Research Article

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# Comparative biochemical features of wild-type and purple cashew (*Anacardium occidentale* L.)

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## Abstract

The comparative biochemical features of both the wild-type and purple-coloured cashew apple varieties are presented. The total soluble sugar content in purple cashew apples was higher (13.96%) than that in normal cashew apples (6.78%). Compared with purple cashews, wild-type cashew apples have a high titratable acidity (0.224%) as they contain more ascorbic acid (342.85 mg/100 g) than purple cashew apples (228.57 mg/100 g). The total polyphenol content of purple fruit leaves (8.04 mg GAE/g), peels (4.532 mg GAE/g), and pulp (2.067 mg GAE/g) was higher than that of wild-type cashews. Additionally, the flavonoid content (9.423 mg/g in leaves, 4.923 mg/g in apple peels, and 3.688 mg/g in cashew pulp) was higher in the purple cashew than in the wild-type cashews. Chlorophyll a, b, and total chlorophyll contents in wild-type cashew leaves (0.287 mg/g, 0.176 mg/g, and 0.463 mg/g, respectively) were greater than those in purple cashew leaves. However, the chlorophyll concentration in the fruit was found to be very minimal. Although the carotenoid content of the fruit was high in the wild-type cashew (22.83 g/100 g), the carotenoid concentration in the purple cashew leaves (83.475 g/100 g) was greater than that in the normal cashew leaves. Analysis of the anthocyanin contents suggested that the leaves and peels of plants with the purple genotype had relatively high anthocyanin contents (38.499 mg cyanidin-3-glucoside equivalents/kg (C3GE/kg) and 25.87 mg C3GE/kg) compared to those of plants with the wild-type cashews (0.157 and 0.951 mg C3GE/kg, respectively). These biochemical constituents of purple cashew suggest its potential application in the development of cashew apple-based nutritional products.

***Keywords:*** purple cashew; colourant; pigments; anthocyanins

## Introduction

Cashew (*Anacardium occidentale* L.) is a common cash crop widely propagated in regions with high temperatures and humidity. Cashew is native to Brazil but is produced on a large scale in India and Vietnam. The genus *Anacardium* has approximately 60 genera and 400 related species in terms of fruit-related morphological features. These related species are found naturally in Brazil (Oliveira *et al.,* 2020). The primary product of the cashew tree is the cashew nut (the true fruit), which has a high lipid content (48.7 g/100 g) and is rich in proteins. Cashew consists of both fruit and nut parts, both of which can be used as food and for medicinal purposes due to the antioxidant activity of flavonoids and phenolics (Jeyavishnu *et al.,* 2021). Cashew apple waste is a valuable source from which pectin can be extracted. Pectin is used in the manufacturing of jams, jellies, marmalades, preserves, and similar products (Rao *et al.*, 2020). The cashew apple is highly acidic, with a pH ranging from 3.5 to 4.8. This acidity makes it a particularly rich source of vitamin C compared to most other tropical fruits. The vitamin C content in cashew apple is between 200–241 mg/100 g, which is approximately 3–5 times greater than that in citrus fruits. This high vitamin C content is used to fortify juices of other fruits characterised by low ascorbic acid contents. Raw cashew fruit contains 5% water, 30% carbohydrates, 44% fat, and 18% protein.

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As stated earlier, cashew is valued for its nuts and other by products (Jeyavishnu *et al.,* 2021). The genetic diversity within the cashew family provides a rich resource for conservation and crop improvement programs. Among the various types, pigmented cashew stands out due to its unique purple colouration covering the entire plant. This distinctive feature serves as a morphological marker and contributes significantly to genetic divergence in both quantitative and qualitative traits (Anand *et al.,* 2015; Veena *et al.*, 2023). However, comprehensive biochemical information on this purple variant of cashew is lacking. Hence, in this study, we documented the important features of the natural purple cashew mutant, which is distinct from the wild type cashew. We analysed the profiles of pigments such as chlorophyll, carotenoids, and anthocyanins in the leaves and apples of both normal and purple cashew, followed by an analysis of biochemical features, including the antioxidant profiles of wild-type and purple cashew apple.

## Materials and Methods

Wild-type cashew apple and purple cashew apple were collected from fields near Manjeshwar, Kasaragod District, Kerala, by the officials of the Extension Training Centre, Manjeshwar, and brought to the ICAR Central Plantation Crops Research Institute (ICAR-CPCRI), Kasaragod (Fig 1). Biochemical analysis included the determination of pigments, titratable acidity, ascorbic acid, total flavonoids and phenolics, total sugars and reducing sugars, and the pH of the colorants of purple cashew apple.

### Sample preparation

Two grams of sample (fruit, peel, and leaves) was extracted with 10 mL of aqueous ethanol (80:20, v/v) using an ultrasonic bath (Model LUC104, M/s Labocon Scientific Limited, Leicester, UK) for 30 minutes in the dark at 60 °C. The extract was centrifuged at 5000 × *g* for 10 minutes. The supernatant was collected, and the extraction process was repeated at least twice. The collected supernatants were combined and evaporated to dryness using a rotary evaporator. After drying, the extracts were dissolved in 5 mL of water and stored in a deep freezer (-20 °C) until further biochemical analysis. The total sugars, reducing sugars, free amino acids, phenolics, flavonoids, and antioxidant capacity of the extracts were determined following appropriate biochemical methods, as mentioned below. For the estimation of chlorophyll and carotenoid contents, 80% acetone was used as the extractant, and the samples were centrifuged to collect the supernatant. For the estimation of anthocyanin, approximately 1 g of sample was extracted with acidified methanol containing 0.1N HCl (Lee *et al.*, 2005).

### Determination of biochemical components

The phenol–sulfuric acid method was used to determine the total soluble sugar content in the extract (DuBois et al., 1956). The method described by Nelson–Somogyi was used for the determination ofreducing sugar content (Somogyi, 1952). The vitamin C content was determined by the 2,6-dichlorophenol-indophenol (DCPIP) method (AOAC 967.21, 2006). The Folin-


**Fig. 1. (a) Purple cashew leaf (b) Purple cashew apple (c) Wild-type cashew leaf (d) Wild-type cashew apple**

Ciocalteu (FC) assay, widely used in the analysis of total phenolic content, was used for analysing fruit, leaf and peel samples of cashew (Singleton *et al.,*1999). The resulting values areexpressed as mg gallic acid equivalent (mg GAE) per 100 g fresh weight. The aluminium chloride/sodium nitrite method described by Zhishen *et al.* (1999) was used for the estimation of the total flavonoid content (TFC) of cashew. Catechin (0–50 µg) was used as a standard, and the results are expressed as mg of catechin equivalent (CE) per 100 g of fresh sample. The total antioxidant potential of the cashew samples was estimated via a two-pronged approach of measuring the radical scavenging activity via the DPPH method and estimating the reducing power via the FRAP method. The DPPH (1,1'-diphenyl-2-picrylhydrazyl) radical scavenging activity was determined by following the method described by Brand-Williams *et al.* (1995). Trolox served as the standard, and the DPPH radical scavenging activity (S%) was calculated using the following equation:

S%= [(Acontrol–Asample)/Acontrol]×100;

where Acontrol is the absorbance of the blank control (containing all reagents except for the sample extract):

A sample is the absorbance of the test sample. The $SC_{50}$ concentration of the sample (*i.e.,* the concentration of the tested samples required to scavenge 50% of the DPPH radical) was calculated and expressed as µmol TE (Trolox equivalent)/100 g of sample.

The FRAP assay was conducted according to the method described by Benzie & Strain (1996). Thereaction mixture comprised sodium acetate buffer (300 mM, pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3$ solution at a ratio of 10:1:1. The reaction mixture (of 2 mL) was mixed with a known volume of sample, and the strength of colour development was measured at 593 nm after 30 min in aUV–Vis spectrophotometer.

**Determination of monomeric anthocyanins**

The pH-differential method employed for the estimation of monomeric anthocyanin content was followed for the cashew apple extracts (Lee *et al.*, 2005). The reaction was carried out using two buffer systems: (i) buffer-potassium chloride, pH 1.0 (0.025 M), and (ii) sodium acetate buffer, pH 4.5 (0.4 M). The concentrated cashew apple extracts were appropriately diluted, and a 0.1 mL aliquot of the diluted extract was transferred to a 10 mL volumetric flask and made up to 10 mL using the respective buffer. The spectrophotometric absorbance was measured at 510 and 700 nm. Total anthocyanins were calculated as cyanidin-3-glucoside equivalents (C3GE) as follows:

$$\text{Total anthocyanin} = \frac{\text{A x MW x DF x 10\^3}}{€ \text{ x1}}$$

where A=(A520 nm-A700 nm) pH 1.0-(A520 nm-A700 nm) pH 4.5

MW (molecular weight) =449.2 g/mol for cyanidin-3-glucoside (cyd-3 glu)

DF=dilution factor

1=path length in cm

€=26900 molar extinction coefficient in $mol^{-3}$ for $cyanidin^{-1}$glucoside (cyd-3-glu)

10^3 = conversion from g to mg.

**pH stability of the colorants**

The pH stability of the purple-coloured cashew apple-derived anthocyanin-based extract was measured over 5 days in 0.05M citrate buffer with a variable pH ranging from 1 to 10 by adding 0.1 M HCl and 0.1 M NaOH to different tubes. The absorbance of the extract at different pH levels was observed and measured at 510 nm and 450 nm using an UV–visible spectrophotometer.

**Results and Discussion**

The pigmented cashew is a significant genetic variant with potential as a parent in hybridisation programs aimed at producing superior varieties with appealing purple colouration. Additionally, it can serve as an ornamental tree, adding aesthetic value to landscapes. Proximate analysis of cashew apple and nut features identified CARS 8 as a suitable genotype for cultivation in Bastar region of Chhattisgarh (Rametke *et al.*, 2020). Furthermore, prior studies on the biochemical variations of this purple cashew genotype have provided preliminary information regarding its pigments and proximate

composition (Veena *et al.,* 2023). Nevertheless, this study offers a comprehensive analysis of various biochemical parameters, including the antioxidant potential and nutritionally important anthocyanin contents of the purple cashew variant.

## Sugar and acid contents

The sugar and acid profiles of the purple and wild-type cashew apples are presented in Table 1. Estimation of sugars showed that the wild-type cashew apple exhibited lower total sugars (6.78%) than the purple cashew apple (13.9%). However, the purple cashew fruit showed a lower percentage of reducing sugars, approximately 3.08%, compared to the 5.20% found in the wild type. The titratable acidity of the two cashew varieties (normal cashew and purple cashew apple) was determined by titrating against a base (0.01 N NaOH). Furthermore, the wild-type cashew apple has a higher titratable acidity (of approximately 0.224%) compared to the purple cashew, (0.197%). Consequently, the wild-type cashew fruit extract had greater ascorbic acid content (342.85 mg/100g) than purple cashew apple (228.57 mg/100 g).

**Table 1 Biochemical parameters of wild-type and purple cashew**

| Biochemical parameters | Wild-type | Purple |
|---|---|---|
| Total soluble sugar (%) | 6.78 | 13.96 |
| Total reducing sugar (%) | 5.20 | 3.08 |
| Titratable acidity (%) | 0.224 | 0.197 |
| Ascorbic acid (mg/100 g) | 342.85 | 228.57 |

## Phenolics and antioxidant potential

The total polyphenol contents of purple cashew leaves, peels, and pulp were greater (8.04, 2.07, and 4.53 mg GAE/g) than those of wild-type cashews (7.79, 1.62, and 3.47 mg GAE/g), respectively. Similarly, the total flavonoid contents of the purple cashew apple (9.42, 3.69, and 4.92 mg QE/g) were greater than those of the wild-type (6.77, 2.19, and 3.25 mg QE/g), respectively. The total antioxidant activity among the different sample extracts was determined using ferric reducing antioxidant power (FRAP) and DPPH assays. In accordance with the total polyphenol and total flavonoid contents, the FRAP activity of the purple cashew apple extract was high (73.01 mg TE/g). The radical scavenging activity, determined using the 2, 2-dipheny l-1-picrylhydrazyl (DPPH) assay, revealed that the fruits of purple cashews showed relatively high activity (22.13 mg/g), whereas the leaves of the wild type showed significantly high antioxidant potential (23.08 mg/g) (Table 2). Analysis of antioxidants in cashews during the ripening process revealed that unripe fruits had a greater content of phenolics and antioxidants than ripe fruits, suggesting that the ripening process causes the conversion of phenolics and antioxidants into other metabolites (Gordon *et al.*, 2012).

**Table 2. Total phenolics and antioxidant potential of wild-type and purple cashew**

| Biochemical parameters | Wild-type | | | Purple | | |
|---|---|---|---|---|---|---|
| | Peel | Fruit | Leaf | Peel | Fruit | Leaf |
| Total phenolic content (mg GAE/g) | 3.47 | 1.62 | 7.79 | 4.53 | 2.07 | 8.04 |
| Total flavonoid content (mg QE/g) | 3.25 | 2.19 | 6.77 | 4.92 | 3.69 | 9.42 |
| FRAP (mg Trolox equivalent/g) | ND | 60.85 | 6.79 | ND | 73.01 | 40.38 |
| DPPH Antioxidant activity (mg/g) | ND | 19.86 | 23.08 | ND | 22.13 | 19.57 |

## Pigments profile

Both the genotypes of cashew exhibited varied pigment profiles and biochemical compositions. According to the pigment profile, the total chlorophyll content in wild-type cashew leaves was significantly higher (0.4635 mg/g) than that in the leaves of purple cashew (0.172 mg/g). However, the chlorophyll content in the fruit was found to be negligible in wild type and purple cashews (0.133 mg/g and 0.0258 mg/g, respectively). The carotenoid content in purple cashew leaves was higher (83.475 µg/100 g) than that in normal cashew leaves (46.867 µg/100 g), whereas in purple cashew fruits, the carotenoid content was relatively low (20.84 µg/100 g). The anthocyanin content was greater in purple-cashew leaves and fruit peels (approximately 38.499 mg cyanidin-3-glucoside equivalents/kg and 25.874 mg cyanidin-3-glucoside equivalents/kg, respectively) than in normal cashew leaves and peels (0.157 and 0.951 mg C3GE/kg, respectively) (Table 3).

**Table 3. Pigment characteristic features of wild-type and purple cashew**

| Pigments | Wild-type | | | Purple | | |
|---|---|---|---|---|---|---|
| | Leaf | Fruit | Peel | Leaf | Fruit | Peel |
| Total chlorophyll (mg/g) | 0.4635 | 0.1333 | ND | 0.172 | 0.0258 | ND |
| Total carotenoids content (µg/100 g) | 46.867 | 22.835 | ND | 83.475 | 20.84 | ND |
| Anthocyanin content (mg C3GE/kg) | 0.157 | 0.379 | 0.951 | 38.499 | 0.487 | 25.87 |

Earlier studies on the pigmentation of yellow and red-peeled cashew apples revealed comparably low concentrations of total carotenoids (0.69–0.73 mg/100g FW) (Schweiggert *et al.*, 2016). However, the orange-peeled samples (2.2 mg/100 g FW) had higher carotenoid levels. The presence of other non-carotenoid pigments in red-peeled cashew apples has also been reported (Schweiggert *et al.,* 2016). In contrast, red-peeled cashew apples had a greater carotenoid content than yellow-coloured cashew apples, suggesting inherent variations in the pigments of cashew apples (Assunção and Mercadante, 2003). In addition, the colourants exhibited pH stability across a pH range of 3-6 beyond which a slow discolouration was observed.

## Conclusion

The comparative biochemical features of two cashew genotypes, the locally available cashew apple (wildtype) and the purple cashew apple, are presented herein. The sugar and ascorbic acid contents of the wild-type cashew apple are high. The antioxidant-conferring phytochemical constituents (phenolics and flavonoids) were more abundant in purple cashew apple than in wild-type cashew apple. Consequently, the antioxidant assay revealed that the FRAP activity was high, especially for the purple cashew apple. An assay based on DPPH activity showed that the fruit of the wildtype exhibited relatively high activity, whereas the leaves of the purple cashew showed significantly high antioxidant potential. Regarding the pigment profile, the total chlorophyll content in wild-type cashew leaves was greater than that in purple cashew leaves; however, the chlorophyll content in the fruit was negligible. The carotenoid content in purple cashew leaves was greater than that in normal cashew leaves, whereas in purple cashew leaves, the carotenoid content was lower. Nevertheless, the anthocyanin content of the purple cashew leaf and fruit peel was significantly greater than that of the wildtype, suggesting its potential for the development of nutraceutical products.

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