

## Antioxidant Activity, Ascorbic Acid, and Beta Carotene of Sumatran Red Tampoi (*Baccaurea costulata*) and Rambai (*Baccaurea motleyana*) Fruits

Erika Pardede<sup>1</sup>♥, Elisa Julianti<sup>2</sup>, Ferlist Rio Siahaan<sup>3</sup>, Claudia Virani Harefa<sup>1</sup>

<sup>1</sup>Technology of Agricultural Products Study Program, Universitas HKBP Nommensen, Medan, Indonesia

<sup>2</sup>Food Science and Technology Study Program, Universitas Sumatera Utara, Medan, Indonesia

<sup>3</sup>Agroecotechnology Study Program, Universitas HKBP Nommensen, Medan, Indonesia

♥Corresponding author: erikapardede@uhn.ac.id

**Article history:** submitted: August 5, 2024; accepted: October 31, 2024; available online: November 25, 2024

**Abstract.** This study evaluated the antioxidant activity, ascorbic acid, and beta-carotene levels in two underutilized species of Sumatran *Baccaurea* Lour. The fruit color of red tampoi or *Baccaurea costulata* (Miq.) Müll. Arg is orange, while rambai or *Baccaurea motleyana* (Müll. Arg) Müll. Arg has a pale-yellow color with an “a” value of  $29.22 \pm 0.51$  and  $2.67 \pm 0.58$ , respectively, measured with a hand chromameter. The half-maximum inhibitory concentration (IC<sub>50</sub>) of the free radical scavenging activities of both fruits was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. The IC<sub>50</sub> of *B. costulata* ( $51.63 \pm 3.42$  µg/g) indicated that its fruit juice has a strong antioxidant property, while *B. motleyana* ( $76.95 \pm 1.28$  µg/g) has a moderately antioxidant characteristic. A high antioxidant activity of *B. costulata* was followed by a high amount of ascorbic acid ( $55.86 \pm 1.73$  mg/100g) and beta carotene ( $150.77 \pm 2.16$  µg/g), in comparison to those of *B. motleyana*, which has a lower amount of ascorbic acid ( $37.30 \pm 2.34$  mg/100g) and beta carotene ( $25.36 \pm 1.37$  µg/g). It was also found that there was a moderate correlation between scavenging capacities (expressed as the reciprocal of the calculated IC<sub>50</sub> value), a strong positive correlation with beta-carotene ( $r^2 = 0.90$ ), and a moderately positive correlation with ascorbic acid ( $r^2 = 0.77$ ). It can be concluded that both *Baccaurea* fruits are nutritious foods due to their high ascorbic acid and beta-carotene content, and they also possess high antioxidant properties. Both ascorbic acid and beta-carotene contributed significantly to the antioxidant activity.

**Keywords:** antioxidant activity; free radical scavenger; phytonutrient; underutilized fruits

### INTRODUCTION

The genus of *Baccaurea* Lour, which consists of more than 47 species, belongs to *Phyllanthaceae* family. Those are native to a wide range of regions stretching from Indonesia to the Malay Peninsula, Thailand, and the Himalayan region (Saswita *et al.*, 2023). *Baccaurea* is a naturally occurring fruit-bearing plant that grows in forests, and whose fruits are regarded as underrated tropical fruits. In North Sumatera, Indonesia, those fruits are traded in markets during their season, and the most common types were *B. motleyana* (rambai), *B. polyneura* (jentikan), *B. lanceolata* (limpasu), and *B. macrocarpa* (tampoi). However, they are eventually left over and thrown away because they are not well known to the people. Despite their well-established ecological and economic benefits elsewhere, this is not the case in Sumatera (Harianja *et al.*, 2021).

People generally consume the pulp when the *Baccaurea*'s fruit is ripe. During physiological development, the color of the

peel and pulp of *Baccaurea* species changes, and the color varies depending on the pigments which give fruits their bright colors of yellow, orange, and red. The pulp color of ripe *B. polyneura* is orange, while *B. macrocarpa* is deep red, and *B. motleyana* is pale yellow. The taste of *Baccaurea* Lour species ranges from sour to sweet, which differs according to the acid content of the fruit. The ripe fruit of *B. motleyana* contains a variety of organic acids, such as citric, tartaric, malic, and oxalic acids. The fruit is also rich in various types of phytochemicals, which have been proven to have health benefits. Various *Baccaurea* species have been believed to be widely used in alternative medicine traditionally such as anti-diarrheal, anti-diabetic, anti-atherosclerotic, analgesic, anthelmintic, antiviral, etc. activities (Charu *et al.*, 2021; Khoo *et al.*, 2016; Mokhtar *et al.*, 2014).

In addition to having medicinal qualities, *Baccaurea* fruit is rich in protein, iron, vitamin C, and vitamin A among other essential nutrients, and they are comparable

as in commercial fruits. Several *Baccaurea* species viz., *B. macrocarpa*, *B. lanceolata*, and *B. motleyana* contained high level of ascorbic acid (Hossain *et al.*, 2021; Masriani & Fadly, 2022; Salusu *et al.*, 2020). Ascorbic acid, commonly known as vitamin C or ascorbate, is an essential micronutrient for humans that is essential for bone and collagenous structures. Vitamin C contributes to immune defense and provides health benefits for its strong antioxidant and anti-inflammatory properties (Bhoot *et al.*, 2023; Macan *et al.*, 2019). *Baccaurea* is also high in carotene as found in *B. polyneura*, *B. ramiflora*, and *B. motleyana* (Debnath *et al.*, 2022; Pardede & Julianti, 2023; Rohilla, 2023). Carotenoids are essential pigments in the photosynthetic organs. Beta-carotene, together with alpha-carotene, are the two primary forms of carotene. Beta-carotene occurs as an orange pigment, which can be found in yellow, orange, and red-colored fruits and vegetables. Carotenoids are important for human health as precursors of vitamin A and could scavenge reactive oxygen species, which play a role in the prevention of cancer (Maoka, 2020).

There is an increasing interest in nutrition and a healthy diet, including searching for naturally occurring antioxidants, which are assumed to have protective effects due to their properties as free radical scavengers. Beta-carotene and ascorbic acid are naturally occurring antioxidant, along with polyphenols. They have been reported as a potential to reduce risk of developing chronic and aging-related diseases. It is well known that beta-carotene and beta-cryptoxanthin function antioxidants. *B. macrocarpa* and *B. lanceolata* grown in Baufort, Sabah, Malaysia, *B. racemose*, *B. pubera*, and *B. polyneura* growing in North Sumatera exhibit antioxidant activities (Bakar *et al.*, 2014; Pardede & Julianti, 2023; Permatasari *et al.*, 2022; Shaharuddin *et al.*, 2021). Owing to their nutritional properties and phytochemical content, they are regarded as functional foods that offer health benefits beyond those of basic nutrition.

There have been limited studies concerned on Sumatran underutilized fruits, therefore the aim of the present work was to evaluate and compare the beta-carotene, ascorbic acid, and antioxidant activity of two species of Sumatran *Baccaurea*, named rambai (*Baccaurea motleyana* Müll. Arg.) and red kapul (*Baccaurea costulata* Müll. Arg). Because of the important roles of beta-carotene and ascorbic acid as potential antioxidants, the correlation of the compounds with antioxidant activities was also investigated.

## METHODS

### Materials

The *Baccaurea* fruits were collected from the local market in Toba region, North Sumatera, Indonesia, and were then promptly transported to the laboratory. Following the collection of the free seed fluids, the compositional indices, color, ascorbic acid, beta-carotene, and antioxidant activity were examined. The official AOAC method was utilized to ascertain the moisture content. Using a hand refractometer, the total soluble solid (TSS) was calculated and expressed as °Brix. Fruit pulp's color was examined using a Merk Minolta Type CR400 chromameter to determine its L, a, and b values. The acid content, expressed as mg/100 g of citric acid in fruit juice, was calculated using the titration method (Pardede & Julianti, 2023)

### Ascorbic acid

The ascorbic acid level was ascertained by volumetric titration with 2,6-dichloroindophenol (Nielsen, 2017). To simplify, dissolve 50 mg of sodium salt in 2,6-dichlorophenol indophenols, then add 50 mL of water containing 40 mg of sodium bicarbonate. The mixture was diluted with 200 mL of distilled water and then used as a dye. Into a 50-mL Erlenmeyer flask, 5 mL of a 3% metaphosphoric acid-acetic acid was added, and then add 5.0 mL of a 10% ascorbic acid standard solution to the flask. Slowly add indophenol solution to standard ascorbic acid solution until a light but distinct rose-pink

color appears and persists, and calculate the volume of dye used. The dye factor was calculated using the following **Equation 1**.

$$\text{Dye factor} = 0.5 / \text{Titre} \dots\dots\dots 1)$$

Pipet into a 100-mL Erlenmeyer flask 50 mL of metaphosphoric acid-acetic acid solution and 2 mL of juice sample. The mixture was homogenized for two minutes, filtered, and centrifuged at 4000 rpm for 15 minutes. The supernatant was collected and put into a 100-mL volumetric flask, and the volume was adjusted to the mark with metaphosphoric acid to make up the final volume of 100 mL. Five mL of the aliquot was titrated against the 1,2-dichloro-6-indophenol dye solution until a rose-pink color that persisted for about 15 seconds was obtained. The ascorbic acid content of the samples was calculated using **Equation 2**.

$$\text{Ascorbic acid (mg/ml)} = [\text{Titre} \times \text{DT} \times \text{Vm} (\text{mL}) \times 100] / [\text{Ve} (\text{mL}) \times \text{M}(\text{g})] \dots\dots 2)$$

- DF = Dye factor
- Vm = Volume made up (mL)
- Ve = Volume extract (mL)
- M = Sample weight (g)

### Beta-carotene

The assay was modified from (Hagos *et al.*, 2022). First, a beta-carotene standard solution was prepared for the UV-visible spectrophotometer method. In brief, a 10 µg/mL standard stock solution of beta-carotene was prepared by dissolving 2.5 milligrams of beta-carotene, 2.5 mL of chloroform, and petroleum benzene to make up 250 mL in a volumetric flask. From this stock solution, serial dilutions were made to obtain 0.5, 1, 1.5, 2, and 2.5 µg/mL of beta-carotene. The absorbance of standard solutions was scanned using a UV-visible spectrophotometer in the range of 450 nm. The standard graph was established by plotting concentration against the absorption

peak maximum obtained at 450 nm and used as the calibration curve.

As for the sample preparation for beta-carotene analysis, a 5 g sample was weighted in a glass test tube. Then, 75 mL of 12% potassium hydroxide was added to it and allowed to stand for 15 minutes before adding 15 mL of petroleum benzene. It was shaken for 30 seconds to promote phase separation. Following the addition of 3 mL of sodium sulfate (5% solution) and 15 mL of petroleum benzene, the mixture was then shaken again. Then, 2.5 mL of the supernatant was collected and introduced to a conical flask with 7.5 mL of petroleum benzene. Three milliliters of cold acetone were combined with 1.5 milliliters of the mixture in a test tube. The absorbance of the extract was determined at 450 nm wavelength in a UV-Vis spectrophotometer. Beta-carotene content in the sample (µg/g) was calculated using a calibration curve.

### Antioxidant activity (IC<sub>50</sub>)

The assay was modified from (Baliyan *et al.*, 2022). In brief, serially diluted several concentrations of fruit juice in methanol (50, 100, 150, 200, and 250 µg/mL) were prepared in sterile test tubes. One milliliter of each serially diluted fruit juice was put in an Eppendorf tube, to which one milliliter of DPPH solution was added. Methanol was then added to each to make up a final volume of 5 mL. As for the blank, only the extraction solvent was added to one milliliter of DPPH. All samples were mixed evenly by shaking and allowed to stand at 37°C for 30 minutes. Their absorbance was read at 517 nm with an UV-visible spectrophotometer. The scavenging activity was calculated using **Equation 3**.

$$\text{DPPH radical scavenging activity (\%)} = [(\text{Ao} - \text{As}) / \text{Ao}] \times 100\% \dots\dots 3)$$

Note:

Ao = Abs. Blank is the absorbance of the control (all reagents except fruit juice)

As = Abs. Sample is the absorbance of the sample solution (all reagents with fruit juice).

The standard graph was established by plotting the DPPH scavenging activity percentage as a function of the concentration. The equation derived from the graph was used to define the DPPH antiradical activity (IC<sub>50</sub>) of the various samples

#### Data analysis

Quantitative data are presented as mean values with the respective standard deviation. The correlation of the compound with antioxidant activities was calculated using the MS Excel Program.

## RESULTS AND DISCUSSION

### Compositional Indices

The moisture content of the rambai pulp (*Baccaurea motleyana* Müll. Arg) was 84.59 g/100g, which was comparable to the figure of 83.7% recorded by (Debnath *et al.*, 2022). It was higher, however, than tampoi merah (*Baccaurea costulata* (Miq) Müll. Arg.) (74.4

g/100g). Both are in a similar range to other *Baccaurea*, as reported previously: *Baccaurea* fruits (*B. polyneura*, *B. lanceolata*, and *B. macrocarpa*) contained moisture of 76.73 g/100g, 92.4 g/100g, and 61.9 g/100g, respectively (Bakar *et al.*, 2014; Pardede & Julianti, 2023)

As reported in **Table 1**, *B. costulata* contained a higher total soluble solid (TSS) (25.43 °Brix) than *B. motleyana* (14.83 °Brix). In respect of titratable acidic (TA), both species of *Baccaurea* were similar and reached 4.10 g/100g to 4.41 g/100g, expressed in g citric acid in 100 g FW. The TSS/TA ratio of *B. motleyana* was lower than that of *B. costulata*. The low TA value and low TSS value of *B. motleyana* seem to explain the low sugar/acid ratio (3.36) of this fruit. On the other hand, the TSS of *B. costulata* is higher, while the TA is lower than that of *B. motleyana*, resulting in a higher sugar/acid ratio. These facts explain the very high sour/weakly sweet taste of the *B. motleyana* fruit, while the *B. costulata* fruit has a sweet taste at the ripe stage.

**Table 1.** Compositional indices of *B. costulata* and *B. motleyana*

Composition indices	<i>B. costulata</i>	<i>B. motleyana</i>
Moisture content (%)	74.45±0.35	84.59±0.25
Total soluble solid (°Brix)	25.43±0.06	14.83±0.29
Titratable acid (g.100g <sup>-1</sup> ) *	4.10±0.87	4.41±0.51
TSS/TA	6.20	3.36

\*Total acid (TA) expressed in g citric acid in 100 g FW

Fruit acidity is mainly regulated by organic acids, and titratable acidity was normally used as a predictor of sourness, while TSS (°Brix) predicted sweetness. *Baccaurea* species have a wide variation in taste, from acidic to sweet. The interaction of sweetness and sourness determines taste perception. Sweetness and sourness suppress each other. Sucrose suppressed the sourness intensity ratings of citric acid and tartaric acid (Junge *et al.*, 2020), while citric acid also suppressed the sweetness of sucrose (Junge *et al.*, 2023). In fruits, sugars such as glucose, sucrose, and fructose contribute to their

sweetness. In fruits, sugars such as glucose, sucrose, and fructose contribute to their sweetness. It has been found to be the major sugar in *B. motleyana* Müll. Arg is glucose. On the other hand, the acidity of most ripe, fleshy fruits is mainly due to the presence of organic acids that contribute to their sour taste. The main organic acids found in most fruits are malate acid (in grapes, tomatoes, and in particular apples) and citric acid in citrus (Huang *et al.*, 2021)

The accumulation of organic acid is varied according to species, cultivar, genetic, and agri-environmental factors (Etienne *et*



*al.*, 2013). Nutrient availability, such as nitrate, affected the synthesis of organic acids. (Zhang *et al.*, 2020) identified a key regulator protein, MdBT2, played an important role in nitrate signalling. High nitrate fertilizer suppressed the malate accumulation in apples and produced a mild and sweet apple. It has been suggested that suggested a pH-associated gene, called CrMYB73, was positively correlated with citric acid accumulation, while citric acid was the main organic acid in *B. ramiflora* (Chen *et al.*, 2022) and in *B. motleyana* Hook. F. (Mokhtar *et al.*, 2014). Regarding organic acid metabolism, research indicated that regarding the transport mechanism of organic acids as well as the substances involved, much remains to be explored (Huang *et al.*, 2021). The study on the taste compounds of *B. ramiflora* Lour indicates that the main primary metabolites, such as *L*-sorbose, *D*-

(+)-glucose, citric acid, *L*-phenylalanine, oleamide, and  $\alpha$ -eleostearic acid, together with secondary metabolites such as phenolic compounds, play a significant role in determining the taste. The differences in composition and concentration of carbohydrates, organic acids, fatty acids, and phenolic compounds might be the primary causes of the differences in taste between the white- and pink-fleshed variants of *B. ramiflora* (Chen *et al.*, 2022).

#### Color indexes.

The color indexes of the two samples are given in Table 2. *Baccaurea motleyana* had a positive hue angle ( $86.21 \pm 1.83$ ), which indicates that the color of *B. motleyana* tends to be yellow, while the hue of *B. costulata* ( $50.59 \pm 0.9$ ) indicates that the color of *B. costulata* tends to be more red.

**Table 2.** Color parameters of *B. costulata* and *B. motleyana*

	L	a	b	Hue
<i>Baccaurea motleyana</i>	51.00±088	2.67±058	23.22±0.39	86.21±1.83
<i>Baccaurea costulata</i>	34.89±1.35	29.22±0.51	33.66±1.15	50.59±0.97

L: Lightness, a: Red-Green range, b: Blue-yellow range

The hue of color angle is expressed in degrees from 0 to 360, indicating the color as red = 0, yellow = 90, green = 180, and blue = 270 (Susmitha *et al.*, 2022). The degree of redness and yellowness was indicated by both a positive “a” value and a negative “b” value, respectively. The “a” value of *B. costulata* ( $29.22 \pm 0.51$ ) is greater than the value of *B. motleyana* ( $2.67 \pm 0.58$ ), proving that the degree of redness of the pulp color of *B. costulata* was greater than that of *B. motleyana*. Similarly, for the “b” value, the yellowness of *B. costulata* was higher than that of *B. motleyana*. This color index proved the whitish-to-pale yellow color of *B. motleyana* and the yellow-to-red color of *B. costulata*. Moreover, the L-value represents lightness in color. The color of *B. costulata* (L = 34.89) is darker than that of *B. motleyana* (L = 51.00). We could detect the difference if we were viewing those two

colors (**Figure. 1**). It is commonly known that the pigment called anthocyanin gives fruit its red color, while carotene gives it its yellow color.

Genus *Baccaurea* Lour., which consists of various species, has a variety of fruit pulp colors, which vary from milky white to blood red (Mokhtar *et al.*, 2014). The overall color perception of fruit pulp is determined by the pigment compounds contained in the pulp. Both investigated *Baccaurea* species contained a high amount of beta-carotene (Table 3), which indicates that the color of fruit pulp is affected by beta-carotenoid, a primary form of carotenoids (Maoka, 2020). The contribution of flavonoids and anthocyanidin to the overall color of fruit pulp should also be recognized (Charu *et al.*, 2021), although they were not tested in this study. (Hu *et al.*, 2016) clearly revealed the roles of MdMYB1, a positive regulator of

anthocyanin biosynthesis, which transcriptionally activates proton pumps and secondary transporters in modulating cellular

pH and vacuolar accumulation of anthocyanins and malate.



**Figure 1:** Fruits of *B. montleyana* and *B. costulata*

The composition of pigment compounds can vary significantly across different fruit species as well as within species, given that the genetic background determines the ability of fruits to produce certain pigments. However, the production of plant secondary metabolites, including flavonoids, was also strongly influenced by environmental conditions as part of stress coping mechanisms. Environment stress will affect the metabolism, including the pigmentation process in plants (Espley & Jaakola, 2023).

Water supply and direct exposure to light are very important for the biosynthesis of carotenoids and anthocyanin. Water deficiency or drought stress can reduce both anthocyanin and carotenoids (Jiang *et al.*, 2020), although (Espley & Jaakola, 2023) in their review revealed that in some cases, water deficiency led to an increase in beta-carotene and lycopene. Little to moderate light stress can be beneficial to some fruits, for example, increasing the levels of anthocyanin and carotenoids in apples. In cases of temperature stress, it has been shown that high temperatures repress transcription of MYB10, which inhibits anthocyanin biosynthesis; however, the opposite has been found in some cases where anthocyanin and carotenoids decrease under cold stress. Aside from genetic factors, the severity and duration of environmental stress received by plants dictate the overall composition of pigment in plants (Espley & Jaakola, 2023).

### Ascorbic acid

*Baccaurea costulata* has a higher ascorbic acid concentration of  $55.86 \pm 1.73$  mg/100g in comparison to *B. montleyana*, which had  $37.30 \pm 2.34$  mg.100g<sup>-1</sup> (FW). (Table 3). It was shown that compared to strawberries, which are considered to have the highest content of ascorbic acid, whose value generally ranges from 5 to 50 mg/100 g fresh weight (FW), both of those underutilized fruits have a considerable amount of ascorbic acid (Skrovankova *et al.*, 2015). Both *Baccaurea* fruits contained considerably high levels of ascorbic acid, which was significant enough to meet the minimum requirement of 10 mg daily for preventing the clinical signs of specific deficiency-scurvy in adults.

### Beta carotene

In this study, a considerable amount ( $25.36 \pm 1.37$  µg/g (w/w)) of beta-carotene (a major carotenoid) was detected in the whitish-colored fruit of *B. motleyana*. Meanwhile, beta-carotene accumulated by the orange-red-colored fruit of *B. costulata* reached  $150.77 \pm 2.16$  µg/g (w/w), which was much higher than that of *B. montleyana*. In comparison to other species of *Baccaurea*, both investigated *Baccaurea* species had higher beta-carotene contents than *B. polyneura* ( $18.55$  µg/g w/w) (Pardede & Julianti, 2023) and were comparable to *B. lanceolata* ( $0.67$  mg/g dry sample) and *B.*

*macrocarpa* (0.69 mg/g dry sample) (Bakar et al., 2014). Both *Baccaurea* are also comparable to carrot, a carotene-rich yellow-orange vegetable, which contains 57.58 µg/g beta-carotene (Orjiakor et al.,

2023). Together with ascorbic acid, beta-carotene is a powerful naturally antioxidant that has the ability to scavenge free radicals.

**Table 3.** Ascorbic acid, beta-carotene and antioxidant activity of *B. costulata* and *B. motleyana*

	Ascorbic acid mg/100g	Beta carotene mg/g (w/w)	IC <sub>50</sub> (DPPH) µg/g
<i>Baccaurea motleyana</i>	37.30±2.34	25.36±1.37	76.95±1.28
<i>Baccaurea costulata</i>	55.86±1.73	150.77± 2.16	51.63±3.42
<i>Baccaurea polyneura</i> *	54,85	185.55	34.17

\* Values are expressed as means of triplicate determination ± standard deviation.

\*\* (Pardede and Julianti, 2023)

### Antioxidant activity

The DPPH method was used to calculate the antioxidant capacity, which is then interpreted using the IC<sub>50</sub> value, or the concentration at which 50% of the scavenging activity is present. Hence, a sample's scavenging activity decreases with increasing IC<sub>50</sub> value. a compound could be considered to have strong antioxidant activity when its IC<sub>50</sub> is less than 50 µg/mL (Fatmawaty et al., 2019). In our investigation, the IC<sub>50</sub> values for *B. montleyana* and *B. costulata* were determined to be 76.95±1.28 and 51.63±3.42 µg/g, respectively (Table 3). It means that the strong antioxidant property of *B. costulata* outperformed *B. montleyana* in terms of scavenging activity among those two species. It is higher than their relatives previously observed for *B. macrocarpa* (97.37±0.2 g/ml FW) (Bakar et al., 2014) and *B. lanceolata* (94.36±0.02 g/mL FW), both of which were grown in Baufort, Sabah, Malaysia. Meanwhile, *B. costulata* has more scavenging capacity than *B. polyneura* (34.17 µg/g) growing in North Sumatera (Pardede & Julianti, 2023)

The measurement of antioxidant scavenge activity in the DPPH-test was based on the neutralization of the DPPH-radical formed in organic solvent by electrons donated from the antioxidant substance in the sample. Some researchers suggest modifications and apply various adjustments

to the analytical protocol to improve its sensitivity and minimize the interference with possible compounds that may occur in order to increase the reliability of the results. The DPPH method was still applied due to its simple technique, easy to perform, quick, reproducible, and adequate for the comparison evaluation of compounds and extracts (Munteanu & Apetrei, 2021).

In addition to the DPPH method, several protocols are available to use to assess capacity action as an ROS scavenge in vivo system, using the oxygen-radical-absorbance capacity (ORAC), total radical-trapping antioxidant parameter (TRAP)-related protocols, and ABTS assays. The ORAC and TRAP methods determine the capacity of an antioxidant to quench free radicals by donating a hydrogen atom (H). Meanwhile, ABTS assay evaluation is based on the ability of antioxidant molecules to quench the long-lived ABTS (applies 2, 2'-azino-bis-3-ethylbenz-thiazoline-6-sulfonic acid) radical cation (Pérez-Gálvez et al., 2020). Due to the different reaction mechanisms involved in different assays, differences in performance may arise.

It was found that a higher DPPH value than the ones obtained by ABTS and FRAP, and these antioxidant activity values varied among the 12 tested plant species (Chaves et al., 2020). Meanwhile, Sridhar & Charles (2018) revealed that the IC<sub>50</sub> values of DPPH

showed merely a correlation with ABTS ( $r = 0.63$ ) and FRAP ( $r^2 = 0.60$ ) when applied to grapes (Sridhar & Charles, 2018). In contrast, it was found strong positive correlation ( $r^2 > 0.75$ ) between the FRAP and ABTS assays, while the DPPH assay showed only weak correlations with the FRAP and ABTS assays ( $r^2 < 0.5$ ) when assessing the antioxidant capacity of lignin (Rumpf *et al.*, 2023). Therefore, it is important to select the proper antioxidant activity quantification method, considering the medium's objectives and conditions.

In this study, we discovered that there is a moderate correlation between ascorbic acid ( $r^2 = 0.77$ ) and scavenging capacities (expressed as the reciprocal of the calculated  $IC_{50}$  value) in *Baccaurea* but a strong positive correlation between beta-carotene ( $r^2 = 0.90$ ) and scavenging capacities by combining data from the current study and the previous study of (Pardede and Julianti, 2023) on *B. polyneura*. Based on current study, there is a moderate correlation between ascorbic acid ( $r^2 = 0.77$ ) and scavenging capacities (expressed as the reciprocal of the calculated  $IC_{50}$  value) in *Baccaurea* but a strong positive correlation between beta-carotene ( $r^2 = 0.90$ ) and scavenging capacities by combining data from the current study and the previous study on *B. polyneura* (Pardede and Julianti, 2023).

The findings indicated that ascorbic acid and beta-carotene significantly contribute to antioxidant activities. Free radicals, or reactive oxygen species (ROS), are produced during normal metabolism and also when inflammation in cells occurs. Excess free radicals are often associated with oxidative stress-related tissue damage and the pathogenesis of inflammation, which has been implicated in various diseases such as cancer, cardiovascular disorders, and neurological disorders. Antioxidants such as ascorbic acid and carotenoid act to neutralize the free radical, or ROS, by donating electrons to neutralize the ROS and discontinue the radical propagation chain (Edge & Truscott, 2018; Pérez-Gálvez *et al.*, 2020).

Antioxidant capacity was also contributed by the total phenolic content of *Baccaurea* fruit, although there was ambiguity regarding their correlation. The total phenolic content and the DPPH assay of *B. lanceolata* and *B. macrocarpa* showed a moderate correlation ( $r^2 = 0.794$ ) and the flavonoid content ( $r^2 = 0.796$ ) (Bakar *et al.*, 2014), meanwhile *B. racemosa* pulp have no significant correlation with their values of DPPH free radical scavenging activity (Permatasari *et al.*, 2019).

## CONCLUSION

The results of the study show that *B. costulata* and *B. montleyana* exhibit strong and moderate antioxidant activity, respectively, in addition to being rich in ascorbic acid and beta-carotene. Special emphasis was placed on the orange flesh of *B. costulata*, which contained higher levels of beta-carotene as well as ascorbic acid. Additionally, there was a strong association between the amounts of beta-carotene and ascorbic acid and their capacity to scavenge free radicals. According to this novel study, both underused fruits, typically thrown away, are rich in antioxidants and should be considered to be used in culinary products while enhancing their commercial value.

## ACKNOWLEDGEMENTS

Thanks to Universitas HKBP Nommensen for funding this research, and to the Food Technology Laboratory at Department of Food Technology, Universitas Sumatera Utara, Indonesia where this research was conducted.

## REFERENCES

- Bakar, M. F. A., Ahmad, N. E., Karim, F. A., & Saib, S. (2014). Phytochemicals and antioxidative properties of borneo indigenous liposu (*Baccaurea lanceolata*) and tampoi (*Baccaurea macrocarpa*) fruits. *Antioxidants*, 3(3),



- 516–525.  
<https://doi.org/10.3390/antiox3030516>
- Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. *Molecules*, 27(1326). <https://doi.org/10.3390/molecules27041326>
- Bhoot, H. R., Zamwar, U. M., Chakole, S., & Anjankar, A. (2023). Dietary Sources, Bioavailability, and Functions of Ascorbic Acid (Vitamin C) and Its Role in the Common Cold, Tissue Healing, and Iron Metabolism. *Cureus*. <https://doi.org/10.7759/cureus.49308>
- Charu, T. K., Chowdhury, N. S., Fatema. Ismat Benta, Liya, F. I., & Salsabil, L. (2021). Traditional and Pharmacological Reports of The Genus *Baccaurea*. A Review. *American Journal of Biomedical Science & Research*, 11(6), 494–508. <https://doi.org/10.34297/ajbsr.2021.11.001683>
- Chaves, N., Santiago, A., & Alías, J. C. (2020). Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used. *Antioxidants*, 9(1). <https://doi.org/10.3390/antiox9010076>
- Chen, J., Wu, F., Wang, H., Guo, C., Zhang, W., Luo, P., Zhou, J., Hao, W., Yang, G., & Huang, J. (2022). Identification of key taste components in *Baccaurea ramiflora* Lour. fruit using non-targeted metabolomics. *Food Science and Human Wellness*, 12(1), 94–101. <https://doi.org/10.1016/j.fshw.2022.07.027>
- Debnath, P., Ahmad, S. K., Mahedi, R. A., Ganguly, A., & Sarker, K. K. (2022). Bioactive compounds and functional properties of Rambai (*Baccaurea motleyana* Müll. Arg.) fruit: A comprehensive review. *Food Science and Nutrition*, 10(1), 218–226. <https://doi.org/10.1002/fsn3.2661>
- Edge, R., & Truscott, T. G. (2018). Singlet oxygen and free radical reactions of retinoids and carotenoids—A review. In *Antioxidants* (Vol. 7, Issue 1). MDPI. <https://doi.org/10.3390/antiox7010005>
- Espley, R. V., & Jaakola, L. (2023). The role of environmental stress in fruit pigmentation. In *Plant Cell and Environment* (Vol. 46, Issue 12, pp. 3663–3679). John Wiley and Sons Inc. <https://doi.org/10.1111/pce.14684>
- Etienne, A., Génard, M., Lobit, P., Mbéguié-A-Mbéguié, D., & Bugaud, C. (2013). What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. In *Journal of Experimental Botany* (Vol. 64, Issue 6, pp. 1451–1469). <https://doi.org/10.1093/jxb/ert035>
- Fatmawaty, Anggreni, N. G. M., Fadhil, N., & Prasasty, V. D. (2019). Potential in Vitro and in Vivo Antioxidant Activities from *Piper crocatum* and *Persea americana* Leaf Extracts. *Biomedical and Pharmacology Journal*, 12(2), 661–667. <https://doi.org/10.13005/bpj/1686>
- Hagos, M., Redi-Abshiro, M., Chandravanshi, B. S., & Yaya, E. E. (2022). Development of Analytical Methods for Determination of  $\beta$  - Carotene in Pumpkin (*Cucurbita maxima*) Flesh, Peel, and Seed Powder Samples. *International Journal of Analytical Chemistry*, 2022. <https://doi.org/10.1155/2022/9363692>
- Harianja, A. H., Sinaga, A. M., Hawari, F. A., & Fauzi, R. (2021). The importance of the utilization of forest fruits in Batak Toba community. *Indonesian Journal of Forestry Research*, 8(1), 1–12. <https://doi.org/10.20886/IJFR.2021.8.1.1-12>
- Hossain, M. M., Rahim, M. A., & Haque, M. R. (2021). Biochemical properties of some important underutilized minor fruits. *Journal of Agriculture and Food*

- Research*, 5.  
<https://doi.org/10.1016/j.jafr.2021.100148>
- Hu, D. G., Sun, C. H., Ma, Q. J., You, C. X., Cheng, L., & Hao, Y. J. (2016). MdMYB1 regulates anthocyanin and malate accumulation by directly facilitating their transport into vacuoles in apples. *Plant Physiology*, 170(3), 1315–1330.  
<https://doi.org/10.1104/pp.15.01333>
- Huang, X. Y., Wang, C. K., Zhao, Y. W., Sun, C. H., & Hu, D. G. (2021). Mechanisms and regulation of organic acid accumulation in plant vacuoles. In *Horticulture Research* (Vol. 8, Issue 1). Springer Nature.  
<https://doi.org/10.1038/s41438-021-00702-z>
- Jiang, W., Li, N., Zhang, D., Meinhardt, L., Cao, B., Li, Y., & Song, L. (2020). Elevated temperature and drought stress significantly affect fruit quality and activity of anthocyanin-related enzymes in jujube (*Ziziphus jujuba* Mill. cv. 'Lingwuchangzao'). *PLoS ONE*, 15(11 November).  
<https://doi.org/10.1371/journal.pone.0241491>
- Junge, J. Y., Andersen, G. H., & Kidmose, U. (2023). Suppression of sweetness: evidence for central mechanism for suppression of sweetness from sucrose by citric acid. *Chemical Senses*, 48.  
<https://doi.org/10.1093/chemse/bjad036>
- Junge, J. Y., Bertelsen, A. S., Mielby, L. A., Zeng, Y., Sun, Y. X., Byrne, D. V., & Kidmose, U. (2020). Taste interactions between sweetness of sucrose and sourness of citric and tartaric acid among Chinese and Danish consumers. *Foods*, 9(10).  
<https://doi.org/10.3390/foods9101425>
- Khoo, H. E., Azlan, A., Kong, K. W., & Ismail, A. (2016). Phytochemicals and Medicinal Properties of Indigenous Tropical Fruits with Potential for Commercial Development. In *Evidence-based Complementary and Alternative Medicine* (Vol. 2016). Hindawi Limited.  
<https://doi.org/10.1155/2016/7591951>
- Macan, A. M., Kraljević, T. G., & Raić-malić, S. (2019). Therapeutic perspective of vitamin C and its derivatives. In *Antioxidants* (Vol. 8, Issue 8). MDPI.  
<https://doi.org/10.3390/antiox8080247>
- Maoka, T. (2020). Carotenoids as natural functional pigments. In *Journal of Natural Medicines* (Vol. 74, Issue 1). Springer.  
<https://doi.org/10.1007/s11418-019-01364-x>
- Masriani, & Fadly, D. (2022). Nutritional profiles of *Baccaurea macrocarpa* fruit. *Food Research*, 6(2), 202–208.  
[https://doi.org/10.26656/fr.2017.6\(2\).273](https://doi.org/10.26656/fr.2017.6(2).273)
- Mokhtar, S. I., Chee Leong, P., Ee Ven, L., Ain Abd Aziz, N., & Seri Intan Mokhtar, A. (2014). Total Phenolic Contents, Antioxidant Activities and Organic Acids Composition of Three Selected Fruit Extracts at Different Maturity Stages. *J. Trop. Resour. Sustain. Sci*, 2, 40–46.
- Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. In *International Journal of Molecular Sciences* (Vol. 22, Issue 7). MDPI AG.  
<https://doi.org/10.3390/ijms22073380>
- Nielsen, S. S. (2017). *Vitamin C Determination by Indophenol Method* (pp. 143–146).  
[https://doi.org/10.1007/978-3-319-44127-6\\_15](https://doi.org/10.1007/978-3-319-44127-6_15)
- Orjiakor, S. N., Obiora, C. U., Ezembu, E. N., Okocha, S. K., Ezegbe, C. C., Ezech, M. C., & Orjika, G. C. (2023). Improving the Beta-carotene Content of Carrots through Process Optimization. *Journal of Advances in Food Science & Technology*, 10(4), 34–41.  
<https://doi.org/10.56557/jafsat/2023/v10i48398>

- Pardede, E., & Julianti, E. (2023). Phytochemicals and antioxidative properties of borneo indigenous liposu (*Baccaurea lanceolata*) and tampoi (*Baccaurea macrocarpa*) fruits. *Gunung Djati Conferences Series*, 18, 94–100.
- Pérez-Gálvez, A., Viera, I., & Roca, M. (2020). Carotenoids and chlorophylls as antioxidants. In *Antioxidants* (Vol. 9, Issue 6, pp. 1–39). MDPI. <https://doi.org/10.3390/antiox9060505>
- Permatasari, L., Riyanto, S., & Rohman, A. (2019). *Baccaurea racemosa* (Reinw. ex Blume) Müll. Arg. pulp: a potential natural antioxidant. *Food Research*, 3, 713–719. [https://doi.org/https://doi.org/10.26656/fr.2017.3\(6\).165](https://doi.org/https://doi.org/10.26656/fr.2017.3(6).165)
- Rohilla, S. (2023). A review on bioactive compounds and health benefits of *Baccaurea ramiflora*. In *Food Bioengineering* (Vol. 2, Issue 4, pp. 339–349). John Wiley and Sons Inc. <https://doi.org/10.1002/fbe2.12069>
- Rumpf, J., Burger, R., & Schulze, M. (2023). Statistical evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins. *International Journal of Biological Macromolecules*, 233. <https://doi.org/10.1016/j.ijbiomac.2023.123470>
- Salusu, H. D., Ariyani, F., Nurmarini, E., & Rasyid Zarta, A. (2020). Kandungan Vitamin C pada Tiga Jenis Buah-Buahan Genus *Baccaurea* Vitamin C Content in Three Species of *Baccaurea* Genus Fruits. *Buletin LOUPE*, 2, 12.
- Saswita, H. M., Syamsuardi, & Nurainas. (2023). Inventory of the Genus *Baccaurea* spp. (*Phyllantaceae*) Review. *International Journal of Progressive Sciences and Technologies (IJPSAT)*, 40(2), 264–269.
- Shaharuddin, S., Husen, R., & Othman, A. (2021). Nutritional values of *Baccaurea pubera* and comparative evaluation of SHS treatment on its antioxidant properties. *Journal of Food Science and Technology*, 58(6), 2360–2367. <https://doi.org/10.1007/s13197-020-04748-0>
- Skrovankova, S., Sumczynski, D., Mlcek, J., Jurikova, T., & Sochor, J. (2015). Bioactive compounds and antioxidant activity in different types of berries. In *International Journal of Molecular Sciences* (Vol. 16, Issue 10, pp. 24673–24706). MDPI AG. <https://doi.org/10.3390/ijms161024673>
- Sridhar, K., & Charles, A. L. (2018). Comparative study of DPPH, ABTS, FRAP, and ORAC assays for determination of dose-dependent antioxidant activities of commercial grape cultivars in Taiwan. *International Symposium of Agriculture Biotechnology*. <https://www.researchgate.net/publication/327792929>
- Susmitha, T., Bagchi, T. B., Deb, B. S., Biswas, T., Adak, T., Banerjee, H., & Pal, S. (2022). Evaluation of colour, texture and nutritional properties of Pigmented Rice Based Fermented Steamed Food- Idli. *Food Chemistry Advances*, 1. <https://doi.org/10.1016/j.focha.2022.100021>
- Zhang, Q. Y., Gu, K. Di, Cheng, L., Wang, J. H., Yu, J. Q., Wang, X. F., You, C. X., Hu, D. G., & Hao, Y. J. (2020). BTB-TAZ domain protein MDBt2 modulates malate accumulation and vacuolar acidification in response to nitrate. *Plant Physiology*, 183(2), 750–764. <https://doi.org/10.1104/pp.20.00208>