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Flavonoids isolated from the fresh sweet fruit of *Averrhoa carambola*, commonly known as star fruit

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WBKKVLAYDMACSC-HPHGPOCDSA-N*Chemical compounds studied in this article:*
(+)-Epicatechin (PubChem CID: 182232)
Dihydrokaempferol (aromadendrin) 3-*O*-glucoside (PubChem CID: 10478918)
Taxifolin 3′-*O*-glucoside (PubChem CID: 14282774)
Galangin 3-*O*-rutinoside (PubChem CID: 98464939)
Isorhamnetin 3-*O*-rutinoside (PubChem CID: 17751019)

ABSTRACT

Thirteen flavonoids were isolated from the fresh sweet fruit of *Averrhoa carambola* L. (Oxalidaceae), commonly known as star fruit, and their structures were determined by spectroscopic and chemical methods. 8-Carboxymethyl-(+)-epicatechin methyl ester, pinobanksin 3-*O*-β-D-glucoside, and carambolasides M–Q were undescribed structures. (+)-Epicatechin, aromadendrin 3-*O*-β-D-glucoside, helicioside A, taxifolin 3′-*O*-β-D-glucoside, galangin 3-*O*-rutinoside, and isorhamnetin 3-*O*-rutinoside were reported from this species for the first time. Pinobanksin 3-*O*-β-D-glucoside and carambolasides M–Q showed more potent 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical cation scavenging activity (IC_{50} = 5.3–2.3 μM) than L-ascorbic acid (10.5 μM). Further, (+)-epicatechin, pinobanksin 3-*O*-β-D-glucoside, isorhamnetin 3-*O*-rutinoside, and carambolasides O–Q exhibited weak porcine pancreatic lipase inhibitory activity.

1. Introduction

The fruit of *Averrhoa carambola* L. (Oxalidaceae), commonly known as star fruit or carambola, is popular in Southeast Asia countries and China with commercial value. There are mainly two types of fruit in taste, sweet or sour. Sweet star fruit can be eaten out of hand or sliced and used in salads or as garnish in cocktail drinks and beverages. Sour star fruit is often eaten with sugar (Lim, 2012). It was reported that the total flavonoid contents of four star fruit cultivars in southern China were 104–235.1 mg catechin equivalent per 100 g fresh weight (Pang et al., 2016). However, with respect to their structures from star fruit, only cyanidin 3-*O*-β-D-glucoside, cyanidin 3,5-di-*O*-β-D-glucoside (Gunasegaran, 1992), procyanidin B_2, (−)-epicatechin, and isoquercitrin (Pang et al., 2016; Gunawardena et al., 2015) were reported. Our previous studies on dried star fruit in sour taste yielded ten phloretin C-glycosides, carambosides A–J, and three known flavonoid glycosides, carambolaflavone, hovertichoside C, and isovitexin 2″-*O*-α-L-rhamnoside (Yang et al., 2015, 2016). The objective of this study was to clarify the structures of flavonoids present in fresh sweet star fruit. As a result, thirteen flavonoids were obtained, including undescribed a flavan-3-ol (**1**), a dihydroflavon-3-ol glucoside (**3**), five phloretin C-glycosides (**9**−**13**), and six known compounds, which were not previously reported from this species. This paper describes the isolation, structural elucidation, and evaluation of antioxidant and pancreatic lipase inhibitory activities of these flavonoids.

2. Results and discussion

The 95% aqueous ethanol extract of fresh star fruit in sweet taste was separated by solvent fractionation, column chromatography (CC), and liquid chromatography (LC) to afford compounds **1**–**13** (Fig. 1).

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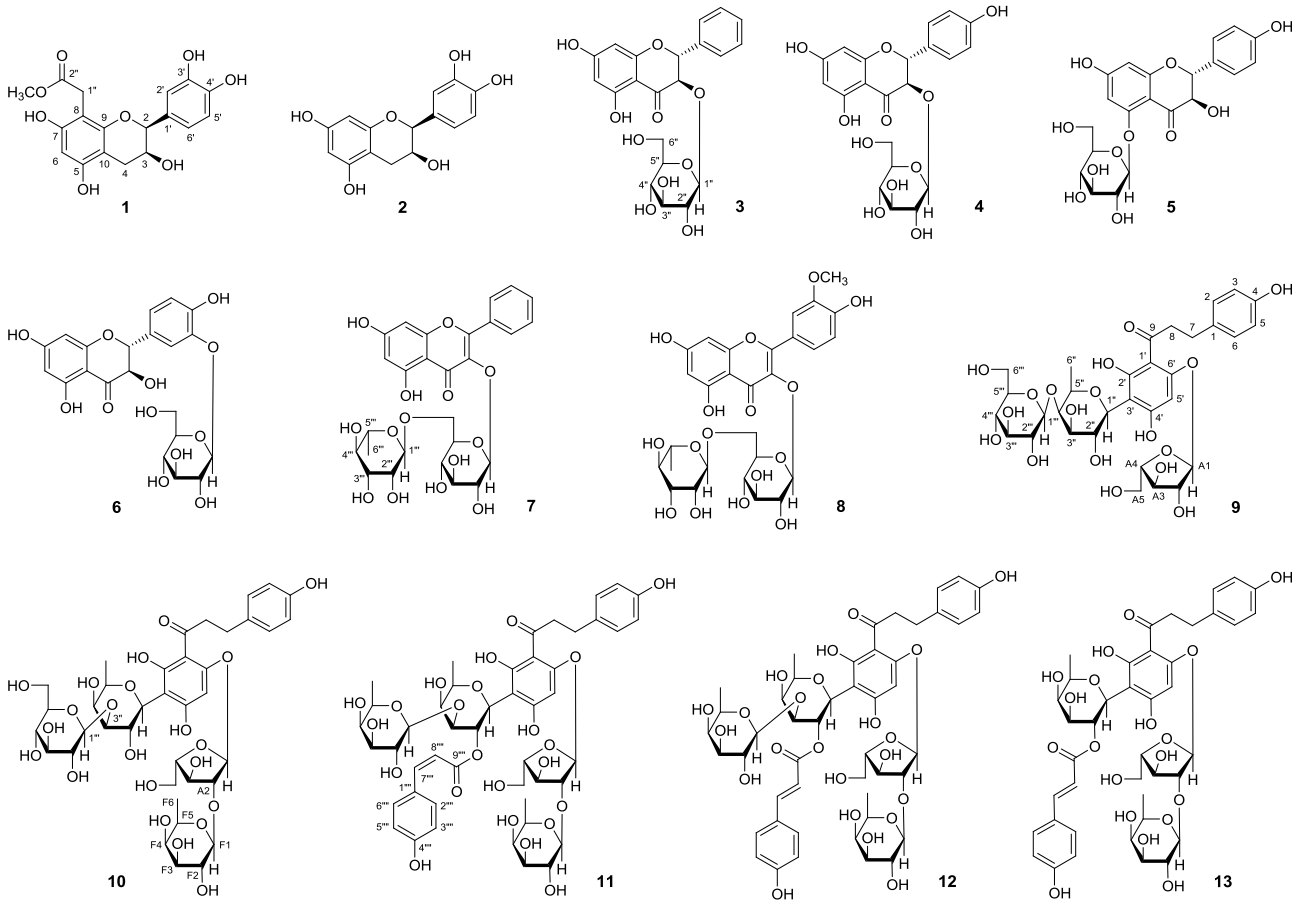

Fig. 1. Structures of compounds **1–13**.

2.1. Structural elucidation

Compound **1** was deduced to have the molecular formula $C_{18}H_{18}O_8$ from its high resolution electrospray ionization mass spectrometry (HRESIMS) and NMR data. The ^{1}H and ^{13}C NMR spectra exhibited signals of a singlet proton (H-6), three ABX-coupled aromatic protons (H-2′, H-5′, and H-6′), twelve aromatic carbons (C-5–10 and C-1′–6′), in addition to four protons and three carbons assignable for a –OCH–OCH–CH_2– part. These signals were typical of an epicatechin moiety with C-6 or C-8 being substituted (Xu et al., 2010). In the hetero-nuclear multiple bond correlation (HMBC) spectrum, the correlations from two protons of an isolated methylene (H_2-1″) to C-7, C-8, C-9, and a carboxyl carbon (C-2″), and from three singlet protons of a methoxyl group to C-2″ ascertained the presence of an acetic acid methyl ester moiety and its connection to C-8 via carbon bond (Tian et al., 2014). However, the optical rotation (α_D) value of **1** was positive, and its electronic circular dichroism (ECD) spectrum showed positive Cotton effects at 208 (+4.3), 219 (+2.2), and 278 (+0.3) nm ($\Delta\varepsilon$), which were opposite to those of (−)-epicatechin (Yanagida et al., 2016). Further, the ECD calculation result (Fig. 2) also revealed the 2*S*, 3*S* absolute configurations. Thus, compound **1** was identified as 8-carboxymethyl-(+)-epicatechin methyl ester.

Compound **3** was determined the molecular formula $C_{21}H_{22}O_{10}$ based on its NMR and HRESIMS data. The ^{1}H and ^{13}C NMR spectra demonstrated signals of five aromatic protons assignable for a mono-substituted phenyl (H-2′–H-6′) and two *ortho*-coupled aromatic protons (H-6 and H-8) for the other phenyl, in addition to signals of two oxygenated methines (OCH-2 and OCH-3), a carbonyl carbon (C-4), and a β-glucosyl moiety. The HMBC correlations from H-2 to C-3, C-4, C-9, C-1′, and C-2′/6′, H-3 to C-2, C-4, C-10, and C-1′, as well as from H-1″ to C-3 and H-3′ to C-1″ clarified the presence of 3,5,7-trihydroxydihydroflavone aglycone (Biva et al., 2016) and the connection of glucosyl moiety to C-3. The coupling constant of 9.8 Hz between H-2 and H-3 clarified their *trans* relative configurations. Consistence of its CD spectrum with that of (2R, 3R)-dihydrokaempferol 3-*O*-β-D-glucoside (Gödecke et al., 2005) and its ECD calculation result (Fig. 2) revealed the 2R, 3R absolute configurations. Acid hydrolysis of **3** released D-glucose [retention time (t_R) = 21.2 min], which was identified by comparison of its HPLC t_R value with those of authentic D-(+)-glucose (t_R = 21.3 min) and L-(−)-glucose (t_R = 19.3 min) (Fig. S33). Hence, compound **3** was established as pinobanksin 3-*O*-β-D-glucoside.

The molecular formula $C_{32}H_{42}O_{18}$ of compound **9** was decided from its HRESIMS and NMR data. The ^{1}H and ^{13}C NMR and hetero-nuclear singular quantum correlation (HSQC) spectra (Table 1) showed signals of two aliphatic methylenes (CH_2-7 and CH_2-8), twelve aromatic carbons (C-1–6 and C-1′–6′), and a carbonyl carbon (C-9), which were characteristic of a dihydrochalcone skeleton. Excluding the signals assigned for the skeleton, comparison of the δ values of remaining seventeen carbons and the δ and J values of remaining protons with those of carambolasides C and Ia, which were previously obtained from dried star fruit in sour taste (Yang et al., 2015, 2016), declared the presence of a β-fucosyl, a β-glucosyl, and an α-arabifuranosyl moiety. In the HMBC spectrum, the correlations from H-1″ to C-2′, C-3′, and C-4′, H-1‴ to C-4″, H-4″ to C-1‴, and H-A1 to C-6′ clarified the direct connection of C-1″ to C-3′ and the connections of C-1‴ to C-4″ and C-A1 to C-6′ via oxygen bridges. Acid hydrolysis of **9** yielded D-glucose (t_R = 21.2 min) and L-arabinose (t_R = 23.7 min), which were determined by comparison of their HPLC t_R values with authentic sugars (Fig. S34). The β-fucosyl moiety connected to phloretin aglycone through carbon bond from this species was previously determined to possess D absolute configuration

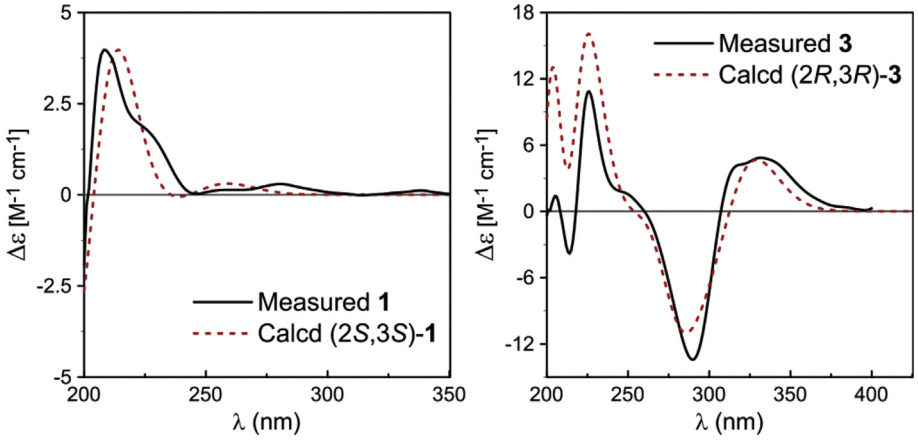

Fig. 2. Comparison between the measured and M06/TZVP calculated ECD spectra of compounds **1** in MeOH (σ = 0.30 eV, shift = ± 0 nm) and **3** in MeOH (σ = 0.30 eV, shift = +20 nm).

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compounds **9** and **10** in CD_3OD.

H/C	9		10	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		133.7		134.0
2	7.06, d (8.4)	130.4	7.09, d (8.4)	130.5
3	6.68, d (8.4)	116.1	6.70, d (8.5)	116.3
4		156.4		156.6
5	6.68, d (8.4)	116.1	6.70, d (8.5)	116.3
6	7.06, d (8.4)	130.4	7.09, d (8.4)	130.5
7	2.89, t (7.4, 2H)	30.7	2.91, m (2H)	30.7
8	3.46, dt (17.4, 7.4)	47.1	3.46, m	47.4
	3.38, dt (17.4, 7.4)		3.37, m	
9		206.6		205.9
1′		106.5		105.4
2′		165.5		165.9
3′		106.7		107.0
4′		164.5		164.7
5′	6.23, s	95.9	6.18, s	96.3
6′		161.4		161.5
1″	4.83, d (9.8)	76.2	4.87, d (9.6)	75.4
2″	4.06, t (9.8)	71.9	4.31, t (9.6)	69.8
3″	3.65, dd (9.8, 3.0)	76.8	3.73, dd (9.6, 3.0)	86.0
4″	3.96, d (3.0)	83.9	4.00, d (3.0)	73.0
5″	3.78, q (6.4)	76.0	3.78, q (6.4)	75.7
6″	1.36, d (6.4, 3H)	17.6	1.29, d (6.4, 3H)	17.2
1‴	4.59, d (7.7)	106.4	4.61, d (7.7)	105.4
2‴	3.33, m	76.2	3.29, dd (9.0, 7.7)	76.0
3‴	3.29, m	78.1	3.31, m	77.9
4‴	3.35, m	71.4	3.32, m	71.3
5‴	3.40, m	78.5	3.37, m	77.7
6‴	3.87, dd (11.9, 2.2)	62.7	3.86, dd (11.9, 1.8)	62.5
	3.71, dd (11.9, 5.4)		3.71, dd (11.9, 4.9)	
A1	5.60, d (1.6)	108.4	5.79, br s	107.0
A2	4.07, dd (5.9, 1.6)	87.4	4.29, dd (4.3, 1.5)	92.4
A3	4.01, dd (5.9, 3.6)	78.2	4.16, d (7.3)	76.5
A4	4.26, dd (3.6, 1.6)	83.3	4.02, m	85.1
A5	3.77, dd (12.0, 4.2)	62.7	3.80, dd (12.4, 2.8)	62.1
	3.65, dd (12.0, 2.6)		3.67, dd (12.4, 5.0)	
F1			4.15, d (7.5)	105.4
F2			3.47, m	72.8
F3			3.37, m	72.1
F4			3.35, m	74.9
F5			3.23, q (6.4)	72.0
F6			0.98, d (6.4, 3H)	16.6

δ: chemical shift in ppm, *J*: coupling constant, s: singlet, br s: broad singlet, d: doublet, br d: broad doublet, dd: doublet of doublet, t: triplet, q: quartet, m: multiplet.

by ECD calculations (Yang et al., 2015). Accordingly, compound **9** was identified as phloretin 3′-*C*-(4-*O*-β-D-glucosyl)-β-D-fucosyl-6′-*O*-α-L-arabinofuranoside and named carambolaside M.

Compound **10** was assigned the molecular formula $C_{38}H_{52}O_{22}$ according to its HRESIMS and NMR data. The ^{1}H and ^{13}C NMR spectra exhibited signals assignable for a phloretin aglycone, two β-fucosyls, a β-glucosyl, and an α-arabinofuranosyl moiety (Table 1). With the aid of the ^{1}H–^{1}H correlation spectroscopy (COSY), HSQC, and HMBC spectra, the protons and carbons of four sugar moieties were assigned. Further, the HMBC correlations from H-1″ to C-2′, C-3′, and C-4′, H-1‴ to C-3″, H-3″ to C-1‴, H-A1 to C-6′, H-F1 to C-A2, and H-A2 to C-F1 revealed the connections of sugar moieties to be as shown (Fig. 1). Acid hydrolysis of **10** afforded D-glucose (t_R = 21.2 min), L-arabinose (t_R = 23.7 min), and D-fucose (t_R = 29.1 min) (Fig. S35). Consequently, compound **10** was determined as phloretin 3′-*C*-(3-*O*-β-D-glucosyl)-β-D-fucosyl-6′-*O*-(2-*O*-β-D-fucosyl)-α-L-arabinofuranoside and named carambolaside N.

The HRESIMS and NMR data of compound **11** made us assign $C_{47}H_{58}O_{23}$ as its molecular formula. Besides the signals assignable for a phloretin aglycone, three β-fucosyl moieties, and an α-arabinofuranosyl moiety (Table 2), the remaining nine carbons (C-1⁗–9⁗), four *para*-coupled aromatic protons (H-2⁗/6⁗ and H-3⁗/5⁗), and two olefinic protons with *J* values of 12.8 Hz (H-7⁗ and H-8⁗) were characteristic of a *cis-p*-coumaroyl moiety (Sahakitpichan et al., 2014). Acid hydrolysis of **11** gave L-arabinose (t_R = 23.763 min) and D-fucose (t_R = 29.246 min) (Fig. S36). The connections of four sugar moieties and a coumaroyl moiety were accomplished by analyses of the HSQC and HMBC spectra and comparison of their NMR data with those of **10** and carambolaside G (Yang et al., 2016). Hence, compound **11** was identified as phloretin 3′-*C*-(2-*O*-*cis-p*-coumaroyl-3-*O*-β-D-fucosyl)-β-D-fucosyl-6′-*O*-(2-*O*-β-D-fucosyl)-α-L-arabinofuranoside and named carambolaside O.

Compound **12** had the same molecular formula as **11** deduced from its HRESIMS and NMR data. Comparison of the ^{1}H and ^{13}C NMR data of two compounds (Table 2) clarified the presence of a *trans-p*-coumaroyl moiety (Xu et al., 2013). Therefore, compound **12** was identified as phloretin 3′-*C*-(2-*O*-*trans-p*-coumaroyl-3-*O*-β-D-fucosyl)-β-D-fucosyl-6′-*O*-(2-*O*-β-D-fucosyl)-α-L-arabinofuranoside and named carambolaside P.

Compound **13** was determined the molecular formula $C_{41}H_{48}O_{19}$ based on its HRESIMS and NMR data, $C_6H_{10}O_4$ less than **12**. Comparison of the ^{1}H and ^{13}C NMR data of two compounds (Table 2) found the deficiency of a β-fucosyl moiety connected to C-3" (Yang et al., 2016). Accordingly, compound **13** was established as phloretin 3′-*C*-(2-*O*-*trans-p*-coumaroyl)-β-D-fucosyl-6′-*O*-(2-*O*-β-D-fucosyl)-α-L-arabinofuranoide and named carambolaside Q.

Yang et al. (2016) reported the structures of carambolasides I, Ia, J, and Ja isolated from sour fruit of *Averrhoa carambola* as containing an α-L-rhamnopyranosyl moiety. In the latter study we re-examined these structures and proposed that the sugar was actually a β-D-fucopyranosyl

Table 2
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compounds **11–13** in CD_3OD.

H/C	11		12		13	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		133.9		134.0		134.0
2	7.00, br s	130.5	6.90/6.99, br s	130.4	6.90/7.01, br s	130.4
3	6.66, d (8.8)	116.1	6.63/6.66, d (8.4)	116.3	6.63/6.66, d (8.6)	116.3
4		156.3		156.5		156.5
5	6.66, d (8.8)	116.1	6.63/6.66, d (8.4)	116.3	6.63/6.66, d (8.6)	116.3
6	7.00, br s	130.5	6.90/6.99, br s	130.4	6.90/7.01, br s	130.4
7	2.76, br s (2H)	30.3	2.74/2.64, br s (2H)	30.2	2.75/2.63, br s (2H)	30.3
8	3.39/3.14 br s (2H)	47.2	3.36/3.09, br s (2H)	47.5	3.36/3.08, br s (2H)	47.5
9		206.1		206.1		206.2
1′		105.7		105.6		105.5
2′		165.2		165.0		165.1
3′		105.7		105.8		105.6
4′		165.2		164.8		165.1
5′	6.20, s	96.1	6.17 (s)	96.1	6.17, s	96.2
6′		161.4		161.7		161.6
1″	5.04, d (9.5)	73.8	5.13, d (9.8)	73.9	5.08, d (9.6)	73.9
2″	5.66, br s	71.3	5.67, br s	71.6	5.51, br s	73.0
3″	3.94, br d (9.6)	81.8	4.01, dd (9.0, 3.0)	82.4	3.85, m	74.6
4″	3.99, d (3.0)	73.2	4.02, d (3.0)	73.3	3.79, m	73.5
5″	3.84, q (6.4)	76.2	3.90, q (6.4)	76.2	3.82, q (6.3)	76.3
6″	1.32, d (6.4, 3H)	17.2	1.34, d (6.4, 3H)	17.2	1.33, d (6.3, 3H)	17.1
1‴	4.32, d (7.5)	105.5	4.37, d (7.6)	105.6		
2‴	3.46, dd (9.7, 7.5)	72.2	3.48, dd (9.8, 7.6)	72.2		
3‴	3.40, dd (9.7, 3.2)	74.7	3.39, dd (9.8, 3.0)	74.6		
4‴	3.57, d (3.2)	72.9	3.56, d (3.0)	72.9		
5‴	3.56, q (6.4)	72.0	3.61, q (6.4)	71.9		
6‴	1.25, d (6.4, 3H)	16.8	1.26, d (6.4, 3H)	16.9		
1⁗		127.5		127.2		127.2
2⁗	7.35, br s	133.6	7.37, d (8.4)	131.2	7.38, d (8.4)	131.2
3⁗	6.66, d (8.8)	115.7	6.82, d (8.4)	116.8	6.82, d (8.4)	116.9
4⁗		159.6		161.2		161.2
5⁗	6.66, d (8.8)	115.7	6.82, d (8.4)	116.8	6.82, d (8.4)	116.9
6⁗	7.35, br s	133.6	7.37, d (8.4)	131.2	7.38, d (8.4)	131.2
7⁗	6.67, d (12.8)	144.4	7.44, d (15.9)	146.3	7.46, d (15.9)	146.3
8⁗	5.60, d (12.8)	116.7	6.07, d (15.9)	115.2	6.09, d (15.9)	115.3
9⁗		167.0		168.2		168.3
A1	5.93, br s	106.7	5.74, br s	107.0	5.73, br s	107.1
A2	4.33, br s	92.1	4.18, dd (4.6, 1.8)	92.9	4.17, br s	92.9
A3	4.18, dd (6.9, 3.7)	76.7	4.12, dd (7.9, 4.6)	76.5	4.11, dd (7.8, 4.7)	76.8
A4	3.98, m	84.4	3.97, m	84.4	3.97, m	84.5
A5	3.64, dd (12.8, 4.9)	62.2	3.64, dd (12.4, 4.8)	61.9	3.64, dd (12.3, 4.8)	62.0
	3.76, br d (12.8)		3.79, br d (12.4)		3.84, br d (12.3)	
F1	3.98, d (6.8)	105.2	3.98, d (7.7)	105.1	3.96, d (7.5)	105.7
F2	3.42, dd (9.7, 6.8)	72.2	3.42, dd (9.2, 7.7)	72.1	3.41, dd (9.7, 7.5)	72.2
F3	3.25, dd (9.7, 3.2)	74.9	3.24, dd (9.2, 3.2)	74.8	3.24, br d (9.7)	74.8
F4	3.41, d (3.2)	72.8	3.40, d (3.2)	72.7	3.41, d (2.8)	72.8
F5	2.90, br s	71.9	2.89, br s	71.7	2.89, br s	71.7
F6	0.80/0.99, br s (3H)	16.8	0.79/0.99, br s (3H)	16.9	0.78/0.97, br s (3H)	16.9

moiety. This revision was based on acid hydrolysis to release D-fucose rather than L-rhamnose (Fig. S37) as well as the NMR data that showed good consistence with those of D-fucosyl moiety in compounds **11–13**. Further, the diglycosyl moiety, methyl 2-*O*-β-D-fucosyl-α-L-arabinofuranoside, were isolated from sour star fruit (Yang et al., 2018). Hence, the structures of carambolasides I, Ia, J, and Ja were revised as shown in Fig. 3. The authors were sorry for this mistake.

The known compounds were identified as (+)-epicatechin (**2**) (Foo et al., 1998; Yanagida et al., 2016), aromadendrin 3-*O*-β-D-glucoside (**4**) (Baderschneider and Winterhalter, 2001; Gödecke et al., 2005), helicioside A (**5**) (Wang et al., 2009; Morimura et al., 2006), taxifolin 3′-*O*-β-D-glucoside (**6**) (Baderschneider and Winterhalter, 2001), and isorhamnetin 3-*O*-rutinoside (**8**) (Liu et al., 2010) by interpretation of their spectroscopic data and comparison of the data with reported values.

2.2. Antioxidant activity

Compounds **1–13** were evaluated for antioxidant activity by three *in vitro* assays. As shown in Table 3, eleven of them except for **1** and **7** demonstrated more potent ABTS radical cation scavenging activity with the IC_{50} values ranging from 7.1 to 0.9 μM than L-ascorbic acid (10.5 μM). In addition, the IC_{50} values of (+)-epicatechin (**2**) scavenging DPPH radicals and FRAP were 22.5 μM and 17.1 mmol/g, respectively, which were more potent than L-ascorbic acid (39.5 μM and 11.3 mmol/g), while the others were inactive (IC_{50} > 100 μM and < 2 mmol/g).

2.3. Pancreatic lipase inhibitory activity

The complex of ethyl acetate soluble fraction and ethanol eluate of the *n*-butanol soluble fraction from fresh sweet fruit was evaluated for porcine pancreatic lipase inhibitory activity, and its IC_{50} value was 68.9 μg/ml. Accordingly, we screened for this activity of the obtained compounds at the concentrations of 50 and 100 μM, and their inhibitory rates were shown in Table 4. Compounds **2**, **3**, **8**, and **11–13** exhibited weak activity with the IC_{50} values ranging from 99.6 to 71.5 μM, which were much higher than orlistat (1.6 μM), a clinic drug for obesity.

3. Conclusion

This study revealed diverse chemical structural types of flavonoids present in fresh star fruit in sweet taste, including flavan-3-ol, dihydroflavon-3-ol, flavonol, and dihydrochalcone. All the flavonoids showed potent ABTS radical cation scavenging activity, especially the (+)-epicatechin, taxifolin 3′-*O*-β-D-glucoside, aromadendrin 3-*O*-β-D-glucoside, carambolaside M, P, and Q, their IC_{50} values were less than 3 μM. In addition, (+)-epicatechin, pinobanksin 3-*O*-β-D-glucoside, isorhamnetin 3-*O*-rutinoside, carambolasides O, P, and Q showed comparable porcine pancreatic lipase inhibitory activity to that of combined ethyl acetate soluble fraction and ethanol eluate of the *n*-butanol soluble fraction. The above results led us to conclude that these flavonoids were major contributors to the antioxidant and porcine pancreatic lipase inhibitory activities of star fruit.

4. Experimental

4.1. General experimental procedures

OR values were recorded on a Perkin-Elmer 343 polarimeter (Perkin-Elmer, Waltham, MA, USA). ECD spectra were run on a JASCO J-810 spectropolarimeter (Tokyo, Japan). UV spectra were acquired on a Perkin-Elmer Lambda 650 UV/Vis spectrophotometer. NMR spectra were obtained on a Bruker Ascend-500 spectrometer using solvent peaks as reference. HRESIMS spectra were measured on a Bruker maXis mass spectrometer. ESIMS spectra were measured on an MDS SCIEX API 2000 LC-MS/MS spectrometer (Applied Biosystems, Forster, CA, USA). HPLC was performed on an LC3000 set connected to a UV3000 scanning spectrophotometer detector (Beijing ChuangXin TongHeng Sci. & Tech. Co., China) and the columns used were Cosmosil 5C18-MS-II, 5 μm, 250 mm × 4.6 mm i.d. for analysis and 250 mm × 20 mm i.d. for preparation (Nacalai Tesque Inc., Kyoto, Japan). Medium pressure

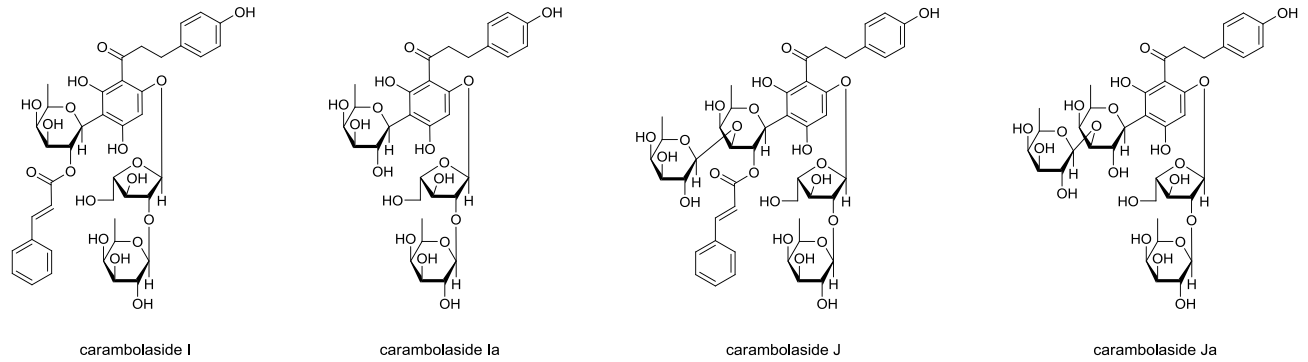

Fig. 3. Revised structures of carambolasides I, Ia, J, and Ja.

Table 3
Antioxidant activity of compounds **1–13**.

Compound	ABTS (IC_{50}, μM)	DPPH (IC_{50}, μM)	FRAP (mmol/g)
1	15.6 ± 0.7	> 100	1.7 ± 0.1
2	0.9 ± 0.1	22.5 ± 2.0	17.1 ± 1.3
3	3.4 ± 0.0	> 100	0.6 ± 0.0
4	2.5 ± 0.0	> 100	0.4 ± 0.1
5	6.0 ± 0.1	> 100	1.6 ± 0.0
6	1.3 ± 0.1	> 100	0.7 ± 0.1
7	11.7 ± 0.1	> 100	0.6 ± 0.0
8	7.1 ± 0.1	> 100	1.7 ± 0.1
9	2.3 ± 0.0	> 100	0.2 ± 0.0
10	3.8 ± 0.1	> 100	0.1 ± 0.0
11	5.2 ± 0.0	> 100	0.2 ± 0.0
12	2.9 ± 0.1	> 100	0.1 ± 0.0
13	2.7 ± 0.0	> 100	0.2 ± 0.0
L-Ascorbic acid	10.5 ± 0.1	39.5 ± 0.5	11.3 ± 0.1

Values represent means ± SD (n = 3).

Table 4
Porcine pancreatic lipase inhibitory activity of compounds **1–13**.

Compound	Inhibitory rate (%)		IC_{50} (μM)
	50 μM	100 μM	
1	19.1 ± 0.1	56.6 ± 0.2	96.4 ± 2.8
2	33.8 ± 0.1	50.7 ± 0.1	98.4 ± 5.8
3	28.5 ± 0.1	59.1 ± 0.1	92.1 ± 3.6
4	25.6 ± 0.0	46.4 ± 0.0	
5	25.7 ± 0.1	47.1 ± 0.0	
6	14.5 ± 0.0	34.6 ± 0.1	
7	14.5 ± 0.1	38.4 ± 0.0	
8	49.0 ± 0.0	62.2 ± 0.1	78.7 ± 8.2
9	25.1 ± 0.2	46.1 ± 0.0	
10	24.7 ± 0.2	47.3 ± 0.0	
11	17.8 ± 0.1	55.1 ± 0.0	97.1 ± 2.9
12	21.3 ± 0.1	52.9 ± 0.1	93.6 ± 1.7
13	27.3 ± 0.2	51.7 ± 0.0	99.6 ± 6.1
Orlistat			1.6 ± 0.1

Values represent means ± SD (n = 3).

liquid chromatography (MPLC) was performed on an EZ Purifier (Lisure Science, Suzhou, China) and the column used was Chromatorex RP-18 SMB100, 20–45 μm, 400 mm × 25 mm i.d. (Fuji Silysia Chemical, Aichi, Japan). Silica gel (100–200 mesh, Qingdao Haiyang Chemical Co., China), Amberlite XAD-7HP macroporous resin (Sigma-Aldrich), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used for CC. TLC was conducted on pre-coated silica gel $HSGF_{254}$ plates (Jiangyou Silica Gel Development Co., Yantai, China) and visualized by UV and then spraying 10% sulfuric acid in EtOH (v/v) followed by heating.

D-(+)-Glucose, L-(−)-glucose, D-(+)-fucose, and L-(−)-fucose were from Aladdin Industrial Corp., Shanghai, China. D-(−)-Arabinose and L-(+)-arabinose were from J&K Scientific Co., Beijing, China. ABTS, DPPH, porcine pancreatic lipase type II crude (EC3.1.1.3, L3126), and orlistat (O4139) were from Sigma-Aldrich (Shanghai) Trading Co., China. L-Ascorbic acid was from Shanghai Boao Biotech Co., China.

4.2. Plant material

Fresh ripe star fruits in sweet taste were collected from an orchard in Xiaozhou Village (113°35′56.02″E, 23°06′70.44′N), Haizhu District, Guangzhou, in December of 2012. The species was botanically authenticated to be *Averrhoa carambola* L. (Oxalidaceae) by Prof. Huagu Ye in the Herbarium of South China Botanical Garden, Chinese Academy of Sciences. A voucher specimen (No. 121201) was deposited at our laboratory.

4.3. Extraction and isolation

The fresh fruits (105 kg) were cut manually to pieces and immediately soaked in 95% EtOH thrice and 2 d per time. The solution was filtrated and condensed under vacuum to give brown syrup, which was diluted with water to 11.5 l and then sequentially partitioned with EtOAc (7.5 l × 4) and *n*-BuOH (7.5 l × 4). The combined solutions were evaporated under vacuum to afford EtOAc soluble (77.4 g) and *n*-BuOH soluble (590.2 g) fractions. The latter fraction was dissolved in water and passed through a XAD-7HP column eluted with water to remove free sugars and then with 95% EtOH to yield an EtOH eluate (128.1 g) after dried under vacuum. The complex of EtOH eluate and EtOAc soluble fraction was subjected to silica gel CC eluted with $CHCl_3$–MeOH (v/v, 1:0, 16 l → 95:5, 22.4 l → 9:1, 38.4 l → 0:1, 16 l) to provide fractions 1–9 according to their TLC profiles. Fraction 8 (2.7 g) was separated by MPLC using aq. methanol as mobile phase to furnish fractions 8-1–8-13. Fraction 8–10 was separated by Sephadex LH-20 CC eluted with methanol to give compound **3** (30 mg). Fraction 9 (150.2 g) was subjected to silica gel CC eluted with $CHCl_3$–MeOH to yield fractions 9-1–9-8. Fraction 9–2 (1.25 g) was separated by MPLC using aq. MeOH as mobile phase to offer fractions 9-2-1–9-2-17. Fraction 9-2-5 was purified by HPLC using 20% aq. MeOH as mobile phase at the flow rate of 5 ml/min to furnish compound **2** (t_R = 70 min, 104 mg). Fraction 9-2-8 was separated by LH-20 CC and then purified by HPLC using 15% aq. CH_3CN as mobile phase at 6 ml/min to afford compound **4** (t_R = 69 min, 9 mg). Fraction 9-2-9 was separated by LH-20 CC and then purified by HPLC using 14% aq. CH_3CN as mobile phase at 5 ml/min to furnish compound **1** (t_R = 73 min, 3 mg). Fraction 9–3 (6.13 g) was separated by MPLC to give fractions 9-3-1–9-3-24. Fraction 9-3-17 was purified by HPLC using 41% aq. MeOH as mobile phase at 6 ml/min to yield compound **7** (t_R = 109 min, 6 mg). Fraction 9-3-19 was separated by LH-20 CC and purified by HPLC using 25% aq. MeOH as mobile phase at 6 ml/min to give compound **6** (t_R = 68 min, 3 mg). Fraction 9-3-22 was separated by LH-20 CC and then purified by HPLC using 14% aq. CH_3CN as mobile phase at 6 ml/min to furnish

compound **5** (t_R = 89 min, 3 mg). Fraction 9–4 (8.51 g) was separated by MPLC to yield fractions 9-4-1–9-4-19. Fraction 9-4-6 was separated by LH-20 CC and then purified by HPLC using 34% aq. MeOH as mobile phase at 6 ml/min to yield compound **8** (t_R = 42 min, 11 mg). Fraction 9–6 (23.5 g) was separated by MPLC to afford fractions 9-6-1–9-6-17. Fraction 9-6-7 was purified by HPLC using 36% aq. MeOH as mobile phase at 5 ml/min to yield compound **9** (t_R = 79 min, 70 mg). Fraction 9-6-12 was separated by LH-20 CC eluted with MeOH to give compounds **11** (90 mg) and **13** (4 mg). Fraction 9–7 was separated by MPLC to afford fractions 9-7-1–9-7-16. Fraction 9-7-6 was separated by LH-20 CC and then purified by HPLC using 18% aq. CH_3CN as mobile phase at 6 ml/min to furnish compound **10** (t_R = 69 min, 44 mg). Fraction 9-7-12 was purified by HPLC using 55% aq. MeOH as mobile phase at 6 ml/min to give compound **12** (t_R = 70 min, 140 mg).

4.3.1. 8-Carboxymethyl-(+)-epicatechin methyl ester (1)

White amorphous powder; $[\alpha]_D^{20}$ +19.5 (*c* 0.20, MeOH); UV (MeOH) λ_{max} nm (log ε) 205 (4.16); ECD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 208 (+4.3), 219 (+2.2), and 278 (+0.3); HRESIMS *m/z* 385.0891 [M + Na]$^+$ (calcd for $C_{18}H_{18}NaO_8^+$, 385.0894); ^{1}H NMR (CD_3OD, 500 MHz) δ 4.83 (1H, d, *J* = 1.4 Hz, H-2), 4.17 (1H, ddd, *J* = 4.6, 3.0, 1.4 Hz, H-3), 2.89 (1H, dd, *J* = 16.7, 4.6 Hz, H-4), 2.76 (1H, ddd *J* = 16.7, 3.0 Hz, H-4), 6.03 (1H, s, H-6), 6.94 (1H, d, *J* = 1.8 Hz, H-2′), 6.75 (1H, d, *J* = 8.1 Hz, H-5′), 6.78 (1H, dd, *J* = 8.1, 1.8 Hz, H-6′), 3.64 (1H, d, *J* = 16.8 Hz, H-1″), 3.59 (1H, d, *J* = 16.8 Hz, H-1″), and 3.61 (3H, s, OCH_3); ^{13}C NMR (CD_3OD, 125 MHz) δ 79.9 (C-2), 67.4 (C-3), 29.3 (C-4), 156.7 (C-5), 96.1 (C-6), 155.7 (C-7), 101.5 (C-8), 155.2 (C-9), 99.8 (C-10), 132.3 (C-1′), 115.1 (C-2′), 145.7 (C-3′), 146.0 (C-4′), 115.9 (C-5′), 119.1 (C-6′), 29.4 (C-1″), 176.3 (C-2″), and 52.3 (OCH_3).

4.3.2. Pinobanksin 3-O-β-D-glucoside (3)

Yellowish amorphous powder; $[\alpha]_D^{20}$ +19.6 (*c* 0.28, MeOH); UV (MeOH) λ_{max} nm (log ε) 230 (4.08); ECD (MeOH) nm ($\Delta\varepsilon$) 214 (−4.6), 225 (+11.2), 290 (−13.5), and 333 (+4.8); HRESIMS *m/z* 457.1108 [M + Na]$^+$ (calcd for $C_{21}H_{22}NaO_{10}^+$, 457.1105); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.41 (1H, d, *J* = 9.8 Hz, H-2), 4.98 (1H, d, *J* = 9.8 Hz, H-3), 5.94 (1H, d, *J* = 2.4 Hz, H-6), 5.93 (1H, d, *J* = 2.4 Hz, H-8), 7.54 (2H, dd, *J* = 7.8, 1.5 Hz, H-2′, 6′), 7.41 (2H, d, *J* = 7.8, 1.5 Hz, H-3′, 5′), 7.39 (1H, t, *J* = 7.8 Hz, H-4′), 3.80 (1H, d, *J* = 7.8 Hz, H-1″), 3.22 (1H, dd, *J* = 9.2, 7.8 Hz, H-2″), 3.09 (1H, t, *J* = 9.2 Hz, H-3″), 3.25 (1H, dd, *J* = 9.8, 9.1 Hz, H-4″), 2.96 (1H, ddd, *J* = 9.8, 5.7, 2.3 Hz, H-5″), 3.73 (1H, dd, *J* = 12.0, 2.3 Hz, H-6″), and 3.58 (1H, dd, *J* = 12.0, 5.8 Hz, H-6″); ^{13}C NMR (CD_3OD, 125 MHz) δ 83.5 (C-2), 77.3 (C-3), 195.7 (C-4), 165.5 (C-5), 97.5 (C-6), 169.1 (C-7), 96.4 (C-8), 164.0 (C-9), 102.6 (C-10), 137.7 (C-1′), 129.0 (C-2′, 6′), 129.5 (C-3′, 5′), 130.0 (C-4′), 102.7 (C-1″), 74.4 (C-2″), 77.5 (C-3″), 71.1 (C-4″), 78.2 (C-5″), and 62.5 (C-6″).

4.3.3. Carambolaside M (9)

Yellowish amorphous powder; $[\alpha]_D^{20}$ +20.0 (*c* 0.45, MeOH); UV (MeOH) λ_{max} nm (log ε) 203(4.31), 224 (4.30), 286 (4.13); HRESIMS *m/z* 737.2272 [M + Na]$^+$ (calcd for $C_{32}H_{42}NaO_{18}^+$, 737.2263); ^{1}H and ^{13}C NMR data, see Table 1.

4.3.4. Carambolaside N (10)

Yellowish amorphous powder; $[\alpha]_D^{20}$ −28.8 (*c* 0.25, MeOH); UV (MeOH) λ_{max} nm (log ε) 224 (4.17) and 286 (4.00); HRESIMS *m/z* 883.2836 [M + Na]$^+$ (calcd for $C_{38}H_{52}NaO_{22}^+$, 883.2842); ^{1}H and ^{13}C NMR data, see Table 1.

4.3.5. Carambolaside O (11)

Yellowish amorphous powder; $[\alpha]_D^{20}$ −22.2 (*c* 1.2, MeOH); UV (MeOH) λ_{max} nm (log ε) 203 (4.45), 224 (4.42), and 287 (4.35); HRESIMS *m/z* 1013.3283 [M + Na]$^+$ (calcd for $C_{47}H_{58}NaO_{23}^+$, 1013.3261); ^{1}H and ^{13}C NMR data, see Table 2.

4.3.6. Carambolaside P (12)

Yellowish amorphous powder; $[\alpha]_D^{20}$ −126.0 (*c* 0.45, MeOH); UV (MeOH) λ_{max} nm (log ε)203 (4.46), 225 (4.46), and 288 (4.43); HRESIMS *m/z* 1013.3276 [M + Na]$^+$ (calcd for $C_{47}H_{58}NaO_{23}^+$, 1013.3261); ^{1}H and ^{13}CNMR data, see Table 2.

4.3.7. Carambolaside Q (13)

Yellowish amorphous powder; $[\alpha]_D^{20}$ −160.5 (*c* 0.13, MeOH); UV (MeOH) λ_{max} nm (log ε) 204 (4.52), 224 (4.53), and 288 (4.50); HRESIMS *m/z* 867.2693 [M + Na]$^+$ (calcd for $C_{41}H_{48}NaO_{19}^+$, 867.2682); ^{1}H and ^{13}C NMR data, see Table 2.

4.4. Determination methods of sugar absolute configurations

Acid hydrolysis of undescribed compounds **3** and **9–13** release monosaccharides, which were esterified and then subjected to HPLC analysis following our previous procedures (Xiao et al., 2016).

4.5. ECD computation

The computational ECD spectra of compounds **1** and **3** were obtained according to our previous methods (Yang et al., 2015) with slight modification (Supplementary data).

4.6. Antioxidant activity assay

Antioxidant activities of compounds were evaluated by the ABTS radical cation scavenging assay, DPPH radical scavenging assay, and ferric reducing antioxidant power (FRAP) assay following our previously described procedures (Ma et al., 2014; Zhou et al., 2014). Their IC_{50} values were calculated based on three independent experiments.

4.7. Pancreatic lipase inhibition assay

This assay was accomplished following our previously described procedures (Jia et al., 2017). The IC_{50} values were calculated based on three independent experiments.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.phytochem.2018.06.007. These data include MOL files and InChiKeys of the most important compounds described in this article.

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