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Original Article

Analysis of aroma compounds and nutrient contents of *mabolo* (*Diospyros blancoi* A. DC.), an ethnobotanical fruit of Austronesian Taiwan



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ABSTRACT

Diospyros blancoi A. DC. is an evergreen tree species of high-quality wood. *Mabolo*, the fruit of this plant, is popular among the natives in Taiwan, but its potential in economic use has not been fully explored. *Mabolo* has a rich aroma. Of the 39 different volatile compounds isolated, its intact fruit and peel were found to both contain 24 compounds, whereas the pulp contained 28 compounds. The most important aroma compounds were esters and α -farnesene. Our data show that *mabolo* is rich in dietary fiber (3.2%), and the contents of other nutrients such as malic acid, vitamin B2, vitamin B3, folic acid, pantothenic acid, and choline chloride were 227.1 mg/100 g, 0.075 mg/100 g, 0.157 mg/100 g, 0.623 mg/100 g, 0.19 mg/100 g, and 62.52 mg/100 g, respectively. Moreover, it is rich in calcium and zinc; the contents of which were found to be 42.8 mg/100 g and 3.6 mg/100 g, respectively. Our results show that *D. blancoi* has the potential to be bred for a novel fruit.

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1. Introduction

Diospyros blancoi, the subject of this study, is an Ebenaceae plant that can grow up to 20 m high or more. There are scientific names of different origins referring to this evergreen plant, with the three most commonly used being *Diospyros discolor* Willd., *Diospyros philippensis* (Desr.) Gürke and *D. blancoi*

A. DC. Knapp and Gibert [1] see *D. discolor* as the single correct name for the plant, whereas in the book *Flora of Taiwan* [2], it is referred to as *D. philippensis*. However, as early as in 1971, Howard [3] had already legitimized *D. blancoi* as the plant's scientific name, and thus it is the one that we use in this study.

In the Filipino language Tagalog, *D. blancoi* is called *kamagong*, and the fruit is known as *mabolo*, meaning “hairy” [4]. The wood of this plant is extremely dense, hard, and dark in color,

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consequently in Taiwan it is known as “Taiwan's black ebony” and considered a valuable timber species. In the Philippines, *kamagong* timber is also known as “iron wood” since it is durable and considered unbreakable. It is widely distributed in the Philippines [5], and is native to eastern and southern Taiwan [2,6]. On a northeastern Taiwanese island known as Turtle Island, a tree with a diameter at breast height (DBH) of 210 cm was discovered, and this may be the northernmost part of the world where this plant is found. The species, known as *mao-shi* in Taiwan [2], produces a fruit with a fluffy exterior, which is the same reference as *mabolo* in the Philippines.

D. blancoi is an important ethnobotanical plant in the Austronesian society. In China, the earliest mentioning of *D. blancoi* was in “*Dong Fan Ji*” by Chen [7] in 1603 during the Ming Dynasty. A travel report recorded in Taiwan, the article describes the western coast of Taiwan and the customs of Siraya tribal life, highlighting the consumption of important edible fruits and vegetables such as coconut, bergamot, sugarcane, as well as *mabolo* (Fig. 1). Taiwanese ethnobotanist Han-Wen Zheng [8] mentioned that *mabolo* is commonly found in the eastern and southern Taiwanese residential courtyards of Kebalan, Pangcah, Payuan, and Pinuyumayan. Tao people plant *mabolo* in private lands and taro fields. In Pinuyumayan culture, the lifecycle of *mabolo* is used as an indicator for the annual work calendar. This includes instructions stating that weeding should be done while the plant is flowering, millet should be collected when its fruit begins to form, or upland rice should be collected when fruits are harvested. Moreover, the elderly or sick people are honored with the mature fruit [8].

Seedless *mabolo* has occasionally been found in the field. With only the female plant found on Turtle Island, it suggests that a lack of pollen causes approximately 90% of the fruit to be seedless. Seeded fruits contain an average of three seeds, and the relatively low number of seeds is indicative of the existence of parthenocarpic varieties of *D. blancoi*. Our recent studies show that the seedless *mabolo* was also a result of artificially induced production [9].

D. blancoi maintains high diversity of fruit size within populations (Fig. 1B) and can be considered a good breeding resource. However, as it exhibits a low level of domestication, selecting elite trees and establishing initial lines that can be used for a potential traits screen becomes very important. The aims of this study are to highlight the importance of *mabolo*

and promote the fact that *D. blancoi* can potentially be an important economic fruit [9].

2. Methods

2.1. Analysis of aroma compounds

Fresh ripe fruits that had fallen on the ground (random samples) were collected from the Kenting area. The fruit was subjected to three types of analysis. For the first analysis, the intact fruit was used; for the second analysis, the fruit peel (approximately 50 g) was used; and for the third analysis, the pulp of the fruit was used after the peel and seeds were removed. Each analysis was performed in triplicate. The samples were placed in the sample cylinder to extract aroma compounds using the headspace solid-phase micro-extraction (SPME) method for 30 minutes, which used polydimethylsiloxane/divinylbenzene (PDMS/DVB; 65 μm) fiber for adsorption. Gas chromatography-mass spectrometry (GC-MS) was performed with the Thermo GC Focus Series and Trace DSQ-MS (Thermo Fisher Scientific Inc., Waltham, MA, USA). A BPX5 (30 m length, 0.25 mm inside diameter, 0.25 μm film thickness) column (Thomas Scientific Inc., Swedesboro, NJ, USA) was used with helium (99.999%) as carrier gas at a flow rate of 1.0 mL/min, and the injector was set in the splitless mode. The injection port was set at 220°C, the MS interface was maintained at 210°C, and the ion trap was set at 200°C. The operating temperature range was 55–130°C, increased at a rate of 3°C/min; 130–210°C, increased at a rate of 2°C/min; and finally held at 210°C for an additional 2 minutes.

The ionization energy of electron impact (EI) was 70 eV and the scanning range was between 33 m/z and 400 m/z. The mass spectra obtained were compared with the mass spectra in the NIST 02 version mass spectral database (National Institute of Standards and Technology, Washington, DC, USA). The match of the mass spectra fragmentation was over 850, and 10% or more probability was the basis for the identification of compounds. The concentrations of compounds were calculated based on the waveform area of the chromatogram. The process was repeated, and if the variation was more than 10%, then the samples were resampled, and the data were analyzed until consistent results were obtained.

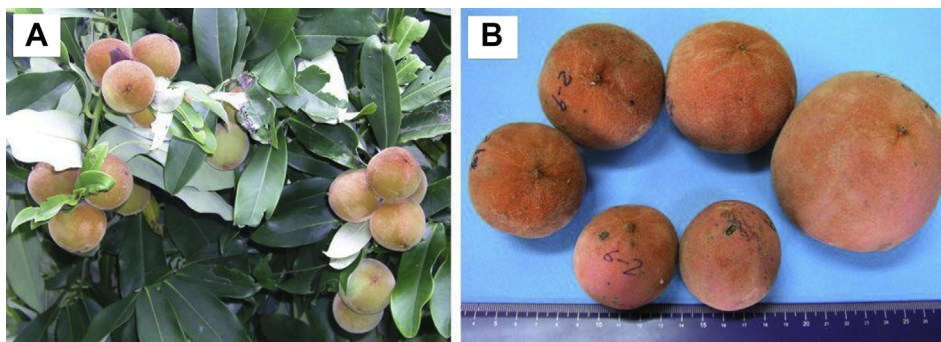


Fig. 1 – The *Diospyros blancoi* A. DC. (A) bears abundant fruit (*mabolo*), and (B) varies greatly in size within populations. The ruler units are centimeters.

2.2. Analysis of nutrient contents

Thirty fruits were collected as samples from the Kenting area (Taiwan), and the pulp of each fruit was weighed after peeling and the removal of seeds. The dry weight of the pulp was determined after freeze-drying for 1 week before the pulp was powdered and stored at -80°C . The process was repeated after conducting an analysis. If the variation between the two data was within 10%, the average of the two datasets was considered as the result. If the variation was more than 10%, the process was continued until a consistent result was obtained. The following analyses were performed according to the following protocols/methods after some modifications to the mentioned methods: moisture content = (fresh weight – dry weight) * 100%; calorie = 9 (crude fat) + 4 (crude protein) + 4 (carbohydrate); carbohydrate = 100 – moisture – ash – crude protein – crude fat; and calories from fat = 9 (crude fat).

Analysis of ash by ashing, was carried out according to Chinese National Standard (CNS) 5034-N6115 [10]. Dietary fiber analysis was carried out by enzymatic gravimetry, according to the Association of Analytical Communities (AOAC) official method 985.29 [11]. Crude protein analysis was carried out by digestion-titrimetry, according to CNS 5035-N6116 [12]. Crude fat analysis was carried out by ether extract, according to CNS 5036-N6117 [13], trans fat by GC, according to the AOAC official method 985.21 [11], and saturated fatty acid by GC, according to the AOAC official method 996.06 [11]. Glucose, fructose, sucrose, lactose, and maltose analysis was performed by high-performance liquid chromatography (HPLC), according to CNS12634-N6223 [14]. Cholesterol analysis was performed by GC, according to the AOAC official method 994.10 [11]. Vitamin A analysis was performed by HPLC, according to CNS12725-N6230 [15], vitamin C by HPLC, according to Odrizola-Serrano et al [16], vitamin E by LC, according to the AOAC official method 992.03 [11], β -carotene by HPLC, according to Chen et al [17], vitamin D3 by HPLC, according to Klejdus et al [18], vitamin K1 by HPLC, according to Jakob and Elmadfa [19], vitamin B1 by fluorometry, according to the AOAC official method 953.17 [11], vitamin B2 by fluorometry, according to Hoffmann-LaRoche [20], vitamin B3 by colorimetry, according to the AOAC official method 961.14 [11], vitamin B6, according to the AOAC official method 985.32 [11], vitamin B12 by titrimetry, according to the AOAC official method 952.20 [11], vitamin H by HPLC, according to BS EN15607 [21], folic acid according to the AOAC official method 944.12 [11], pantothenic acid according to the AOAC official method 945.74 [11], and inositol by GC-flame ionization detector (FID), according to International Classification for Standards (ICS) 67.040 C53 [22]. Choline chloride was analyzed by HPLC, according to Chen et al [23], citric acid, malic acid, fumaric acid, propionic acid, acetic acid, tartaric acid, succinic acid, and formic acid by LC, according to Hyun et al [24]. Sodium was analysed by atomic absorption (AA)-spectrophotometry, according to CNS12869-N6231 [25], calcium, iron, chromium, potassium, magnesium, manganese, nickel, phosphorus, zinc, cobalt, molybdenum, titanium, selenium by AA-spectrophotometry, according to the AOAC official method 985.35 [11]. Total tannin content was analysed by Folin–Ciocalteu colorimetry, according to Li et al [26].

3. Results and discussion

3.1. Analysis of aroma compounds

We were able to detect 39 different volatile aroma compounds (Table 1). Twenty-four compounds were detected in the intact fruit. The five major compounds (each constituting > 5% of the total content of volatile compounds) detected in the intact fruit were butyl benzoate (25.57%), hexyl butyrate (16.46%), benzyl butyrate (14.71%), hexyl benzoate (13.78%), and α -farnesene (9.60%). Collectively, these compounds constituted 80.12% of the total content of volatile compounds. The volatile compound composition of the peel was similar to that of the intact fruit, with only minor differences observed. Twenty-four different compounds were detected in the peel, of which the seven major compounds (each constituting > 5% of the total content of volatile compounds) were butyl benzoate (31.54%), hexyl butyrate (16.98%), α -farnesene (11.73%), benzyl butyrate (11.50%), butyl 2-methylbutyrate (7.48%), hexyl benzoate (6.02%), and butanoic acid, 2-methyl-hexyl ester (5.02%). Collectively, these compounds constituted 90.27% of the total content of volatile compounds. Twenty-eight volatile compounds were detected in the pulp, of which the six major compounds (each constituting > 5% of the total content of volatile compounds) were hexyl butyrate (29.37%), benzyl butyrate (15.08%), α -farnesene (13.62%), phenylethyl butyrate (9.24%), hexyl hexanoate (8.33%), and butyl benzoate (6.39%). Collectively, these compounds constituted 82.03% of the total content of volatile compounds. The main aroma compounds in the intact fruit, pulp, and peel were esters and α -farnesene. Not only for *mabolo*, the most important volatile compounds of many fruits are esters. For example, esters accounted for 78–92% of whole volatile compounds of apples [27], and there were 33 kinds of esters in the total 58 kinds of identified volatile compounds of passion fruit juice [28].

Mabolo has a rich aroma [29–35]. The strong aroma, which is due to the peel, is described by Smith and Oliveros-Belardo [29] as a smell that can increase appetite, while Morton [4] describes it as a cheese-like odor. As the strong aroma is not preferred by some people, it is recommended that the skin is peeled and the fruit is placed in the refrigerator for several hours before consumption. Selection of a line with a less intense aroma is of importance in order to cater to public taste. It has been suggested by Morton [4] that strains producing fruit with purple skin had better flavor. The fresh sweet aroma of the ripe *mabolo* soon after the fruit has fallen to the ground turns unpleasant as time progresses. Smith and Oliveros-Belardo [29] suggest that free butyric acid is the source of this unpleasant smell.

Collins and Halim [33] utilized steam distillation to obtain essential oils from *mabolo*, and after analyzing the oils by GC, infrared spectroscopy (IR), and MS, they identified 24 different volatile compounds. Smith and Oliveros-Belardo [29] analyzed the petroleum ether extract of *mabolo* and identified five main constituents, including benzyl salicylate (26.9%), benzyl benzoate (19.2%), cinnamyl benzoate (10.3%), butyl benzoate (6.0%), and benzyl butyrate (4.1%). Wong et al [34] analyzed the dichloromethane extract of *mabolo* and identified 67

Table 1 – Volatile constituents of fruit, pulp, and peel at room temperature.

Compounds	M _w (Da)	Retention time (min)	Relative content (%)		
			Fruit	Pulp	Peel
Hexyl acetate	144	0.45	0.24	0.90	ND
Butanoic acid, 1-methylbutyl ester	158	1.79	0.61	ND	0.84
Benzyl alcohol	108	3.65	ND	ND	0.58
Butyl 2-methylbutyrate	158	3.95	2.56	0.96	7.48
Butanoic acid, 3-methylbutyl ester	158	5.68	ND	0.45	ND
Butanoic acid, 2-methylbutyl ester	158	5.86	ND	0.17	ND
Linalool	154	9.45	ND	0.24	ND
Benzyl nitrile	117	12.78	ND	0.43	ND
Benzyl acetate	150	14.17	2.28	1.54	0.21
Benzoic acid, ethyl ester	150	14.57	0.33	0.57	0.29
Butanoic acid, 3-hexenyl ester, (Z)-	170	15.43	0.24	0.22	2.45
Hexyl butyrate	172	15.83	16.46	29.37	16.98
Acetic acid, octyl ester	172	16.93	0.10	0.27	ND
cis-3-Hexenyl valerate	184	17.98	ND	ND	0.87
Butanoic acid, 2-methyl-, hexyl ester	186	18.24	2.98	1.23	5.02
Hexyl crotonate	170	18.79	ND	0.28	ND
Isopentyl hexanoate	186	19.09	ND	0.58	ND
Phenethyl acetate	164	19.48	ND	0.78	ND
Propyl benzoate	164	20.27	0.44	0.21	0.41
Cinnamyl alcohol	134	22.33	0.10	0.24	0.23
Benzyl butyrate	178	24.10	14.71	15.08	11.50
Eugenol	164	24.37	ND	ND	0.15
Copaene	204	25.11	0.10	ND	0.60
Butyl benzoate	178	25.43	25.57	6.39	31.54
Hexyl hexanoate	200	25.73	1.25	8.33	0.69
Eugenol methyl ether	178	26.72	ND	ND	0.32
Phenylethyl butyrate	192	28.50	0.62	9.24	1.20
Cinnamyl acetate	176	28.90	1.19	0.83	ND
α -Farnesene	204	31.39	9.60	13.62	11.73
Cadinene	204	32.10	0.09	ND	0.36
Hexanoic acid, phenylmethyl ester	206	33.69	4.91	4.21	ND
Elemicin	208	33.86	ND	ND	0.36
Phenylpropyl iso-butyrate	206	34.25	ND	0.44	ND
3-Hexen-1-ol, benzoate, (Z)-	204	35.03	ND	ND	0.82
Hexyl benzoate	206	35.41	13.78	1.76	6.02
Butyric acid, cinnamyl ester	204	38.33	1.61	0.82	0.23
Phenethyl hexanoate	220	38.48	ND	1.00	ND
Cinnamyl isovalerate	218	40.36	0.31	ND	ND
Benzyl benzoate	212	45.04	0.17	ND	ND

Da = daltons; M_w = molecular weight; ND = not detected.

compounds by GC and GC-MS analysis [34]. Esters constituted 88.6% of the total volatile content, wherein the most abundant esters were methyl butyrate (32.9%), ethyl butyrate (10.7%), butyl butyrate (10.2%), and benzyl butyrate (10.0%). When these results are compared with those from Smith and Oliveros-Belardo [29], they are found to vary depending on the analysis method used. Utilizing GC and GC-MS analysis, Pino et al [35] identified 96 different aroma components by distillation of pulp, which included benzyl butyrate (33.9% of the total composition), butyl butyrate (12.5%) and (E)-cinnamyl butyrate (6.8%). This result was similar to that obtained by Wong et al [34]. The main constituents, butyl benzoate and benzyl butyrate, were the same while compared to the result from Smith and Oliveros-Belardo [29] where only benzyl butyrate was the same. In this study, we used fiber at room temperature with headspace SPME and combined this with GC-MS for analysis. The four important aroma components in the intact fruit, pulp, and peel were butyl benzoate, hexyl

butyrate, benzyl butyrate, and α -farnesene. Hexyl butyrate and α -farnesene have not been reported previously, which indicates variability in the results due to the different extraction methods used. The detection of hexyl butyrate and benzyl butyrate indicates that *mabolo* contains butyric acid esters, whereas Smith and Oliveros-Belardo [29] have suggested that the degradation of free butyric acid is the source of the unpleasant smell [29]. This needs to be confirmed by further studies.

3.2. Analysis of nutrient contents

The nutrients quantified from *mabolo* are shown in Table 2. The undetected compounds were transfat, saturated fatty acid, sucrose, lactose, maltose, cholesterol, vitamin A, β -carotene, vitamin D3, vitamin K1, vitamin B1, vitamin B6, vitamin B12, vitamin H, inositol, citric acid, cobalt, molybdenum, titanium, selenium, iron, chromium, manganese, nickel,

Table 2 – Nutrient contents of *mabolo* and comparison with other representative fruits.

Composition	Content	Relative nutrition content of representative fruits ^a				Remark/postscript
		Persimmon PCA	Persimmon PCNA	Apple “Red-Delicious”	Apple “Fuji”	
Moisture (g/100 g)	84.4	81	85	86	87	
Ash (g/100 g)	0.8	0.3	0.4	0.3	0.4	
Calorie (kcal/100 g)	62	68	52	50	46	
Carbohydrate (g/100 g)	13.8	18	13.8	13.4	12.1	
Dietary fiber (g/100 g)	3.2	4.7	1.3	1.6	1.2	
Crude protein (g/100 g)	0.4	0.5	0.5	0.1	0.3	
Calories from fat (kcal/100 g)	5.4	—	—	—	—	
Crude fat (g/100 g)	0.6	0.2	0.1	0.1	0.2	
Glucose (g/100 g)	1.9	—	—	—	—	
Fructose (g/100 g)	2.4	—	—	—	—	
Vitamin C (mg/100 g)	2.2	46	79	2.1	2	
Vitamin E (mg/100 g)	0.59	—	—	—	—	DL- α -tocopherol
Vitamin B ₂ (mg/100 g)	0.075	0	0.03	0	0.01	
Vitamin B ₃ (mg/100 g)	0.157	—	—	—	—	Niacin
Folic acid (mg/100 g)	0.623	—	—	—	—	Vitamin B ₉
Pantothenic acid (mg/100 g)	0.19	—	—	—	—	Vitamin B ₅
Choline chloride (mg/100 g)	62.52	—	—	—	—	
Malic acid (mg/100 g)	227.1	EU regulations require apple juice contains > 3 g/L [36] = 300				
Fumaric acid (mg/100 g)	4.5	—	—	—	—	
Sodium (mg/100 g)	2.0	6	10	4	3	
Calcium (mg/100 g)	42.8	10	9	3	5	High-quality milk 95
Potassium (mg/100 g)	19.6	150	150	100	110	
Magnesium (mg/100 g)	7.7	8	6	4	4	
Phosphorus (mg/100 g)	17	14	19	11	11	
Zinc (mg/100 g)	3.6	0.4	0.1	0	0.1	Oyster 7.1
Tannin acid (mg/100 g)	69.2	—	—	—	—	light green
Tannin acid (mg/100 g)	155.3	—	—	—	—	dark green
Tannin acid (mg/100 g)	213.3	—	—	—	—	ripe

PCA = pollination constant and astringent; PCNA = pollination constant and nonastringent.

^a Data from the Food and Drug Administration, Ministry of Health and Welfare, Taiwan [37].

propionic acid, acetic acid, tartaric acid, succinic acid, and formic acid.

The nutrients in *mabolo* have previously been analyzed in the Philippines and in India, and *mabolo* was found to be an excellent source of iron, calcium, and vitamin B complex [4]. In our study, we found that the calcium content was 42.8 mg/100 g, which is nearly half of what is obtained from milk (95.0 mg/100 g; Table 2). We could not detect iron in our analysis as the iron content was below the detection limit (0.2 ppm). The fact that *mabolo* is considered as a source of iron-nutrition could simply be folklore of the Philippines or India. In other words, the author of that cited literature may have only used a record of ethnobotany. If excluding the possibility of the above, the differences between our results and those from literature review may be attributed to sample contamination, difference of detection methods, different soil conditions for plant growth, or even difference of strains. This needs to be further studied for confirmation. Vitamin B₂ (0.075 mg/100 g), vitamin B₃ (0.157 mg/100 g), folic acid (0.623 mg/100 g), pantothenic acid (0.19 mg/100 g), and choline chloride (62.52 mg/100 g) were all detected in *mabolo*, proving the fruit to be an excellent source of vitamin B complex. Many B vitamins are involved in homocysteine metabolism, and hyperhomocysteinemia has been associated with cardiovascular disease and other age-related diseases [38]. Moreover, *mabolo* is rich in malic acid and zinc. Malic acid can be directly

absorbed by the body to provide immediate energy. Malic acid also aids recovery from fatigue, and is often used to relieve muscle aches, enhance strength, and protect the liver, heart, and skin [39]. Our data show that *mabolo* contains high levels of zinc (3.6 mg/100 g), hence 340–420 g of *mabolo* can provide the recommended daily allowance of zinc [40]. Zinc is helpful for health, as it is required for the functioning of many enzymes and is an essential nutrient for the maintenance of normal gonadal function and for neurogenesis, synaptogenesis and neuronal growth [41,42]. Taking these results into account and considering the contents of vitamin B complex, malic acid, calcium and zinc in *mabolo*, we can now appreciate why the aboriginal Pinuyumayan honor the elderly with *mabolo* or offer it to patients. Moreover, we found that the dietary fiber content of *mabolo* was 3.2 g/100 g, which was higher than that of the “Red-Delicious” apple (1.6 g/100 g), “Fuji” apple (1.2 g/100 g), and pollination constant and non-astringent (PCNA)-persimmon (1.3 g/100 g; Table 2).

In this study, we measured tannins by Folin-Ciocalteu colorimetry. The data obtained can also be interpreted as the total polyphenol content. While tannins in mature *mabolo* are condensed and insoluble, they can still be measured. The total polyphenol content increased from 69.2 mg/100 g to 155.3 mg/100 g as the fruit matured and turned from light green to dark green, and the content in ripe *mabolo* was found to be 213.3 mg/100 g. Phenolic compounds are potent natural

antioxidants, which decrease the generation of reactive oxygen species and scavenge free radicals. Phenolic compounds (phenolic acids, polyphenols, and flavonoids) have been used as antioxidants by humans [43]. They also possess anti-inflammatory and anti-cancer properties, thus aiding the prevention of many diseases [44].

4. Conclusion

Many ethnobotanical plants or wild plants without domestication have high value for human use in that they can provide new food sources such as an excellent edible oil [45] or vegetable for nutritional supplement [46] and so on. Many functional foods or medicinal ingredients are also developed from such plants. In some recent studies, a number of plant species with antioxidants such as polyphenols and flavonoids have been reported [47–53], and plants with other effects such as antitumor [48,54], antibacterial [48], anti-inflammation [46,52], liver protection [53,55], immunomodulatory effects [47] or even containing anticholinesterase activity [50] have been screened out in succession. Such plants with potential of development should undergo considerable research on their economic use, including *mabolo* as it contains good nutrients for health functions and it can be directly eaten as a vegetable or fruit. For vegetables and fruits of further use, it is important that we clearly know their chemical composition and potential biological properties [46]. Compared to the general persimmon, *mabolo* has a rich aroma and a high nutritious value that is good for human health. We start this research project and publicize the preliminary results at the initial stage to engender the interest of researchers around the world and speed up the usage of *D. blancoi* as an important economic fruit tree.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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