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ORIGINAL ARTICLE / ÖZGÜN MAKALE

THE EFFECT OF MATURITY ON PHYTOCHEMICAL CONSTITUENT, ANTIOXIDANT ACTIVITY, AND NUTRIENT COMPOSITION OF *MUNTINGIA CALABURA* FRUITS CULTIVATED IN INDONESIA

MATURİTENİN, ENDONEZYA'DA YETİŞTİRİLEN MUNTINGIA CALABURA MEYVELERİNİN FİTOKİMYASAL BİLEŞENLERİ, ANTİOKSİDAN AKTİVİTESİ VE BESİN KOMPOZİSYONU ÜZERİNDEKİ ETKİSİ

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ABSTRACT

Objective: *Cultivation location and maturity levels could affect Muntingia calabura's bioactive compounds and biological activities. The present investigation evaluated two different maturity stages (young and ripened) of Indonesian M. calabura on their phytochemical constituents (total phenolic [TP] and total flavonoid [TF]), antioxidant activity, and nutrition composition.*
Material and Method: *The TP and TF were measured using the Folin-Ciocalteau reagent and ammonium chloride ($AlCl_3$). Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS). Nutrition composition: total soluble solids (TSS) were determined by the gravimetric method; soluble sugars used anthrone-sulfuric acid colorimetric assays; and vitamin C established 2,6-dichloroindophenol (DCIP) titration.*
Result and Discussion: *The ripened fruit presented the most potent antioxidant activity. DPPH and ABTS IC_{50} values were 28.38 ± 0.84 µg/ml and 29.92 ± 3.05 µg/ml, respectively. In contrast, the young fruit exhibited the highest TP (56.85 ± 1.08 mg/g GAE) and TF (8.45 ± 0.65 mg QE). Our findings additionally suggested that ripened fruit was a good source of nutrients, such as soluble sugar (SS; 12.34 ± 0.76%) and vitamin C (21.88 ± 2.73 mg/g).*
Keywords: *Antioxidant, bioactive compound, fruit, maturation, proximate*

ÖZ

Amaç: *Yetiştirme yeri ve olgunluk seviyeleri, Muntingia calabura'nın biyoaktif bileşenlerini ve biyolojik aktivitelerini etkileyebilir. Bu çalışmada, Endonezya'da yetiştirilen M. calabura'nın iki farklı olgunluk aşaması (genç ve olgun) fitokimyasal bileşenleri (toplam fenolik [TP] ve toplam flavonoid [TF]), antioksidan aktivitesi ve besin kompozisyonu açısından değerlendirildi.*
Gereç ve Yöntem: *TP ve TF, Folin-Ciocalteau reaktifi ve amonyum klorür ($AlCl_3$) kullanılarak*

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ölçüldü. Antioksidan aktivitesi, 2,2-difenil-1-pikrilhidrazil (DPPH) ve 2,2′-azino-bis(3-etilbenzotiyazolin-6-sülfonik) asit (ABTS) kullanılarak değerlendirildi. Besin kompozisyonu: toplam çözünür katılar (TSS) gravimetrik yöntemle; çözünür şekerler, antron-sülfürik asit kolorimetrik testleri ile; ve vitamin C, 2,6-dikloroindofenol (DCIP) titrasyonu ile belirlendi.

Sonuç ve Tartışma: *Olgunlaşmış meyve, en güçlü antioksidan aktiviteyi gösterdi. DPPH ve ABTS IC_{50} değerleri sırasıyla 28.38 ± 0.84 µg/ml ve 29.92 ± 3.05 µg/ml idi. Buna karşılık, genç meyve en yüksek TP (56.85 ± 1.08 mg/g GAE) ve TF (8.45 ± 0.65 mg QE) değerlerini gösterdi. Bulgularımız ayrıca, olgunlaşmış meyvenin çözünür şeker (SS; %12.34 ± 0.76) ve vitamin C (21.88 ± 2.73 mg/g) gibi besinlerin iyi bir kaynağı olduğunu öne sürdü.*

Anahtar Kelimeler: *Antioksidan, biyoaktif bileşen, meyve, vejetasyon süresi*

INTRODUCTION

Free radicals are one of the harmful products of the body's metabolic processes, especially respiration. The body naturally produces endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione-S-transferase (GST) to get rid of free radicals [1,2]. However, uncontrolled free radicals will cause several metabolic syndrome diseases such as inflammatory disease [3], diabetes [4], cardiovascular disease [5], cancer [6], neurodegenerative diseases [7], and Alzheimer's [8]. To neutralize these radicals, additional antioxidants are needed from external sources, such as consuming vegetables and fruits (e.g., cherry fruit) (*Muntingia calabura* L.).

As a member of the Muntingiaceae family, *M. calabura* can be found in countries with tropical and sub-tropical climates, such as Indonesia, Thailand, Malaysia, and the Philippines. In both urban and rural areas, the tree grows wild in gardens and yards [9]. Despite its widespread presence, the community has not used it effectively. The children prefer the *M. calabura* fruit because it is sweet and sour. Ripe fruits are red, whereas young ones are green.

According to Muslimin et al. (2020), *M. calabura* fruit has a very high antioxidant activity of 3.27 mg AAE/g (ascorbic acid equivalent per gram). It has high levels of mineral essentials such as potassium (K), calcium (Ca), and iron (Fe). Furthermore, the fruit is also rich in moisture content [10]. High antioxidant activity is associated with a high vitamin C content of 171.36 mg/100 g and a high carotene content of 1576.97 µg/100 g [11].

The level of fruit ripeness greatly influences the nutritional content of fruit. Ripe fruit contains high levels of phenolics and flavonoids [12]. Meanwhile, young fruit generally has higher levels of tannins and alkaloids, and the taste becomes astringent and bitter [13]. The ripe fruit also has a higher carbohydrate content, making it delicious. This study compared the levels of total phenolic (TP) and total flavonoids (TF) contained in the young and ripe fruits of *M. calabura*. Antioxidant activity was also determined using two test methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). Antioxidants are closely related to vitamin C content and are determined using titration. Furthermore, total soluble solid (TSS) and soluble sugar (SS) levels were also analyzed.

MATERIAL AND METHOD

Plant Material

The fruits of *M. calabura* were harvested at two different development stages (i.e., young and ripened) from Sudiang, Makassar, and South Sulawesi in December 2022 (rainy season). Fruit samples from each maturation stage were sorted based on their morphology and color. The young fruit samples were green, while the ripened ones were red. The sample was cleaned under running water to remove dirt and then washed again with aquadest.

Phenolic and Flavonoid Extraction

Sample extraction was carried out in a dark room based on the method described by Mokhtar et al. [12]. A total of 200 g of fruit were extracted sequentially using methanol and ethyl acetate (0.05% v/v hydrochloric acid/solvent [10:90]), followed by simultaneous sonication for 30 minutes. Both extracts were combined, filtered, and evaporated.

Determination of Total Phenolic

A method from Pakki et al. (2020), the Folin-Ciocalteu method, was adopted to determine the total phenolic (TP) [14]. About 0.1 ml of each extract (10 mg/ml) was added to 1 ml of 50% Folin-Ciocalteu and 1 ml of 7.5% sodium carbonate. After the allowed 15 minutes in a dark room, the mixture was filtered, and water was added to bring the total volume to 10 ml. The optical density (OD) was recorded using a spectrophotometer (Agilent 8453, USA) at λ 730 nm. This experiment was performed in triplicate.

Determination of Total Flavonoids

Total flavonoid (TF) content was measured using aluminum chloride ($AlCl_3$) and was slightly modified from what is described by Mokhtar et al. [12]. An amount of 0.1 ml of each extract was transferred to a 5 ml volumetric flash, and 0.1 ml of $AlCl_3$ (10%) and 0.1 ml of $NaNO_2$ were added. After a 5 minutes incubation, 0.5 ml of NaOH (1 M) was added to it. The OD was recorded using a spectrophotometer (Agilent 8453, USA) at λ 510 nm. This experiment was performed in triplicate.

DPPH Test

The free radical-scavenging ability of the fruit of *M. calabura* was tested by bleaching the stable radical DPPH. The DPPH method was adopted from the method of Mokhtar et al. [12]. Briefly, 0.25 ml of the tested sample was added to 1 ml of DPPH (0.1 mmol) in 5 ml of volumetric flash and added to ethanol. The mixture was incubated for 30 minutes in the dark room. After incubation, the OD was measured at λ 515 nm using a spectrophotometer (Agilent 8453, USA). Vitamin C was used as the reference standard, and the tested sample was replaced with ethanol for the control. This experiment was performed in triplicate.

ABTS Test

The ABTS antioxidant activity was generated according to Wołosiak et al. [15]. An equal volume of substrate solution (ABTS, 7 mM) and oxidant (potassium persulfate, 2.45 mM) was made to react overnight in the dark. An amount of 1 ml of each sample was mixed with 3 ml of ABTS solution, stirred, and incubated for 10 minutes. The OD was measured at λ 734 nm using a spectrophotometer (Agilent 8453, USA). Vitamin C was used as the positive control. This experiment was performed in triplicate.

Total Soluble Solids

The TSS of the fruit of *M. calabura* was used in a membrane filter with a 0.4 µm pore size (Merck, Germany). The fruits were crushed using a blender (Philips), and the liquids were centrifuged at 14.500 rpm for 2 minutes. The filter was collected and passed through Millipore 0.4 µm. The number of dissolved substances was compared with the sample weight to calculate TSS.

Soluble Sugars

Soluble sugar in the fruit was measured using the anthrone colorimetric method, with slight modifications [16]. The fruits were crushed using a blender (Philips), then the liquids were centrifuged at 14.500 rpm for 2 minutes and dried. In a beaker, 0.25 ml of each sample was added to 50 µl of NaOH (2 mol/l) and boiled at 90°C for 5 minutes until room temperature reached. Briefly, 1 ml of transparent layer was mixed with 2.5 ml of anthrone and boiled at 90°C for 10 minutes. The corresponding OD values were measured at λ 620 nm using a spectrophotometer (Agilent 8453, USA). D-glucose was used to create a standard curve.

Vitamin C

The vitamin C in the fruit was analyzed using 2,6-dichlorophenolindophenol (DCPIP). An aliquot of 10 ml of each sample was titrated with 0.2% (w/v) DCPIP until the pink color appeared. Metaphosphoric (w/v) acid (5%) was used as a solvent to prevent degradation during the titration.

Statistic

The data were expressed as mean ± standard of deviation (± SD) and statistically evaluated by unpaired T-test at $p < 0.05$ level of significance. The statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 20 software.

RESULT AND DISCUSSION

People are advised to consume lots of vegetables and fruit to maintain their health because these foods are rich in general metabolite compounds, especially polyphenols, flavonoids, and terpenoids, which have pharmacological properties [17]. The secondary metabolites are generally efficacious as antioxidants, anticancer agents, antihyperglycemic agents, and cofactors. Therefore, it is essential to know the levels of polyphenols and flavonoids in fruits to predict their biological activity. One of the many factors influencing the compound content of a fruit is the level of ripeness [18]. This study compared the TP and TF content of young and ripe *M. calabura* fruits.

Determination of TP levels use gallic acid as a reference standard, while TF levels are compared with quercetin. The TP and TF levels are expressed as gallic acid equivalents (GAE) and quercetin equivalents (QE). A standard curve was obtained for the TP levels using the equation $y = 0.0053x + 0.1677$, yielding an R^2 value of 0.9987. In TF levels, a standard curve was obtained using the regression equation $y = 0.0193x + 0.132$, with an R^2 value of 0.9987 (Figure 1). The R^2 value shows the linearity of a curve; the closer the value to 1, the straighter the line equation is. R^2 is a figure of merit demonstrating the linearity of calibration curves in method validations [19].

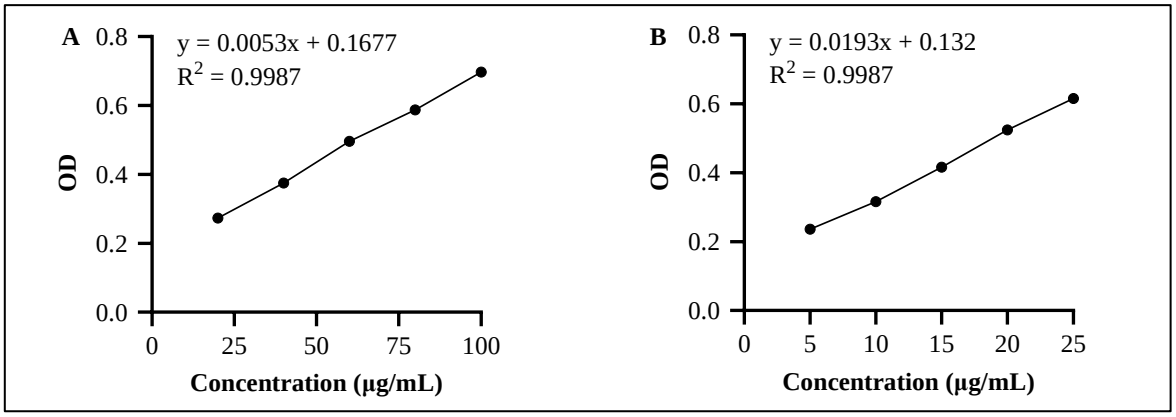

Figure 1. Phytochemical content on TP and TF in the fruit of *M. calabura*. Linear regression of phenolic (A) and flavonoid (B) determination

Our results showed that *M. calabura* fruits' TP and TF depend on their maturity stage. Figure 2 shows that the TP content of young fruits (56.85 ± 1.08 mg/g GAE) was higher than that of ripened fruits (42.29 ± 4.09 mg/g GAE). A similar result was also seen in TF levels; young fruits (6.99 ± 0.80 mg/g QE) had higher levels than ripened fruits (4.80 ± 0.52 mg/g QE). Pereira et al. (2018) reported that ripened *M. calabura* fruits collected from Campinas-SP, Brazil, contained TP of 6.88 ± 0.02 mg GAE/g [20]. Young fruits generally contain many chemical compounds. Fruit ripening necessitates the decomposition of these compounds. In addition, young fruits have high levels of certain active compounds, particularly the tannin group, which decrease as the fruits mature to protect them from predators [21].

Mokhtar et al. (2021) found that young pumpkin fruits have a 3.3-time higher polyphenol content and a 2.8-time higher flavonoid content than ripened ones [12]. The major volatile compounds, such as terpenes β-farnesene and dendrolasin, are concentrated in the ripened fruit of *M. calabura.* Meanwhile, also found in ripened fruits were gallic acid was 5325 µg/g dried weight (DW), and cyanidin-3-O-glucoside was 171 µg/g DW [20].

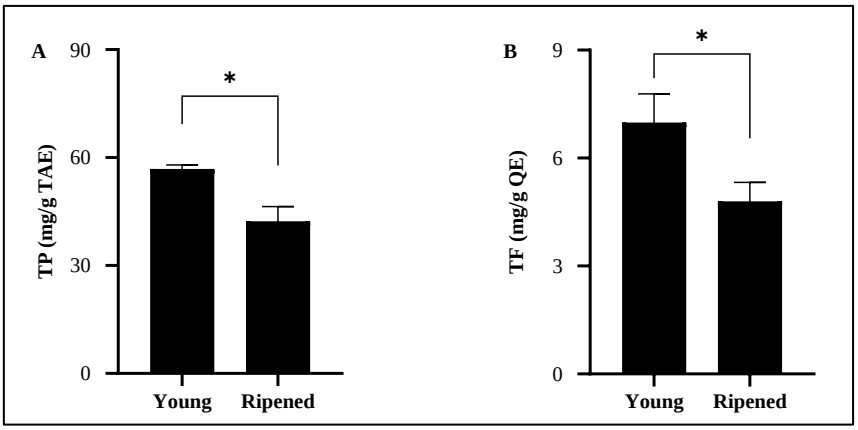

Figure 2. Total phenolic (A) and flavonoids (B) of the fruit of *M. calabura* at 2 stages of ripeness (n=3). *Characters represent significant differences between young and ripened group at $p < 0.05$ by unpaired T-test

One of the primary functions of fruits is to provide antioxidants. Many studies have proven that fruits are good sources of natural antioxidants for the body, which help prevent metabolic syndrome [22,23]. This study compared the antioxidant capacity of young and ripened *M. calabura* fruit using two different methods: DPPH and ABTS. Generally, both young and ripened fruits have antioxidant activity proportional to the concentration used. The higher concentration of the sample resulted in a higher percentage of inhibition. Table 1 shows that ripened fruits have a higher percentage of inhibition at the same concentration as compared to young fruits. At a concentration of 10 µg/ml, young fruits inhibited the activity of DPPH free radicals by 12.88 ± 1.53%, while ripened fruits reached 23.42 ± 0.70%. The ABTS antioxidant also showed the same activities (Table 2).

Table 1. DPPH antioxidant activity of different maturity stages of the fruit of *M. calabura* (n= 3)

Maturity stages	µg/ml	Inhibition (%)			Mean ± SD
		I	II	III	
Young	10	11.99	14.64	11.99	12.88 ± 1.53
	20	23.83	24.06	23.94	23.95 ± 0.11
	40	35.92	37.41	35.16	36.16 ± 1.14
	80	56.50	55.30	57.53	56.44 ± 1.11
Ripened	10	22.62	23.82	23.82	23.42 ± 0.70
	20	37.41	35.03	33.85	35.43 ± 1.81
	40	69.72	74.16	71.02	71.63 ± 2.29
	80	87.62	83.70	80.58	83.97 ± 3.53
Vitamin C	1	10.78	11.99	11.99	11.59 ± 0.70
	2	35.92	34.70	35.89	35.50 ± 0.70
	4	60.92	59.82	62.21	60.98 ± 1.20
	8	90.14	88.49	90.76	89.80 ± 1.17

The antioxidant capacity of an active compound is reflected in its IC_{50} value. IC_{50} is a concentration that can inhibit 50% of free radical activity. The smaller the IC_{50}, the better the effect. The results showed that in both the DPPH and ABTS tests, ripened fruits had the lowest IC_{50} values compared to young fruit. Ripened and young fruit had IC_{50} values against DPPH of 28.38 ± 0.84 µg/ml and 68.77 ± 1.24 µg/ml and ABTS of 29.92 ± 3.05 µg/ml and 67.46 ± 1.22 µg/ml, respectively (Figure

3). The antioxidant activity of fruit of *M. calabura* was also similar to those found in a previous study. According to Nur et al. (2022), ethanol extract of the fruit of *M. calabura is* capable of reducing hydroxyl radicals with an IC_{50} value was 32.06 µg/ml [24]. Hence, another part, like leaves, is capable of scavenger the DPPH free radical and nitric with IC_{50} value range 17.85 ± 5.40 till 19.77 ± 4.05 µg/ml and 19.90 ± 3.21 till 25.29 ± 5.33 µg/ml, respectively [25].

Table 2. ABTS antioxidant activity of different maturity stages of the fruit of *M. calabura* (n= 3)

Maturity stages	µg/ml	Inhibition (%)			Mean ± SD
		I	II	III	
Young	10	3.32	2.98	1.22	2.51 ± 1.13
	20	10.98	15.11	15.11	13.73 ± 2.38
	40	24.96	25.19	30.92	27.02 ± 3.38
	80	66.00	65.83	61.53	64.46 ± 2.53
Ripened	10	7.41	7.64	15.11	10.05 ± 4.38
	20	35.90	36.37	32.50	34.92 ± 2.11
	40	62.40	56.62	56.62	58.55 ± 3.34
	80	86.62	83.68	83.62	84.64 ± 1.71
Vitamin C	1	7.32	7.32	9.23	7.96 ± 1.10
	2	20.40	20.57	23.24	21.40 ± 1.60
	4	48.14	50.52	49.89	49.52 ± 1.24
	8	86.78	87.73	88.22	87.57 ± 0.73

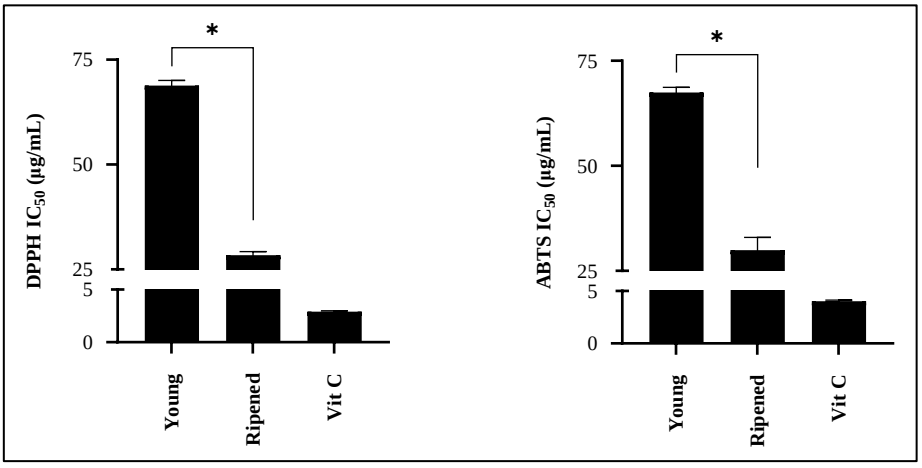

Figure 3. The IC_{50} value of the fruit of *M. calabura* in 2 different maturity stages (n= 3). *Characters represent significant differences between young and ripened group at $p < 0.05$ by unpaired T-test

A negative correlation was observed between the antioxidant capacity of the *M. calabura* fruits' polyphenols and flavonoids at different stages of fruit maturation concerning TP and TF. The polyphenolic concentration decreased during the ripening fruit process, whereas the antioxidant level increased. Phenolic and flavonoid levels were recorded to be significantly higher in the young fruits of *Rubus ellipticus*, *Myrica esculenta*, and *Pyracantha crenulate* [26]. Samaniego et al. (2020) have described that blackberry cultivars' polyphenol, flavonoid, and anthocyanin content decrease with increasing ripening [27]. Dong et al. (2019) demonstrated that the phenolic and antioxidant potency composite values decrease as the degree of fruit maturity increases in *Citrus limon* (L.) Burm. f [28]. As

fruits ripen, their phenols undergo oxidation and contribute to the production of anthocyanins, which accumulate over the course of fruit ripening. Consequently, the phenol concentration in ripened fruits decreases [26].

Thus, specific compounds decrease, and others increase during the fruit ripening process. At this time, TSS levels in the fruit and water content will increase. Based on our results (Table 3), the TTS content of young fruits (49.42 ± 4.92%) was lower than that of ripened fruits (60.75 ± 2.67%). Furthermore, carbohydrate levels in ripened fruit also increase. Increasing carbohydrate levels and simple sugars such as glucose, fructose, and sucrose will increase fruit sweetness. This study showed that SS levels in ripened fruits were much higher than in young fruits.

Table 3. The nutrient composition of the fruit of *M. calabura* in 2 different maturity stages (n= 3)

Parameter	Young	Ripened
Total soluble solid, TSS (%)	49.42 ± 4.92	60.75 ± 2.67*
Soluble sugar, SS (%)	7.13 ± 0.77	12.34 ± 0.76*
Vitamin C (mg/g)	13.38 ± 2.91	21.88 ± 2.73*

*Characters represent significant differences between young and ripened group at $p < 0.05$ by unpaired T-test

Vitamin C levels closely correlate with antioxidant capacity. The level of vitamin C in ripened fruits was much higher than that in young fruits at 21.88 ± 2.73 and 13.38 ± 2.91 mg/g, respectively (Table 3). The antioxidant activity of ripened fruits was higher than that of young fruits, as evidenced by the vitamin C levels. The results obtained are comparable with those reported by Gull et al. (2012), who showed an increasing trend in vitamin C content in guava fruit with maturation [29]. According to Soares et al. (2007), in a study of immature fruit, the amount of vitamin C was 76.8 mg/100 g of the sample, and it became 168.36 mg/100 g at the ripe stages, respectively [30]. Vitamin C, or ascorbic acid, a water-soluble vitamin, plays a role in controlling infections and healing wounds and is a powerful antioxidant that can neutralize harmful free radicals [31,32].

Young to adult people (> 19 years) need 90 mg daily for men and 75 mg for women. Pregnancy and lactation will increase the amount to 85 mg and 120 mg daily, respectively [33]. These studies could confirm our expectation of the benefit value of the fruit of *M. calabura.* Future and in-depth research, including a pharmacological bioassay and randomized clinical trials, is essential to claim the hidden potential medicinal values.

The active compounds, like polyphenols and flavonoids, and the biological activity of the fruit of *M. calabura* depend on the ripeness. In general, ripened fruit has a high vitamin C content and is correlated to antioxidant activity in higher young fruit. The SS content and the TSS also increase in ripened fruit. However, young fruit contains higher polyphenols and flavonoids than ripened fruit. In the future, comprehensive research must be carried out to determine the glycemic index and its potential as a sustainable nutrition and food source.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that ethics committee approval is not required for this study.

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