and incorporation potential within food matrices. This research presents the first integrated evaluation of these aerial parts, facilitating their specific functional roles, including improved antioxidant capacity, mineral supplementation, fibre and bioactive enrichment or inclusion into low glycaemic food systems. The findings of this study seek to provide a scientific basis for leveraging *Cissus quadrangularis* L. as a promising nutrient-rich, underexploited resource that can enhance dietary quality and novel functional food formulations.

MATERIALS AND METHODS

Chemicals and reagents

All solvents, reagents and standards were of analytical grade, primarily sourced from Sisco Research Laboratories Pvt. Ltd. (SRL, Mumbai, India) and Sigma-Aldrich (St. Louis, MO, USA).

Origin and Sample Preparation

The morpho-variant quadrangular stem of veldt grape (*Cissus quadrangularis* L.), characterised by prominent four-winged edges, was selected for this study. Aerial fractions, including leaves and whole stems, were harvested from Tamil Nadu, India (11°17'13.1"N, 78°11'33.0"E) and separated into three fractions: leaf (CQL), stem with peel (CQWP), and stem pulp (CQP). Following collection and separation, the samples were washed, chopped uniformly, and dried at 40°C using a tray dryer (HF 12TD). The dried material was milled into fine powder, sieved for uniform particle size, and stored in airtight containers for further analysis.

Characterization of Veldt grape aerial fractions

Proximate analysis

The proximate profile of aerial fractions of Veldt grape was evaluated using the AOAC method [13]. Moisture was identified by recording the constant weight using a hot air oven (Hi Tech, India; 50–250°C) at 106 ± 3 °C. The protein composition was measured through the standard Kjeldahl method. The carbohydrate content was analysed using the anthrone acid-hydrolysis method with the help of a spectrophotometer (V-730, JASCO, Tokyo, Japan) calibrated at 670nm. The fat was determined with Soxhlet extraction (GESE: 6, GUNA Enterprises, India) by using hexane as solvent. Estimation of fibre content through the acid-hydrolysis method. Ash was evaluated using a muffle furnace (Elite, Sree Andal

Scientific Co., India) at 550 ± 3 °C for 4 hr. The calorie ($\text{kcal } 100\text{g}^{-1}$) and energy ($\text{kJ } 100\text{g}^{-1}$) were calculated using equations 1 and 2, respectively.

$$\text{Energy } (\text{kJ } 100 \text{ g}^{-1}) = 4.184 \times \text{calorie (Eq. 1)}$$

$$\text{Calorie } (\text{kcal } 100\text{g}^{-1}) = (4 \times \text{Protein}) + (9 \times \text{Fat}) + (4 \times \text{Carbohydrate}) \text{ (Eq. 2)}$$

Antinutritional analysis

Phytates, tannin, saponin and oxalates were estimated using the method proposed in the study [14]. For phytates, 0.5 g of the sample with 25 mL of 2.4% HCL was allowed to remain at room temperature for 60 min, then centrifuged for 30 min at 3000 rpm. 3 mL of the sample were mixed with 1 mL of reagent wade (0.03% FeCl. $6\text{H}_2\text{O}$ and 0.3% sulfosalicylic acid in water) with absorbance measured at 500nm using UV spectrophotometer (V-730, JASCO, Tokyo, Japan), and phytate concentration $\text{mg}/100\text{g}$ was evaluated using $y = 0.0021x - 0.0158$, with phytic acid as a standard (Supplementary, Fig. 1a). For tannin, 5g of the sample is dissolved in 50 mL of distilled water and kept for 30 min at ambient temperature by intermediate shaking. 2 mL of extract is mixed with 2.5 mL of saturated Na_2CO_3 and 1 mL of Folin-C reagent and made to 50 mL, followed by measuring at 760nm after 90min incubation. The linear regression equation $y = 0.0073x - 0.0409$ was used to determine the tannin concentration $\text{mg}/100\text{g}$, with tannic acid as standard (Supplementary, Fig. 1b).

For saponin, extraction in a ratio of 1:20 in 80% methanol for 16 hr, followed by centrifugation at 4000g for 10 mins, repeat this and collect the supernatant. Measure the absorbance at 520 nm of 0.2 mL aliquots combined with 0.25 mL of vanillin, 0.05 mL of 80% methanol, and 2.5 mL of 72% sulfuric acid. The linear regression equation $y = 0.0016x + 0.1266$ was used to evaluate the saponin content $\text{mg}/100\text{g}$, with diosgenin as standard (Supplementary, Fig. 1c). For oxalate, 1 mL of sample was extracted with 30 mL of $0.5 \text{ N } \text{H}_2\text{SO}_4$ using a boiled water bath for 15 mins, followed by filtering and adding an equal amount of deionised water. From the filtered extract, 10 mL was mixed with 40 mL of $0.5 \text{ N } \text{H}_2\text{SO}_4$ and heated to 60°C and then titrated against $0.05 \text{ N } \text{KMnO}_4$, by observing the permanent appearance of light pink colour and expressed in $\text{g}/100\text{g}$.

Physiochemical properties

Water activity of prepared samples was measured using a water activity meter at 28 ± 2 °C. pH was evaluated using (Systronics Digital pH meter 335, India) pH meter. Colour of the samples was evaluated with L, a^* , b^* values from the Hunter colourimeter (ColoQuest XE, Hunter Lab, Virginia, USA).

Techno-functional properties

Water absorption capacity (WAC) and oil absorption capacity (OAC) were evaluated, following [15]. For WAC, 5 g of sample was suspended in 25 mL of distilled water within pre-weighed centrifuge tubes. The mixture was agitated for 5 min, then centrifuged at 1000×g for 15 min. The supernatant was discarded, and the sediment was weighed. For OAC, 0.75 g of sample was suspended in 9 mL of groundnut oil within pre-weighed centrifuge tubes. The tubes were agitated for 1 minute, then left for 30 min, followed by centrifuging at 3000×g for 20 min. Excess oil was removed, and the pellet was weighed.

Foaming capacity (FC) and Foaming stability (FS) were measured using [15] with slight modifications. For FC, the sample was dispersed (5% w/v) in a beaker and subjected to homogenization at 24,000 rpm for 90s. Immediately, the initial height of the sample was measured after homogenization. The same procedure was followed for FS, and the percentage of foam volume was calculated at 10 min after homogenization. Results were expressed as g water/g, g oil/g for WAC and OAC, and % for foaming indices, calculated using equations 3, 4, 5 and 6.

$$\text{Water absorption capacity} = \frac{A_1}{A_0} \text{ (Eq. 3)}$$

$$\text{Oil absorption capacity} = \frac{\text{Weight of wet sediment} - \text{weight of sample}}{\text{Weight of sample}} \text{ (Eq. 4)}$$

$$\text{Foaming capacity \%} = \frac{V_B - V_A}{V_A} \times 100\% \text{ (Eq. 5)}$$

$$\text{Foaming stability \%} = \frac{V_C - V_A}{V_B - V_A} \times 100\% \text{ (Eq. 6)}$$

where: A_1 – wet sediment, A_0 – initial weight and V_A , V_B and V_C – the total volume before, directly after and after 10min homogenization, respectively.

Phytochemical analysis

The folin-ciocalteu method, followed by [16], was employed to determine the total phenol content (TPC) with slight modification. 0.5 mL of sample/gallic acid at different concentrations was added with 2.5 mL (1:10 ratio) of Folin-C and neutralized using 7.5% of 2 mL sodium carbonate. After 30 min of dark incubation, the absorbance was measured at 760 nm. The linear regression equation $y = 0.0089x + 0.0731$ was used to determine TPC. Gallic acid is used as a standard presented in (Supplementary, Fig. 2a), and results were expressed in $\mu\text{g GAE/mL}$.

The total flavonoid content (TFC) was determined using the aluminium chloride method adopted from [17]. A 0.1 mL of 10% aluminium chloride, 1.5 mL of methanol, addition of 2.8 mL of distilled water, with 0.1 mL of 1M potassium acetate. The mixture was thoroughly mixed and incubated for 30 min in the dark, followed by measuring absorbance at 415 nm. The linear regression equation $y = 0.006x + 0.1059$ was used for the calculation of TFC. Quercetin is used as a standard presented in (Supplementary, Fig. 2b), and results were expressed in $\mu\text{g QAE/mL}$.

DPPH antioxidant assay

The antioxidant properties were evaluated using the DPPH radical scavenging method [17]. 4 mL of a 0.1 mM DPPH solution prepared in methanol were mixed with 1 mL of the extract. The mixture was kept in the dark for 30 min, and measured at 517nm absorbance. A control was made with 1 mL of methanol and 4 mL of DPPH solution. The antioxidant activity's IC₅₀ value was determined using linear regression of the data presented in (Supplementary Fig. 3).

Mineral analysis

An ICP-MS system (NexION 2000, PerkinElmer, USA) was used for mineral analysis following the procedure [18]. Before analysis, samples were subjected to closed-vessel microwave digestion. The instrument operated with a 1 mL sample loop in flow switching mode at 1600 W RF power, with a glass cyclonic spray chamber maintained at 2°C and a sample introduction rate of ~ 300 µL/min. The mineral composition (Iron, Calcium, Magnesium, Potassium) was estimated through Inductively Coupled Plasma Mass Spectrometry (ICP-MS) by digesting the samples with strong acid (nitric acid). subsequently, 1 mL of hydrogen peroxide was added, and the mixture was boiled at 95°C for 10 min. The volume was adjusted to 10 mL with ultrapure water and transferred to acid-treated Falcon tubes for mineral analysis. Phosphorus was estimated through the AOAC method [19] using a spectrophotometer (V-730, JASCO, Tokyo, Japan).

GC-FID profiling of fatty acids

The fatty acids were analysed using Gas Chromatography coupled with a Flame Ionisation Detector (GC-FID, Agilent 7890B, Agilent Technologies, USA), following the AOAC standard method 966.01 mentioned in the study [20]. Fatty acids were extracted by hydrolysing the sample with 8 M hydrochloric acid at 80°C for 40 min under continuous shaking, followed by cooling. The hydrolysate was transferred to a mojonnier fat extraction flask using a mixture of ethyl ether and petroleum ether. The solvent was evaporated under a nitrogen stream, and the extracted lipids were then subjected to saponification using sodium hydroxide in methanol. Methylation was performed with 14% boron trifluoride in methanol to convert the fatty acids into fatty acid methyl esters, which were subsequently analysed by FID.

HPLC profiling of amino acids

The amino acid profile of the aerial fractions was determined using the technique described in [20]. Begin with hydrolysis of the samples in 6N hydrochloric acid at 100°C for 24h, followed by neutralization with sodium hydroxide. High-Performance Liquid Chromatography (HPLC-1100, Agilent Technologies, USA) was carried out using the Hewlett-Packard system equipped with a Variable Wavelength Detector (VWD). The mobile phase consisted of two components: Phase A contained 0.018% triethylamine and 20 mM sodium acetate (pH 7.2), while Phase B comprised 20% of 100 mM sodium acetate, 40% methanol, and 40% acetonitrile (pH 7.2). Chromatographic separation was conducted at 40°C with a flow rate of 0.5 mL/min, and detection was performed at 338 nm.

In-vitro protein digestibility

In-vitro protein digestibility was assessed, as described by [21] with slight modifications. A simulated gastrointestinal digestion was applied to 1g of the samples. To simulate stomach digestion, it was first incubated for 3 h at 37°C with 15 mL of 0.1 M HCl containing 1.5 mg pepsin. After neutralizing the resultant solution with 7.5 mL of 0.2 M NaOH, 4 mg of pancreatin was added to 7.5 mL of 0.2 M phosphate buffer (pH 8.0) to mimic intestinal digestion. The mixture was incubated at 37°C for 24 h, followed by the addition of 10 mL of 10% trichloroacetic acid (TCA). The mixture was centrifuged for 20 min at 2000 rpm to separate the larger peptides and undigested protein. IVPD was calculated using the following Eq. 7.

$$IVPD (\%) = \frac{\text{Protein digested}}{\text{Total protein}} \times 100 \text{ (Eq. 7)}$$

Evaluation of Nutritional Quality

Protein quality was evaluated based on the amino acid profile. The ratio of total essential amino acids (TEAA) to total amino acids (TAA) was calculated following [22]. To estimate the overall biological value of protein, the essential amino acid index (EAAI) and Nutritional index were also computed and calculated using equations 8, 9 and 10.

$$\frac{TEAA}{TAA} = \frac{Ile + Leu + Lys + Met + Phe + Thr + Val + His}{(Ala + Asp + Arg + Gly + Glu + His + Ile + Leu + Lys + Met + Phe + Tyr + Thr + Val + His)} \text{ (Eq. 8)}$$

$$EAAI = \sqrt[n]{\frac{100a \times 100b \times \dots \times 100j}{a_v \times b_v \times \dots \times j_v}} \text{ (Eq. 9)}$$

$$\text{Nutritional Index } (\%) = EAAI \times \frac{\text{Protein}}{100} \text{ (Eq. 10)}$$

Where the variable 'n' represents the number of essential amino acids, while a, b, c ... j denotes individual amino acids; a_v , b_v ... j_v indicate their respective contents in the reference protein (egg).

GC-MS profiling of secondary metabolites

Screening of phytoconstituents using GC-MS-QP2010 Plus (Shimadzu), using a modified procedure from [23]. A volume of 1 μ L of the methanolic extract was injected using a split ratio of 30:0 onto an RTX-5 column (60 m $\times$ 0.25 mm, 0.25 μ m film thickness). Helium was employed as the carrier gas at a flow rate of 1 mL/min under an inlet pressure of 53.5 kPa. The oven temperature program began at 50°C, was raised to 280°C at a rate of 8°C per minute, and maintained at the final temperature for 2 min. The injection port and detector temperature were maintained at 200 °C and 280 °C, respectively. The ionization voltage was performed at 70 eV with a scan interval of 0.30 sec; the total run time of GC-MS is 31.00 min. The NIST database was taken as a reference to interpret the identified compounds with the help of retention indices (Ris) and mass spectra (MS).

FTIR analysis of functional compounds

Functional compounds were identified through Fourier-transform infrared spectrophotometry (FTIR, IRTracer 100, Shimadzu, Japan). With 45 scans at 4 cm^{-1} resolution and an integration duration of 2 min across a wavenumber range from 4000 to 500 cm^{-1} .

XRD analysis of crystallinity nature

Crystalline structure was characterized using X-Ray Diffractometer (XRD, PANalytical, Netherlands), with Cu-K α radiation at a wavelength of $1.54\text{ \AA}$ at 45 kV and 40 mA. Diffraction patterns obtained were recorded between 5° and 90° (2θ). The crystallinity index was calculated using the Eq. 11

$$\text{Crystallinity Index } \% = \frac{\text{Area of all crystalline peaks}}{\text{Area of all crystalline peaks and amorphous peaks}} \times 100 \text{ (Eq. 11)}$$

Particle size distribution and Zeta potential

Zeta potential and particle size distribution were analysed using a Zetasizer Nano ZS-90 (Malvern Instruments, Worcestershire, UK) at 25°C , with 135.7 kcps count rate, using a disposable cuvette.

Thermal properties of Veldt grape fractions

Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was performed using STA 2500 Regulus (Netzsch, Germany). Approximately 6 mg of each sample was heated from 30°C to 600°C at $10^\circ\text{C}/\text{min}$, under nitrogen with a $50\text{ mL}/\text{min}$ flow rate. Data were analysed using Proteus software.

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) was performed with DSC 300 Caliris Classic (Netzsch, Germany), following the method by [24]. Precisely 5 mg of each sample was heated from 30°C – 220°C , at $10^\circ\text{C}/\text{min}$, under a nitrogen flow of $20\text{ mL}/\text{min}$. Thermograms and glass transition temperature (T_g) were determined via Proteus software.

Morphological studies

Surface and internal morphology were investigated using high-resolution scanning electron microscopy (HRSEM, Thermo Scientific Apero S) and optical microscopy at different magnifications. For SEM analysis, samples were mounted on an aluminium stub with both-sided adhesive tape and imaged at an accelerating voltage of 20 kV .

Statistical analysis

The statistical analysis was conducted using IBM SPSS Statistics 25. One-way (ANOVA) was performed at the level ($P < 0.05$) to evaluate significant differences. Results were presented as mean $\pm$ standard deviation (SD).

RESULTS AND DISCUSSION

Proximate composition

Proximate composition revealed distinct fraction-specific variations among aerial parts, shown in Table 1. The leaf fraction (CQL) exhibited the highest protein content ($16.82 \pm 0.03\%$) coupled with good caloric value (285.54 ± 1.53 kcal/100 g; 1194.01 ± 0.47 kJ/100 g), suggesting its nutrient-dense profile. This is likely due to the abundance of protein-rich organelles, particularly chloroplasts and associated cytoplasmic enzymes, specifically ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), crucially involved in photosynthetic carbon assimilation [25]. In contrast, elevated levels of fibre ($18.31 \pm 0.05\%$), fat ($10.59 \pm 0.17\%$) and ash ($19.64 \pm 0.05\%$) levels were found in the CQWP despite its comparatively lower protein concentration ($5.59 \pm 0.07\%$) ($P < 0.05$). These attributes may be linked with the presence of peel, high in structural polysaccharides (cellulose and hemicellulose), mineral-bound epidermal tissues, and cuticular lipids that contribute to structural rigidity and defensive mechanisms. Its reduced contribution to caloric value is likely due to lower digestible protein and carbohydrate. The pulp (CQP) distinctly showed the highest carbohydrate content ($41.66 \pm 0.03\%$), along with moderate levels of fibre ($13.86 \pm 0.77\%$), fat ($5.31 \pm 0.03\%$) composition, contributing to balanced caloric value (259.71 ± 1.87 kcal/100g; 1086.30 ± 0.94 kJ/100 g), suggesting that the absence of peel reduces the portion of indigestible fibre and ash, thereby chance of enhancing polysaccharides and energy value, favored balanced nutrient composition.

Notably, the fibre profile of all the fractions was comparable to green leafy vegetables such as spinach (9.61%), chickpea (13.95%), mustard (11.99%) and cauliflower (9.23%), conferring potential health benefits including improved digestion, reduced cholesterol absorption, and better trace element uptake [17, 26]. Moisture content was highest in pulp ($13.33 \pm 0.06\%$), likely due to the dominance of thin-walled parenchyma cells, and mucilaginous polysaccharides aid in water retention in succulent tissues [27]. Collectively, the pulp fraction showed a broader and balanced nutrient profile with a possible palatable dietary application compared to other fractions.

Antinutritional factors

Antinutrients, including tannins, saponins, phytates and oxalates, varied significantly across fractions ($P < 0.05$), as illustrated in Table 1. CQWP showed the highest levels of tannins ($0.1428 \pm 0.06\%$), phytates ($0.1912 \pm 0.22\%$), and saponins ($0.123 \pm 0.09\%$), followed by CQL and CQP. CQL showed moderate levels of tannins, phytates, and saponins, whereas CQP, lacking peel, had the lowest levels: $0.0839 \pm 0.15\%$ (tannin), $0.1496 \pm 0.10\%$ (phytate) and $0.1101 \pm 0.05\%$ (saponin), respectively. Oxalates were the predominant metabolite across fractions, with the highest in CQL ($9.62 \pm 0.08\%$), followed by CQWP and CQP ($7.31 \pm 0.02\%$). Cumulatively, the CQWP demonstrated the highest accumulation of antinutrient load.

Plants employ secondary metabolites (also known as antinutrients) as key biochemical defence mechanisms regulated by phytohormones against environmental stress. The elevated saponin, phytate and tannin levels in CQWP, attributed to higher metabolic activity and are accumulated higher in outer stem and peel tissues, which protect against biotic and abiotic stress [28, 29]. The leaf fraction (CQL) exhibited the highest oxalate levels, reflecting its role in calcium regulations, stress defence and was influenced by temperature, light exposure, and nitrate uptake could effectively stimulate the accumulation of oxalates in the leaf [30, 31]. In contrast, the pulp (CQP) showed lower accumulation, aligning with lower defensive function. Despite the protective roles in plant physiology, these compounds reduce the bioavailability of the minerals in the nutritional aspect [65].

Table 1
Nutritional and antinutritional profile of veldt grape aerial fractions

Parameters	CQL	CQWP	CQP
Centesimal composition (g/100g)			
Protein %	16.82 ± 0.03 ^a	5.59 ± 0.07 ^c	11.32 ± 0.04 ^b
Fat %	8.42 ± 0.06 ^b	10.59 ± 0.17 ^a	5.31 ± 0.03 ^c
Fibre %	9.29 ± 0.01 ^c	18.31 ± 0.05 ^a	13.86 ± 0.77 ^b
Ash %	18.48 ± 0.07 ^b	19.64 ± 0.05 ^a	14.50 ± 0.07 ^c
Moisture %	11.36 ± 0.09 ^b	11.12 ± 0.14 ^b	13.33 ± 0.06 ^a
Carbohydrates %	35.62 ± 0.00 ^b	34.75 ± 0.00 ^a	41.66 ± 0.03 ^c
Calorie (kcal 100g ⁻¹)	285.54 ± 1.53 ^a	256.67 ± 2.16 ^c	259.71 ± 1.87 ^b
Energy (kJ 100g ⁻¹)	1194.01 ± 0.47 ^a	1073.45 ± 0.06 ^c	1086.30 ± 0.94 ^b
Antinutritional factors			
Tannin (mg/100g)	131.50 ± 0.47 ^b	142.83 ± 0.062 ^a	83.86 ± 0.159 ^c
Phytate (mg/100g)	109.56 ± 0.10 ^c	123.05 ± 0.22 ^a	110.05 ± 0.10 ^b
Saponin (mg/100g)	118.28 ± 0.35 ^c	191.21 ± 0.09 ^a	149.61 ± 0.05 ^b
Oxalate (g/100g)	9.62 ± 0.08 ^a	8.11 ± 0.02 ^b	7.31 ± 0.02 ^c

Note: CQL: *Cissus quadrangularis* L. Leaf; CQWP: *Cissus quadrangularis* L. stem pulp with peel; CQP: *Cissus quadrangularis* L. pulp. Subscripts with different letters in a row has a significant difference (P < 0.05) and all the values are expressed in mean ± standard deviation (SD).

Physiochemical properties

Physicochemical properties reveal notable differences ($P < 0.05$) among plant fractions as shown in Table 2. CQP exhibited the highest water activity (0.52 ± 0.00), attributed to its higher soluble fibre and water retention capacity, followed by CQWP (0.46 ± 0.00) and CQL (0.43 ± 0.00). pH values also varied significantly ($P < 0.05$), with CQWP showing the highest pH (9.25 ± 0.02), followed by CQP (8.64 ± 0.01) and CQL (6.31 ± 0.01), likely due to differences in cellular composition and the presence of acidic or alkaline compounds.

Colour analysis showed CQP with the highest lightness (61.13 ± 1.23), followed by CQWP (59.85 ± 0.05), while CQL appeared darkest (44.19 ± 0.01), likely due to higher pigment concentrations, such as chlorophyll and carotenoids, showing a significant difference ($P < 0.05$). All samples had negative a^* values, indicating greenness, most pronounced in CQL (-0.73 ± 0.01), then CQWP (-2.88 ± 0.28), and least in CQP (-4.19 ± 0.19). The b^* values, indicating yellowness, were highest in CQP (20.23 ± 0.39), followed by CQWP (19.29 ± 0.47) and CQL (11.28 ± 0.14). These colour metrics suggest higher levels of green and yellow pigments, potentially influenced by phenolic polymerisation and Maillard reactions during drying [32]. The observed a^* and b^* values exhibited a significant difference ($P < 0.05$) across all samples were correlated with the lightness values, pigment content and surface reflectivity of each aerial fraction.

Techno-functional properties

Techno-functional properties, as shown in Table 2, indicate that CQP had the maximum water WAC and OAC at 3.3 ± 0.05 and 1.9 ± 0.10 , respectively, followed by CQWP and CQL (2.83 ± 0.05 , 1.59 ± 0.01). These differences are likely due to CQP's higher soluble fibre content, as CQWP contains more insoluble fibre. WAC and OAC values of CQWP and CQL were not significantly different ($P > 0.05$), whereas CQP differed significantly. CQP also exhibited the highest foaming capacity (73.11 ± 0.03), likely correlated to elevated carbohydrate content, enhancing air entrapment. This was followed by CQWP and CQL (23.80 ± 0.02), with significant differences ($P < 0.05$) across samples. Despite its moderate foaming capacity, CQWP had the highest stability (11.11 ± 0.01), attributed to fibre accumulation at the air–liquid interface that stabilises foam [33]. In contrast, CQL showed the lowest foaming stability (4.69 ± 0.02), consistent with its lower carbohydrate and fibre content, thereby limiting viscosity and bubble stabilization.

Phytochemical profiling and antioxidant assay

The quantitative phytochemical profiling (phenol and flavonoid) of aerial fractions, shown in Table 2, revealed significant variation ($P < 0.05$). The CQL had the highest total phenolic content (TPC, $213.62 \pm 0.54 \mu\text{g GAE/mL}$) and total flavonoid content (TFC, $262.58 \pm 0.45 \mu\text{g QE/mL}$), followed by CQP and CQWP, showing the lowest levels (TPC: $123.12 \pm 0.33 \mu\text{g GAE/mL}$; TFC: $190.03 \pm 0.04 \mu\text{g QE/mL}$). Among fractions, TFC exceeded TPC, indicating a stronger presence of flavonoids. Antioxidant activity, assessed by the DPPH assay (Table 1), revealed that CQL exhibited the strongest activity, with the lowest IC₅₀ value ($7.83 \pm 0.00 \text{ mg/mL}$), followed by CQWP ($14.39 \pm 0.31 \text{ mg/mL}$) and CQP ($19.23 \pm 0.02 \text{ mg/mL}$).

The high levels of polyphenols (phenols, flavonoids) in the leaves are highly associated with active photosynthetic metabolism, which is linked to the shikimic acid pathway, which synthesises flavonoids [34]. CQP and CQWP, on the other hand, lack flavonoid synthesis, which results in a lower concentration. CQP primarily serves as a source of carbohydrates, whereas CQWP, with elevated fibre accumulation, suggests their role in structural support and defensive mechanisms. The higher levels of flavonoids in all fractions signify that the flavonoid subclasses were crucial for getting rid of free radicals and other redox-related processes. The antioxidant trend shows the same pattern, with CQL showing the highest activity (lowest IC₅₀), followed by CQWP and least evident in CQP. Interestingly, despite its higher polyphenol content, CQP exhibited lower antioxidant performance than CQWP, implying antioxidant effectiveness may depend more on the specific type and structural characteristics of phenolic compounds than on total concentration [35]. Consistent with earlier findings by [17], antioxidant activity is influenced by hydroxyl group patterns, and the degree of polymerization plays a role in the redox behaviour and radical-neutralising efficiency of polyphenolic compounds.

Table 2
Physiochemical, techno-functional and Phytochemical composition of veldt grape aerial fractions

Parameters	CQL	CQWP	CQP
Physiochemical properties			
aW	0.43 ± 0.00 ^c	0.46 ± 0.00 ^b	0.52 ± 0.00 ^a
pH	6.31 ± 0.01 ^c	9.52 ± 0.02 ^a	8.64 ± 0.01 ^b
L	44.19 ± 0.01 ^c	59.85 ± 0.05 ^b	61.13 ± 1.23 ^a
a*	-0.73 ± 0.01 ^c	-2.88 ± 0.28 ^b	-4.19 ± 0.19 ^a
b*	11.28 ± 0.14 ^c	19.29 ± 0.47 ^b	20.23 ± 0.39 ^a
Techno-functional properties			
Water absorption capacity (g/g)	2.83 ± 0.05 ^c	3 ± 0.1 ^b	3.33 ± 0.05 ^a
Oil Absorption Capacity (g/g)	1.59 ± 0.01 ^b	1.33 ± 0.15 ^c	1.9 ± 0.1 ^a
Foaming Capacity (FC%)	23.80 ± 0.02 ^c	34.66 ± 0.01 ^b	73.11 ± 0.03 ^a
Foaming Stability (FS%)	4.69 ± 0.02 ^c	11.11 ± 0.01 ^a	5.26 ± 0.02 ^b
Phytochemical profile			
Total Phenol Content (µg GAE/mL)	213.62 ± 0.54 ^a	123.12 ± 0.33 ^c	188.88 ± 1.10 ^b
Total Flavonoid Content (µg QAE/mL)	262.58 ± 0.45 ^a	190.03 ± 0.04 ^c	213.19 ± 0.05 ^b
IC 50	7.83 ± 0.00 ^c	14.39 ± 0.31 ^b	19.23 ± 0.02 ^a

Note: CQL: *Cissus quadrangularis* L. Leaf; CQWP: *Cissus quadrangularis* L. stem pulp with peel; CQP: *Cissus quadrangularis* L. pulp. Subscripts with different letters in a row have a significant difference (P < 0.05), and all the values are expressed in mean ± standard deviation (SD).

ICP-MS profiling of minerals

Mineral composition revealed fraction-specific accumulation across aerial parts, with calcium (Ca) as the predominant element across all fractions, as described in Fig. 1. The leaf fraction (CQL) recorded the highest concentration of calcium (Ca: 4.20g/100g), followed by CQWP (3.13g/100g) and CQP (2.90g/100g). Conversely, magnesium (Mg) recorded the lowest level overall, with the least concentration observed in leaf fraction (CQL: 0.59g/100g). Individually, the CQL was enriched with iron (Fe) content (Fe: 1.47g/100g), CQP was notably rich in phosphorus (P: 2.87g/100g), while CQWP was

enriched with potassium (K: 2.44g/100g), magnesium (Mg: 0.64g/100g) and highest total mineral load, signifying strong distribution trend in peel-associated fractions.

These accumulation patterns are consistent with total ash trends, highlighting the CQWP fraction with superior mineral density, followed by the leaf and pulp fraction, demonstrating their potential for dietary mineral enrichment strategies such as functional food development and micronutrient fortification. The leaf with a good source of calcium and iron supports its role in photosynthesis, enzymatic actions and structural integrity, offering its known health benefits in bone health, brain function and immune modulations [36, 37]. The pulp's elevated phosphorus content aids in energy metabolism, cellular functions and membrane signalling pathways [36]. Conversely, higher levels of magnesium and potassium in CQWP indicate its morphological specialization and adaptive functions in ionic homeostasis and stress mitigation, with nutritional implications in muscle function and blood glucose regulation [36, 38]. Overall, the mineral distribution pattern CQWP > CQL > CQP indicates that peel-associated tissues act as reservoirs of essential minerals, making it a superior candidate for enhanced micronutrient and fortification applications.

GC-FID profiling of fatty acids

GC-FID-based fatty acid analysis demonstrated a predominant accumulation of unsaturated fatty acids over saturated fatty acids across all fractions. CQL had the highest total fatty acid (5.08 g/100 g) profile, driven by higher levels of polyunsaturated fatty acids (PUFA: 2.06 g/100 g) and monounsaturated fatty acids (MUFA: 1.32 g/100 g), alongside the presence of saturated fatty acids, such as stearic and palmitic acid (1.70 g/100 g). CQP followed the same trend with a dominant presence of unsaturated fatty acids (UFA: 1.99g/100g), comprised of significant alpha-linolenic acid (0.60 g/100 g) with total fatty acid (3.15 g/100 g) composition, including saturated fatty acids (SFA: 1.16 g/100 g). CQWP had a comparable (3.20 g/100 g) total fatty acids to CQP, but a higher proportion of SFAs, particularly lauric acid and myristic acids, with diminished polyunsaturated fatty acids (PUFA: 1.24 g/100 g) and monounsaturated fatty acids (MUFA: 0.63 g/100 g) concentration.

These fatty acids in aerial fractions play specific physiological and structural roles in membrane structuring, composition and energy metabolism. Synthesis of fatty acids in plastids through a series of enzymatic processes starting from acetyl-CoA that occurs in plants [39]. The CQL exhibited a higher concentration of linoleic and oleic acids, essential for maintaining the membrane fluidity and integrity in chloroplasts and thylakoids during the active photosynthetic process [40], which aligns with the higher presence in photosynthetic tissues. These unsaturated fatty acids (UFAs) are nutritionally valuable, particularly contributing to lipid-lowering functions (obesity and overweight management), thereby offering functional food applications [41]. In contrast, the pulp fraction (CQP) exhibited higher PUFA to SFA ratios, having balanced fatty acid composition with significant presence of alpha-linolenic acid, a critical ω -3 PUFA that acts as a precursor for EPA and DHA, known for its cardioprotective and brain health benefits [42]. Further, reduced saturated fatty acids (SFA) concentration enhances the functional profile of CQP. The same trend was followed by CQWP, with a higher proportion of saturated fatty acids (SFA) and a notable presence of medium-chain fatty acids (myristic and lauric acids) underscores the

lipid diversity in epidermal origin that was associated with cuticular waxes that function as a protective defense layer [43]. Although SFAs are observed cautiously, the medium-chain types are effectively involved in development for lipid membrane building blocks and act as a storage mechanism [39].

HPLC profiling of amino acids

HPLC analysis demonstrated fraction-specific variability in amino acid profiles, as shown in Fig. 1a. CQL showed the highest total amino acid profile (12.9 g/100 g), including essential amino acids such as histidine (0.07 g/100g), isoleucine (0.62 g/100g), leucine (0.65 g/100g), lysine (3.88 g/100g), methionine (0.14 g/100g), phenylalanine (0.47 g/100g), threonine (0.08 g/100g) and valine (0.31 g/100g) except tryptophan and non-essential amino acids such as aspartic acid, serine, glutamic acid, glycine, alanine, tyrosine and arginine contributes (6.68g/100g) in a total. Notably, higher concentrations of lysine (3.88g/100g), aspartic (3.32g/100g), and glutamic acids (2g/100g) were observed in the leaf fraction (CQL). The pulp fraction (CQP), contained consistent amount of total amino acid content of 10.3g/100g which comprises of essential amino acids; Histidine (0.55 g/100g), isoleucine (0.34 g/100g), leucine (0.55 g/100g), Lysine (1.98 g/100g), methionine (0.26 g/100g), phenylalanine (0.67 g/100g), threonine (0.29 g/100g) and valine (0.10 g/100g) except tryptophan and non-essential amino acids including, Aspartic acid (0.21 g/100g), Serine (1.45 g/100g), Glycine (0.39 g/100g), Glutamic acid (0.52 g/100g), Alanine (0.70 g/100g), Tyrosine (1.01 g/100g) and Arginine (1.28 g/100g). In contrast, CQWP was the lowest amino acid-yielding concentration (3.49 g/100 g) and displayed reduced levels of both indispensable (1.59g/100g) and dispensable (1.90g/100g) amino acids.

Amino acids play diverse roles in plant physiology, corresponding to developmental regulation, nutrient assimilation, defence-related mechanisms and stress response pathways [44]. CQL, enriched in lysine, glutamic and aspartic acid, contributes to its metabolic flux, active secondary metabolite synthesis under stress conditions [44]. These amino acids also contribute nutritionally, supporting calcium absorption and protein synthesis, while glutamic and aspartic acid aid in metabolic regulations and bone health [45]. The pulp (CQP) exhibited a broader distribution of both essential and non-essential amino acids with higher levels of lysine, serine, tyrosine and arginine involved in nitrogen metabolism, defense precursors and photorespiration. These compounds confer functional food benefits such as cognitive functions, neurotransmitter regulations and immune modulation [46, 47, 48, 49]. On the other hand, CQWP had the lowest amino acid profile, suggesting limited metabolism of protein-rich actions. This may be due to the peel's complex structure, which makes it harder to extract the amino acids. Nonetheless, its higher arginine content implies the role in nitrogen metabolism and stress adaptive ability, while nutritionally contributing as a free radical scavenger, immune function and wound healing properties [44]. Although CQWP is limited as a direct protein source, CQWP is better positioned for non-dietary valorization pathways, including fibre-derived biopolymers and biomaterials [50].

***In vitro* protein digestibility and nutritional quality evaluation**

The nutritional quality estimation (Table 3) further revealed compositional divergences in amino acid distribution and protein quality indices, underscoring their potential utilization in functional food innovations. Among the three, CQP depicted the highest EAAI: 50.95%, and *in vitro* protein digestibility ($53.57 \pm 2.14\%$), indicating biological value and balanced essential amino acid profiles (TEAA/TAA: 0.460; TEAA/TNEAA: 0.852), positioning the potent source in the design of protein-enriched formulations. In contrast, CQWP displayed the lowest nutritional indices, including EAAI: 18.32%, nutritional index (1.02%) and markedly declined digestibility ($19.33\% \pm 0.57$), corresponding to structural integrity and reinforcing its limited utility as a direct protein source. However, it has a higher proportion of non-essential amino acid (TNEAA/TAA: 0.544), and a high fibre source could be exploited in dietary fibre formulations. Interestingly, CQL exhibited a favourable profile, with total amino acids (12.9%), moderate digestibility ($47.55 \pm 1.23\%$), nutritional index (6.88%) and EAAI (40.93%). This suggests that the leaf matrix with a good protein and amino acid profile directly impacts nutritional quality.

Table 3: Nutritional quality evaluation of dietary protein

Amino acids quality evaluation (%)			
Parameters	CQL	CQWP	CQP
TEAA/TAA %	0.482	0.455	0.460
TNEAA/TAA %	0.517	0.544	0.539
TEAA/TNEAA ratio	0.931	0.836	0.852
TNEAA %	6.68	1.9	5.56
TEAA %	6.22	1.59	4.74
TAA %	12.9	3.49	10.3
EAAI %	40.93	18.32	50.95
Nutritional index %	6.88	1.02	5.76
<i>In vitro</i> protein digestibility %	47.55 ± 1.23^b	19.33 ± 0.57^c	53.57 ± 2.14^a

Note: TEAA: Total essential amino acid, TAA: Total amino acid, TNEAA: Total non-essential amino acid, EAAI: Essential amino acid index. CQL: *Cissus quadrangularis* L. Leaf; CQWP: *Cissus quadrangularis* L. stem with peel; CQP: *Cissus quadrangularis* L. pulp. Subscripts with different letters in a row show a significant difference ($P < 0.05$), and *in vitro* protein digestibility values are expressed in mean $\pm$ standard deviation (SD).

GC-MS profiling of secondary metabolites

GC-MS profiling of secondary metabolites using methanolic extracts of tissue-specific fractions identified diverse compounds with distinct and overlapping signatures, matched with the NIST database,

as detailed in Table 4 and Figure 2. Common compounds identified across all fractions included Neophytadiene (20.76-20.80 min), n-Hexadecanoic acid (22.42-22.47 min) and Octadecanoic acid (24.87-26.72 min). Additional shared compounds included dibutyl phthalate (23.53 min), linoleic and linolenic acids (24.58 & 24.66 min), 1,2-Benzenedicarboxylic acid (29.08 min), β -D-Glucopyranose (5.35-5.83 min) and 1,2,3-Benzenetriol (14.76-14.87 min). Distinct phytochemicals were observed across samples. CQL contained the highest proportion of Phytol (52.40%), along with unique compounds such as Glycyl-L-tryptophylglycine, Geranylgeraniol (2.47%), and Spirafolide (1.10%). 4H-Pyran-4-one was shared by CQWP (0.73%) and CQP (2.74%). 2-Pentanone and Capric acid were specific to CQWP. Notably, CQWP showed the highest linoleic and linolenic acid levels (7.42% & 14.02%), followed by CQP (2.75% & 7.82%). Minor phytoconstituents encompass CQWP compounds such as 4H-Pyran-4-one (0.73%), pentadecane (0.52%), tetradecane (0.56%), tridecane (0.41%), octadecane (1.09%), heneicosane (0.90%), and eicosanal (0.21%), all identified at retention time ranges 10.17-27.80 min and consistent with the findings [51, 52]. CQP featured propionic acid (2.26%), pentadecanoic acid (0.21%), 7-hexadecanal (1.30%), 1-docosanal (1.28%), purin-8-ol (0.51%), cholestane-3-one (0.30%), and eicosanal (0.98%) with retention times 12.51-28.84 min. CQL included propanoic acid (0.07%), Lactone G (0.33), tridecane (1.12%), hydroquinone (0.79%), 3-octanone (0.92%), tyrosine (0.06), eicosanoic acid (0.38%), coumarin (0.50%), ethyl iso-allocholate (0.90%), and geranyl linalool isomer B (0.25%) at retention times 4.42–30.03 min.

CQWP showed abundance in phenol, ketone, fatty acid derivatives, terpenoid groups, which are known to contribute to structural, stress tolerance and antioxidative properties. The presence of (α -Linolenic acid, Linolenic acid) suggests enhanced membrane fluidity and oxidative defence, and stress against environmental factors in a plant's physiology [40]. All these compounds offer anti-inflammatory, antioxidant, and cardioprotective functional benefits [42]. Additionally, CQWP contained quercitol and pyrimidinone derivatives, metabolites typically associated with stress signalling and plant growth regulators. Common bioactive compounds across all the fractions, such as Neophytadiene, a diterpene with a defence mechanism, confer functional benefits such as analgesic, antipyretic, and anti-inflammatory properties [53]. Additional shared compounds included dibutyl phthalate, reported to have antimicrobial potential [42]; 1,2-Benzenedicarboxylic acid, a phthalic acid derivative with plasticizer and antimicrobial functions [52]; β -D-Glucopyranose, a sugar compound with preservative potential [51]; and 1,2,3-Benzenetriol, a polyphenolic compound with antioxidant and antibacterial activities [54]

CQL showed the pronounced presence of phytol compared to stem fractions, underscoring its physiological role in chlorophyll metabolism and offering diverse biological activities, including anti-inflammatory, anticancer, and diuretic effects [51]. Other compounds include Glycyl-L-tryptophylglycine, a tryptophan-derived peptide, and Geranylgeraniol and Spirafolide, both terpenoid compounds with antioxidant and neuroprotective roles. 4H-Pyran-4-one, a flavonoid with antimicrobial and anti-inflammatory properties [51], was shared by CQWP and CQP. Capric acid was specific to CQWP, likely due to the higher fatty acid content in the stem peel. Minor active compounds include: CQWP comprises 4H-Pyran-4-one, pentadecane, tetradecane, tridecane, octadecane, heneicosane, and eicosanal are long-chain alkenes that are responsible for cuticular waxes as a defence mechanism [51, 52]. CQP featured

propionic acid, pentadecanoic acid, 7-hexadecanal, 1-docosanal, borneol, purin-8-ol, cholestane-3-one, and eicosanal were derivatives of fatty acids, sterols, aldehydes and terpenoids. CQL included propanoic acid, Lactone G, tridecane, hydroquinone, 3-octanone, tyrosine, eicosanoic acid, coumarin, ethyl iso-allocholate, and geranyl linalool isomer B plays a role in stress, redox balance and photosynthetic functions. Despite their low concentrations, these compounds highlight the phytochemical diversity of veldt grape tissue-specific fractions, underscoring their potential in plants' physiological roles and nutraceutical value.

Table 4: Categorized metabolite profiling of major phytochemicals of veldt grape fractions via GC-MS

Type of metabolite	Name of metabolite	Chemical formula	Retention time	Peak area %	Aerials with the presence of Compound
Ketone	2-Pentanone	C ₅ H ₁₀ O	4.06	4.05	CQWP
Phenol	Pyrogallol	C ₆ H ₆ O ₃	14.76	15.75	CQWP
	1,2,3-Benzenetriol	C ₆ H ₆ O ₃	14.87	16.85	CQP
Pyrimidinone derivative	4(1H)-Pyrimidinone	C ₄ H ₄ N ₂ O	15.12	6.61	CQWP
Polyol/Sugar alcohol	Quercitol	C ₆ H ₁₂ O ₆	15.50	3.85	CQWP
	β-D-Glucopyranose	C ₆ H ₁₂ O ₆	16.06, 16.07	5.35, 5.83	CQWP, CQP
	1,2,3-Propanetriol	C ₃ H ₈ O ₃	7.11	12.64	CQL
Fatty acids	Capric acid	C ₁₀ H ₂₀ O ₂	16.93	1.25	CQWP
	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	22.42, 22.43, 22.47	5.80, 5.88, 2.10	CQWP, CQP, CQL
	9,12-Octadecadienoic acid (ZZ)-	C ₁₈ H ₃₂ O ₂	24.57, 24.58	7.42, 2.75	CQWP, CQP
	9,12,15-Octadecatrienoic acid (Z,Z,Z)-	C ₁₈ H ₃₀ O ₂	24.66, 24.66	14.05, 7.82	CQWP, CQP
	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	24.87, 24.88, 26.72	8.57, 6.55, 1.19	CQWP, CQP, CQL
	9-Octadecanoic acid	C ₁₈ H ₃₄ O ₂	28.47	1.18	CQL
	Dodecanoic acid	C ₁₂ H ₂₄ O ₂	16.93	2.59	CQP
	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	19.83	1.22	CQP
	Neophytadiene	C ₂₀ H ₃₈	20.76, 20.76, 20.80	5.13, 9.08, 8.38	CQWP, CQP, CQL
Terpenoids	Geranylgeraniol	C ₂₀ H ₃₄ O	21.14	2.47	CQL
	Spirafolide	C ₁₂ H ₁₄ O ₄	21.69	1.10	CQL
	Phytol	C ₂₀ H ₄₀	24.29, 24.32	2.79, 52.40	CQP, CQL

Amino acids	L-Isoleucine	$C_6H_{13}NO_2$	6.96	5.51	CQL
	D-Glutamic Acid	$C_5H_9NO_4$	8.16	1.82	CQL
Flavonoid precursors	4H-Pyran-4-one	$C_5H_4O_2$	10.12	2.74	CQP
Nucleoside	Guanosine	$C_{10}H_{13}N_5O_5$	15.35	15.10	CQP
Phthalate and derivative	Dibutyl phthalate	$C_{16}H_{22}O_4$	22.53, 22.53	3.59, 3.77	CQWP, CQP
	1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	29.08, 29.09	11.76, 6.02	CQWP, CQP
Dipeptide	Glycyl-L-tryptophylglycine	$C_{15}H_{19}N_5O_5$	28.52	2.05	CQL
Lactam	2-Pyrrolidinone	C_4H_7NO	26.65	2.51	CQP

Note: CQL: *Cissus quadrangularis* L. Leaf; CQWP: *Cissus quadrangularis* L. stem with peel; CQP: *Cissus quadrangularis* L. pulp

FTIR characterization of functional groups.

FTIR spectral analysis revealed the functional characteristics, focusing on both functional group (4000–1500 cm^{-1}) and fingerprint (1500–500 cm^{-1}) regions, offering insight into their functional group complexity and bio-functional relevance, shown in Figure 3a. Five well-defined wavenumber ranges were identified. First, the broad O–H stretching band observed in the 3270–3330 cm^{-1} range, with the most intense in CQL (3277.06 cm^{-1}), indicating the presence of hydroxyl and phenolic groups associated with cellulose, supporting its higher antioxidant potential, consistent with findings by [55] followed by CQWP (3317.56 cm^{-1}), and a weaker peak in CQP (3327.1 cm^{-1}). Second, strong C–H stretching peaks in the range of 2920–2855 cm^{-1} appeared in CQWP and CQL at 2920.23 cm^{-1} and 2854.65 cm^{-1} , suggesting greater aliphatic hydrocarbon content, likely lipid-fibre network and waxy cuticular constituents, in line with the structural integrity and lignin-rich profile similar to the findings by [58], while CQP at 2918.30 cm^{-1} and 2852.72 cm^{-1} showed lower intensity, suggesting comparatively less lipid content.

Third, the C=C bonds of alkene and aromatic stretching region (1600–1500 cm^{-1}) revealed strong peaks in CQWP (1606.70 cm^{-1}), indicating the lignin signature, corresponding to defensive and mechanical integrity, followed by CQL (1579.70 cm^{-1}) and CQP (1602.85 cm^{-1}), corroborating lignin distribution patterns studied in natural fibres [56]. Fourth, the C–O stretching region (1400–1000 cm^{-1}), corresponding to higher carbohydrate-associated alkoxy bond studied by [57], exhibited high intensity in CQP, validating its higher carbohydrate content. Lastly, the fingerprint region (900–400 cm^{-1}), including a peak around 778 cm , showed C–H out-of-plane bending and demonstrated the presence of saline

residues and inorganic components, particularly in CQWP, linked with its higher mineral content and supported by [58].

Collectively, the FTIR spectral data highlight the compositional specificity: CQL is enriched with phenolics and cellulose-linked hydroxyl groups, contributing antioxidant potential; CQWP possess lignin and lipid-fibre structures corresponding to structural integrity; while CQP is dominated by carbohydrate groups with moderate phenolics. These FTIR molecular signatures closely align with the nutritional and phytochemical composition, paving the way to their application in structurally stable and bioactive functional food matrices.

XRD analysis of crystallinity index.

XRD findings demonstrated crystallinity and amorphous differences, as depicted in Figure 3b. In the 2θ range of 15° – 35° , CQWP exhibited the highest-intensity peaks (7244, 7028, 5182, 4080, 4759), indicating 93% crystallinity. CQL showed moderate peaks (50, 51, 55, 42, 39) in the 15° to 40° range, demonstrating intermediate crystallinity of 46%. CQP, on the other hand, had low-intensity peaks (52, 50, 45, 38) in the 18° – 38° range, suggesting predominantly amorphous structure (80% amorphous), associated with higher polysaccharide and lower mineral presence. Collectively, all fractions exhibited fraction-specific structural orientation.

Crystallinity results were linked with fraction-specific nutritional and structural roles. CQWP's high crystallinity likely reflects calcium oxalate deposition, high mineral and cellulose I content (also evident as crystals in SEM, mineral profile and thermal stability), suggesting potential for bone health and mineral fortification [36, 58]. CQL's intermediate crystallinity corresponds to higher phenolics and cellulose content, offering therapeutic applications against oxidative stress consistent with the study by [55, 58]. In contrast, CQP exhibited predominantly amorphous structure, attributed to its high polysaccharide and low mineral composition (as evident in proximate and morphological studies), supporting its use in functional foods targeting enhanced digestion and palatability. Collectively, XRD findings align with the morphological, thermal and nutritional profile, offering comprehensive insight into the structural attributes of each plant part.

Z-average size, potential and PDI properties of Veldt grape fractions

The colloidal behaviour was characterized using dynamic light scattering (DLS) and zeta potential measurements, revealing fraction-specific differences across aerial parts (CQL, CQP, CQWP) as described in Table 5. The hydrodynamic diameter of pulp fraction (CQP) showed the smallest particle size (229.85 μm) and relatively low polydispersity index (PDI: 0.602), indicating the most uniform particle distribution. This smaller particle size may be attributed to the softer parenchymatous and polysaccharide tissues, which are crushed into finer and homogeneous particles during milling. Conversely, the CQL and CQWP exhibited the larger Z-average sizes (1.626 μm and 1.244 μm , respectively) along with higher PDI values (1.00 and 0.945), indicating significantly higher heterogeneity

and Z-average large particle size. These characteristics may correspond to higher fibre and inherent structural complexity of these tissues, which alleviates uniform fragmentation. This aligns with the report by [59] that the fibre-like crystalline assemblies tend to disassemble poorly in aqueous formulation due to aggregation.

Zeta potential analysis revealed negative electrokinetic potential across all fractions, ranging from – 12.10 mV to – 15.25mV, indicating moderately relative colloidal stability driven by electrostatic repulsion [60]. The elevated negative surface charge was recorded in CQL (-15.25mV), which may be attributed to its higher polyphenolic profile, which contributes ionizable functional groups such as carboxyls and hydroxyls, contributing to stronger repulsive forces between particles [61]. The moderate zeta potential suggests a need for stabilizers or homogenization for long-term suspension in emulsion formulations. Furthermore, CQWP and CQP followed the same negative surface charge trend (intermediate ZP: -14.15 mV; lowest ZP: -12.00 mV, respectively). This is possibly due to surface-active polysaccharides, hydrophobic cuticular waxes, and dietary fibres, yet with less electrostatic stabilization. Collectively, these observations suggest that all fractions have intrinsic colloidal behaviour, highly pronounced in the pulp fraction due to its lower particle size and PDI value.

Functionally, these food matrices offer potential food applications according to the size, stability and dispersibility index. The pulp fraction with its submicron size and moderate electrokinetic potential offers applications in liquid-based functional foods such as smoothies and beverages, where mouthfeel and dispersibility index are crucial [62], though it may require emulsifiers for stability. The CQWP, despite moderate zeta potential and high fibre content, shows high PDI, making it applicable for semi-solid foods such as prebiotic or fibre-enriched gel formulations. The leaf fraction, with the largest and highly polydisperse particles, suggests its application in solid food formulations such as dry mix powders and antioxidant-rich bars, which require size reduction or encapsulation to improve dispersibility. Collectively, their diverse colloidal profiles suggest tailored formulation strategies with suitable matrix types for controlled release and stability [62].

Table 5			
Z-average size, electrokinetic potential and PDI properties of Veldt grape fractions			
Sample	Electrokinetic potential (mV)	PDI	Z. average size (d.µm)
CQL	-15.25 ± 1.48	1 ± 0.00	1.626 ± 0.27
CQP	-12.1 ± 0.98	0.602 ± 0.12	0.229 ± 0.00
CQWP	-14.15 ± 1.20	0.945 ± 0.07	1.244 ± 0.09

PDI: Polydispersity Index; CQL: *Cissus quadrangularis* L. Leaf; CQWP: *Cissus quadrangularis* L. stem with peel; CQP: *Cissus quadrangularis* L. pulp

Thermal properties

Thermogravimetric analysis (TGA)

The thermal stability and decomposition behaviour of aerial fractions were evaluated using TGA, as shown in Fig. 3c. The initial weight loss below 150°C (evaporation) was observed in all the fractions, with higher moisture retention in CQWP due to its fibrous matrix. All the fractions demonstrated good thermal stability till 250°C, aligning earlier findings that cissus stem fibres are resistant to decomposition until 270°C [63], these characteristics suggesting their application in thermal processing methods like baking and extrusion without degradation in the structural matrix.

The major denaturation phase (200– 350°C), which reflects hemicellulose and cellulose degradation, with a more prominent steep was observed in pulp fraction (CQP), reflecting its higher proportion of thermolabile polysaccharides and low fibre content. In contrast, CQL and CQWP exhibited gradual mass loss, reflecting stronger stability and may be associated with lignin, cellulose content consistent with the findings by [58]. Beyond 350°C, progressive degradation of resistant macromolecules such as lignin and stable phenolic complexes occurred, resulting in residual char yields of CQL (34.10%) > CQWP (30.36%) > CQP (24.72%). These residual masses directly reflect inorganic mineral and ash; CQL and CQWP retained higher inorganic content, which correlates with their higher ash and suggests application in micronutrient fortification. Collectively, the TGA thermograms of aerial fractions possess robust thermal stability, which is ideal for application in the high-temperature processing of food product formulations targeting micronutrient and antioxidant bioavailability.

Differential Scanning Calorimetry (DSC)

The thermal transition and behaviour were analysed using DSC, displayed in Fig. 3d. The DSC curves of aerial fractions exhibited endothermic peaks characterised by onset temperatures of 52.2°C, 59.1°C, and 63.5°C and end-set temperatures of 325°C, 360°C, and 365°C, respectively. The samples were heated at 10°C/min. This thermal deformation presented three key transitions: (i) an endothermic transition, (ii) a subtle enthalpic shift and (iii) an exothermic reaction. All fractions showed a primary endothermic reaction at 52–65°C, linked to the evaporation of moisture and waxes, aligned with the study reported around 89°C [58]. The broad peak area was observed in CQL (-169.1 J/g), followed by CQP (-149.6 J/g), and the least in CQWP (-114.1 J/g). This indicates the high-water retention capacity, which may be linked to fibrous, polyphenolic-rich matrices. The thermal inertness below 100°C suggests that these fractions remain structurally stable during preprocessing conditions such as grinding, blanching or pasteurisation.

The second transition observed between 160°C and 210°C, attributed to the initial degradation of thermolabile polysaccharides and proteins, is more pronounced in CQP (-24.71 J/g), aligning with an 80% amorphous matrix and is least evident in highly crystalline CQWP. Meanwhile, CQWP showed delayed and subtler transitions, indicating higher crystallinity and mineral-enriched matrices. The higher temperature exothermic reactions, ranging from 280°C to 380°C, are particularly prominent in CQWP (14.76 J/g), suggesting that the degradation of cellulose, hemicellulose, and fibre components with higher crystalline complexes closely correlates with its higher inorganic content (as evidenced by

mineral analysis, TGA outcomes), and is consistent with the findings of [58]. The trend in thermal transitions is CQWP > CQP > CQL, with later exotherm onset indicating greater stability. This trend aligns with the results from XRD and TGA. It suggests enhanced heat resistance and a robust structural matrix, supporting the application of these aerial fractions in thermally processed foods such as baked goods and extruded products.

Morphological studies

Morphological studies revealed diverse microstructural characteristics across aerial parts, shown in Fig. 4. The (A-CQL) leaf exhibited a highly heterogeneous, moderately rough matrix, with some structural damage attributed to the drying and grinding processes. The presence of fibrous layer structures suggests the presence of cellulose components in the plant leaf matrix, along with small flake-like structures that indicate oxalate crystals. The pulp (B-CQP) stayed rich in soluble fibres and polysaccharides (starch-grains) in round disk-like structures, as reported in [58]. The interconnected polysaccharide fibres provide strength, flexibility, and the detection of oxalate crystals reduces mineral bioavailability. The CQWP in image C demonstrate a rough, rigid fibrous network with shallow pores, indicating both soluble and insoluble fibres with oxalate and acicular/raphide crystals, likely contributing to a plant defense mechanism [6, 58].

The whole stem, shown in images D, E and F, revealed well-defined stomatal complexes and parenchymal cells surrounded by oxalate crystals of raphides, supporting findings by [58, 64]. The clear microstructure of the fibre with complex structural lateral pores supports the rigid composition that can be seen in image E, and is supported by the study [58]. Image F displays the distribution of stomata, and impurities such as waxes were observed in the stem fraction. Further, optical microscopic images, highlighting the acicular oxalate crystals with higher concentration, were found in CQWP (H) than CQP (G), while Image I shows the vascular network of the stem fraction, with phloem elements that are wide and compact, which includes parenchymatous cells, suggesting its role in elasticity and resistance to mechanical stress. Morphological analysis revealed distinct features that align with the XRD patterns and offer integrated insights into their structural–functional attributes and potential dietary applications.

CONCLUSION

This study addresses the critical gap in understanding the nutritional, biochemical, functional, physiochemical, and biophysical characteristics and suitability of *Cissus quadrangularis* L. (Veldt grape) aerial parts, an area underexplored despite the plant's ethnomedicinal relevance. The systematic evaluation of leaf (CQL), pulp (CQP), and stem pulp with peel (CQWP), highlighting their distinct nutritional and biophysical signatures for functional food applications. Findings revealed clear tissue-specific nutritional benefits: CQL enriched with phytochemicals, antioxidants, amino acids, protein, and oxalates with good thermal stability and moderately amorphous structure and protein digestibility; CQWP exhibited superior fibre, mineral load and antinutrient density with impeding digestibility and rigid structural functions; CQP offered a balanced nutritional profile (proximate, amino acids, fatty acids and phytochemicals) and lower antinutrients with a favorable digestibility profile and processing suitability.

Collectively, this comprehensive evaluation of aerial fractions can be aligned with modern functional food design, bridging the gap between indigenous resources and contemporary nutritional sciences. The findings demonstrate the veldt grape as a nutrient-rich, affordable and sustainable plant resource capable of contributing to a diverse dietary profile, micronutrient fortification and enhanced overall wellbeing. Nonetheless, the presence of antinutritional factors necessitates targeted processing techniques to improve the bioavailability of nutrients and broaden food inclusion strategies. Future research should therefore focus on targeted antinutrient reduction and functional food development, validation through in vitro and in vivo product application strategies and evaluate their health benefits using in vitro and in vivo studies to fully assess the functional food potential of this underutilized indigenous species.

Declarations

Data Availability

Data will be made available on request.

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Contribution

Namakkal Bommurasu Nishaant: Conceptualization, Design of work, Data curation, Formal analysis, Methodology, Resources, Writing – original draft. Kottur Senthilkumar Navin Venketeish: Review &

editing. Nagamaniammai Govindarajan: Project administration, Supervision, Validation, Writing – review & editing.

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Ethics declaration

Conflict of interest

The authors declare no conflict of interest.

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Figures

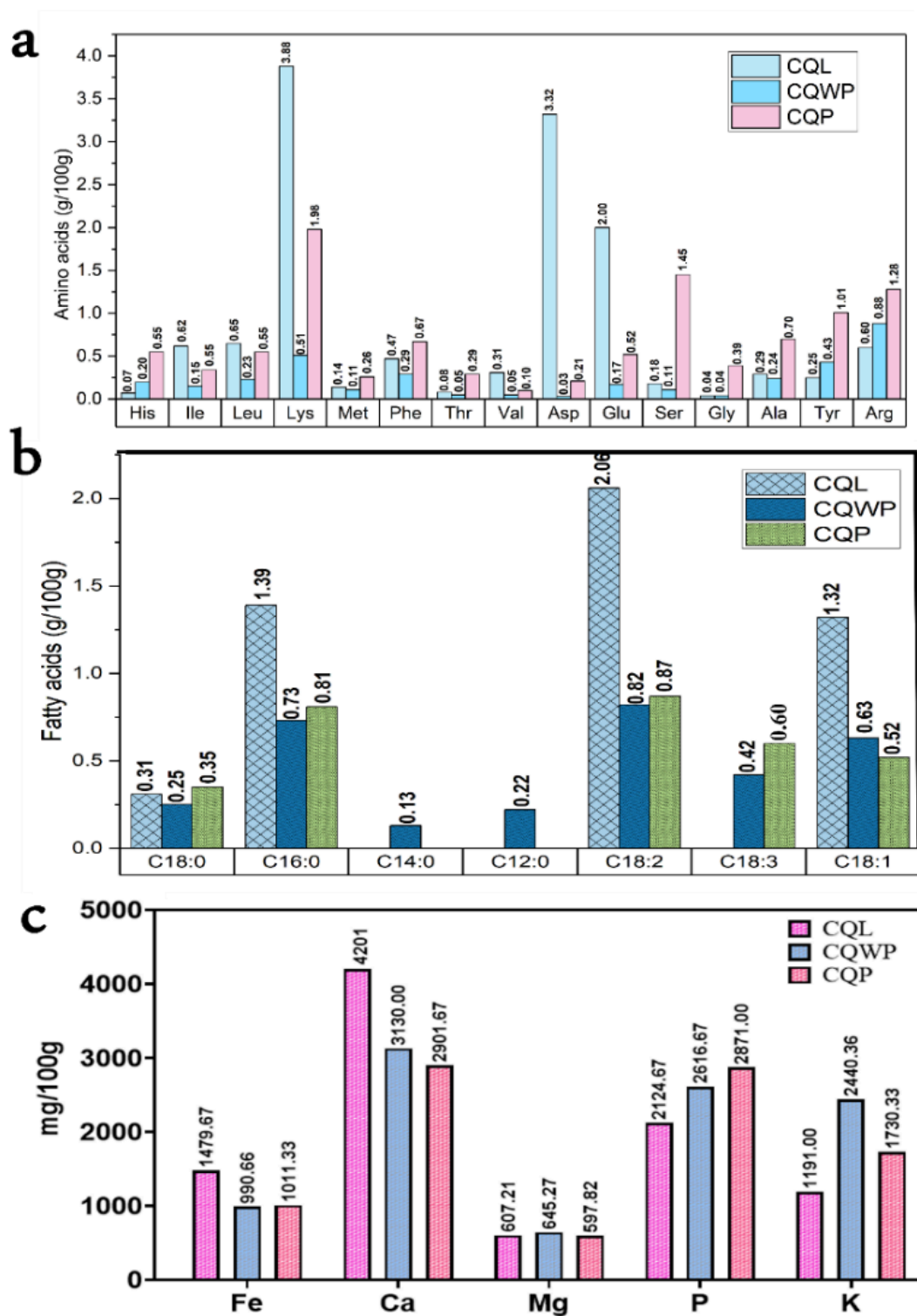


Figure 1

Amino acids, Fatty acids and Mineral profile of Veldt grape aerial fractions

Note: CQL: *Cissus quadrangularis* L. Leaf; CQWP: *Cissus quadrangularis* L. stem with peel; CQP: *Cissus quadrangularis* L. pulp, His (Histidine), Ile (Isoleucine), Leu (Leucine), Lys (Lysine), Met (Methionine), Phe (Phenylalanine), Thr (Threonine), Val (Valine), Asp (Aspartic acid), Glu (Glutamic acid), Ser (Serine), Gly

(Glycine), Ala (Alanine), Tyr (Tyrosine), and Arg (Arginine), C12:0 (Lauric acid), C14:0 (Myristic acid), C16:0 (Palmitic acid), C18:0 (Stearic acid), C18:1 (Oleic acid), C18:2 (Linoleic acid), and C18:3 (Linolenic acid).

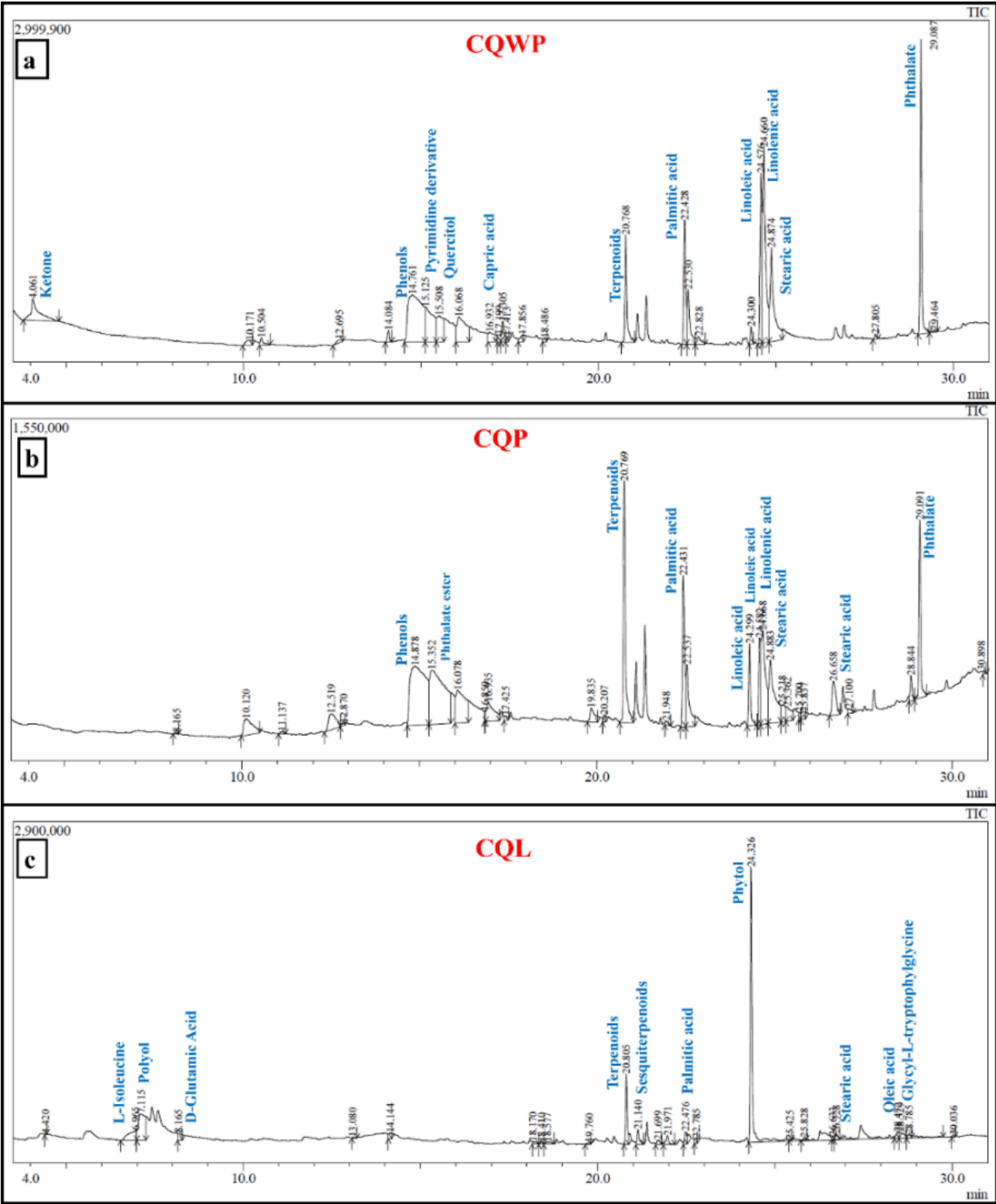


Figure 2

Phytometabolite characterization of veldt grape aerial fractions via GC-MS Chromatogram

Note: a: *Cissus quadrangularis* L. stem pulp with peel (CQWP), b: *Cissus quadrangularis* L. stem pulp (CQP), c: *Cissus quadrangularis* L. leaf (CQL)

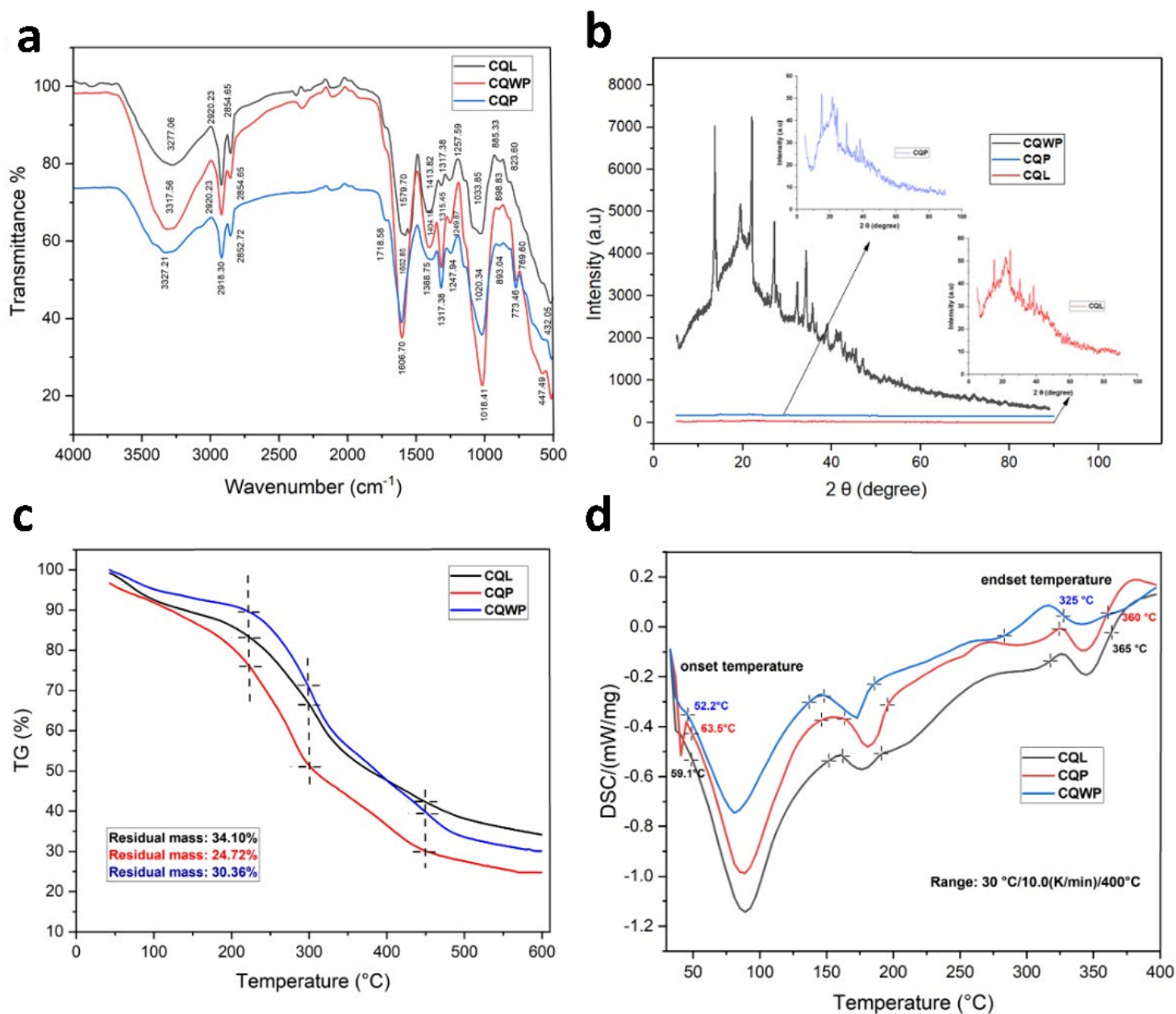


Figure 3

(a) FTIR, (b) XRD, (c) TGA and (d) DSC properties of Veldt grape aerial fractions.

CQL: *Cissus quadrangularis* L. Leaf; CQWP: *Cissus quadrangularis* L. stem with peel; CQP: *Cissus quadrangularis* L. pulp

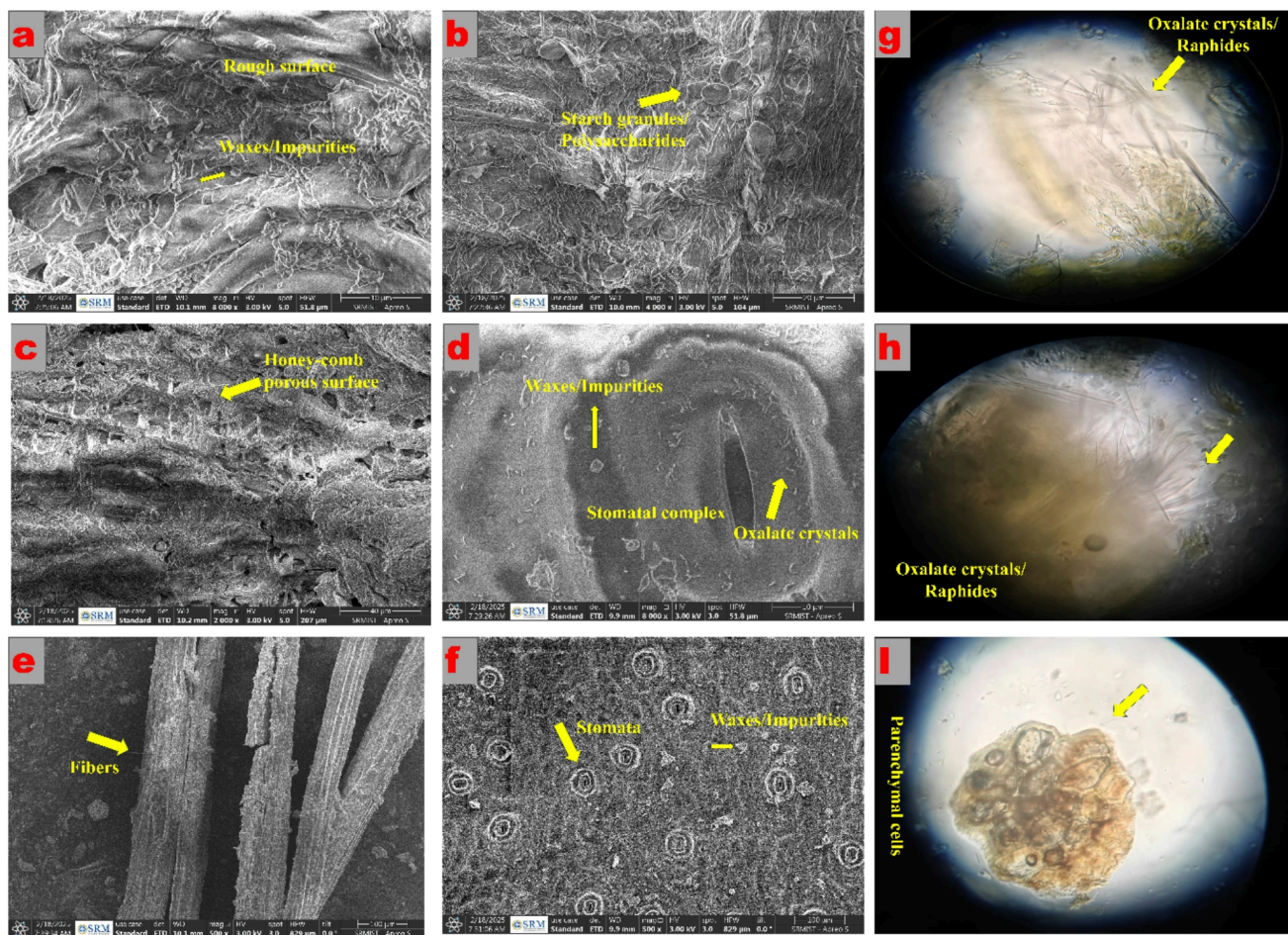


Figure 4

Morphological studies of veldt grape aerial fractions at different magnifications

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