Food and Chemical Toxicology 132 (2019) 110730

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Identification of moracin N in mulberry leaf and evaluation of antioxidant activity

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ARTICLE INFO

Keywords:
Cellular antioxidant activity
Mulberry leaf
NMR
Prenylated phenolics
Resveratrol

ABSTRACT

Mulberry leaf is a newly accepted vegetable for daily diet. It tastes good and has multiple health benefits, including antioxidant and anti-inflammatory activities. However, the chemicals responsible for these health benefits remain unveiled. Prenylated phenolics are characteristic bioactive compounds in mulberry leaf, which are recognized as good antioxidants. In this work, moracin N was purified from mulberry leaf. It showed better antioxidant activities than resveratrol. The EC_{50} value of cellular antioxidant activity was 24.92 μM, and the IC_{50} value against DPPH radical was 40.00 μM. The prenyl group rendered the molecule more membrane affinity which improved the bioavailability. The furan ring was critical for the antioxidant behaviour. The cell viability test revealed that moracin N had a good safety. These results pointed out that moracin N contributed to the antioxidant activity of mulberry leaf.

1. Introduction

Oxidative stress is highly involved in many diseases, such as neurodegenerative disorder, atherosclerosis, cataract, inflammation, cancer, coronary heart disease and Alzheimer's disease (Aruoma, 1998; Diaz et al., 1997). It is generated during cell metabolism and leads to the oxidation of multiple biomolecules, like DNA, lipids and proteins (Fredotović et al., 2017), which can subsequently cause pathological and degenerative processes in human body. Antioxidants, especially natural antioxidants, are getting increasing attention for their abilities to maintain the oxidation-antioxidation balance (Fredotović et al., 2017). By interfering with the propagation of oxidizing chain reaction or by inhibiting the formation of radicals (van Acker et al., 1996), plant phenolics behave as effective antioxidants (Wright et al., 2001).

Fruits and vegetables are rich sources of phenolics. Young leaf of mulberry is consumed as vegetable in Asia. It has specially delicious taste and has been used to make many dietary foods. It is also a functional food to assist treating diabetes mellitus (Jia et al., 1999). Other bioactivities have been illustrated, which include treating fever, protecting liver, lowering blood pressure, anti-obesity, anti-inflammation and antitumor activities (Chang et al., 2016; Katsube et al., 2006). Some phytochemicals in mulberry leaf have been identified, including stilbenoids, phenolic acids and flavonoids (Chang et al., 2016). It is worthy to mention that prenylated phenolics are characteristic chemicals in this vegetable, and this type of chemicals are recognized as phytoestrogens (Yang et al., 2019) and present remarkable bioactivities (El-Beshbishy et al., 2006; Yang et al., 2015).

In this work, through separation and purification by column chromatography, a leading chemical was collected and identified to be moracin N by NMR. In order to investigate the antioxidant potential and structure-activity relationship, a series of non-cellular and cellular assays for antioxidant activity were conducted, including cellular antioxidant activity, oxygen radical absorbance capacity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The cellular antioxidant activity assay provides a comprehensive understanding of how an antioxidant behaves under physiological conditions (Wen et al., 2017), and gives evidence whether prenylation facilitates cell absorption (Chi et al., 2001). Moreover, cytotoxicity of moracin N was also measured. The results would be helpful to understand the health benefits of mulberry leaf.

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https://doi.org/10.1016/j.fct.2019.110730
Received 26 April 2019; Received in revised form 21 July 2019; Accepted 28 July 2019

2. Materials and methods

2.1. Chemicals and reagents

Trolox, 2,7-diacetate di-chlorofluorescin, 2,2′-azobis-amidinopropane (ABAP), 1,1-diphenyl-2-picrylhydrazyl, fluorescein sodium salt, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and fetal bovine serum (FBS) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), ascorbic acid, quercetin, potassium phosphate dibasic, potassium phosphate monobasic, Williams' medium E, Dulbecco's modified Eagle's medium (DMEM), Hank's balanced salt solution (HBSS), and other cell reagents were purchased from CASMART, China. Methanol and acetonitrile of chromatography grade were used for HPLC, and all the other reagents used for exaction and purification were of analytical grade.

2.2. Sample preparation

Morus alba (cultivar 'Yuesang 11') was selected as the material, which is widely grown in southern China as a vegetable. The leaves were collected from a mulberry farm of Sericultural & Agri-Food Research Institute of Guangdong province, China, in September 2017. The leaves were air-dried and then pulverized before extraction.

2.3. Extraction and isolation

The purification procedure was carried out according to literature (Ha et al., 2018). Dried leaf powders (8.9 kg) were extracted for three times with 95% EtOH (25 L) for 5 day at room temperature. The extract was concentrated under reduced pressure to give a dark green solid. The EtOH extract (1200 g) were suspended in water and then sequentially extracted with petroleum ether and ethyl acetate (EtOAc), to yield petroleum ether fraction (230 g) and EtOAc fraction (72.4 g). The EtOAc fraction was subject to silica gel column (100–200 mesh, 2.0 kg) and eluted with chloroform-MeOH (99:1–80:20) to afford 12 subfractions (1–12). Subfraction 8 was separated by C18 column eluted with aqueous MeOH (35%–80%) to afford 14 fractions (8–1 to 8–14). Subfractions 8–12 were further separated by HPLC equipped with C18 column (5-μm particle size) eluted by 65% MeOH to obtain 8-12-3.

2.4. Analysis techniques

A Bruker DRX-500 instrument (^{1}H NMR, 500 MHz; ^{13}C NMR, 125 MHz, Bruker, Rheinstetten, Germany) was used to record spectra. Acetone-*d6* (δ_H 2.05 and δ_C 29.92 ppm) was used as solvent and chemical shift calibration. Electrospray ionization-mass spectrometry (ESI-MS) data was acquired on an MDS SCIEX API 2000 LC/MS apparatus (MDS Sciex, Ontario, Canada). High performance liquid chromatography (HPLC) was carried out with a Shimadzu LC-20AT liquid chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with a Shimadzu UV detector, a Shimadzu-Pack PRC-ODS column (20 × 250 mm, 5 μm) and a Silgreen C18 column (10 × 250 mm, 5 μm). Ultra-performance liquid chromatography (UPLC) was carried out with an Agilent 1260 infinity Quaternary liquid chromatograph (Agilent Technologies, Inc., Santa Clara, USA) with a DAD detector, an Agilent ZORBAX SB-C18 column (3 × 100 mm, 1.8 μm).

2.5. Determination of oxygen radical absorbance capacity (ORAC)

The ORAC values of moracin N, resveratrol, and quercetin were measured by using the method of Huang et al. (2002) and Wen et al. (2015) with some modifications. In brief, the assay was performed in a multimode plate reader (Tecan Spark™, Swiss), by using black-walled 96-well plates. All the reagents and samples were made freshly and diluted with 75 mM phosphate buffer at pH 7.4, and Trolox solutions (6.25, 12.5, 25 and 50 μM) were used as the positive control. First, 20 μL of each diluted sample, Trolox standard, or buffer solution (as blank) were added in each well, followed by 200 μL of 96 nM fluorescein sodium salt. The plates were incubated for 20 min at 37 °C, before finally 20 μL of 119 mM ABAP were added to each well as a free radical initiator solution. The reaction mixture was put into the plate reader immediately, with an excitation wavelength of 480 nm and an emission wavelength of 520 nm. The absorbance was recorded every 90 s for 50 cycles after a 5-s-shaking. The ORAC values were calculated from the linear regression between area under the curve (AUC) and Trolox concentration, and finally expressed as Trolox equivalents.

2.6. Determination of DPPH radical scavenging activity

The scavenging capacities against 2,2-diphenyl-1-picrylhydrazyl (DPPH) of moracin N, resveratrol, and quercetin were evaluated by using the method described previously (Wen et al., 2017) with some modifications. In brief, 200 μM DPPH in methanol was prepared freshly, and ascorbic acid aqeous solutions (6.25, 12.5, 25 and 50 μM) were prepared as the positive control. Samples were dissolved in methanol and diluted appropriately according to their activities in preliminary experiments, and five different concentrations of samples were finally tested. The final concentrations of moracin N were 10–60 μM, 10–400 μM for resveratrol, and 2.5–50 μM for quercetin. 100 μL of the tested sample were mixed with 100 μL of DPPH solution in 96-well plates. The sample mixed with methanol was set as blank. After 30-min incubation at room temperature in dark, the absorbance was measured at 510 nm. The IC_{50} value was calculated on the scavenging activity against DPPH radical.

2.7. Cell cultivation

Human hepatocellular carcinoma (HepG2) cells were obtained from the laboratory of Animal Center, Sun Yat-Sen university, China. HepG2 cells were cultured in William's medium E (WME) along with 5% FBS (Gibco Life Technologies, Grand Island, NY), 50 units/mL penicillin, 2 mM L-glutamine, 100 μg/mL gentamicin, 10 mM Hepes, 50 μg/mL streptomycin, 5 μg/mL insulin, and 0.05 μg/mL hydrocortisone, maintained at 37 °C in a 5% CO_2 incubator. The HepG2 cell between rows 18 and 22 were used for activity tests.

2.8. Cellular antioxidant activity (CAA) assay

The CAA assay was carried out by using the protocol in our own lab (Wen et al., 2017). Briefly, HepG2 cells were inoculated at 6×10^4 cells/well in a 96-well plate. Approximately after seeding at 37 °C for 24 h, the growth medium was then removed and each well was washed with 100 μL of phosphate buffer (PBS). After that, the wells were treated with 100 μL of WME medium containing sample and 50 μM 2,7-diacetate dichlorofluorescin (DCFH-DA). After 1-h incubation, the medium was removed, and the 96-well plates were treated with PBS wash or no PBS wash. After treating with the oxidant-supplemented medium (HBSS with 10 mM HEPES, 600 μM ABAP), the 96-well plates were placed into a multimode plate reader. The fluorescence was recorded every 5 min for 1 h at 37 °C with emission wavelength of 538 nm and excitation wavelength of 485 nm.

After subtraction of background and initial fluorescence value, the area under curve for fluorescence versus time was integrated to calculate CAA value as follows:

$$\textit{Cellular antioxidant activity} = 1 - \frac{\int SA}{\int CA}$$

In this equation, ∫ SA is the integrated area under the fluorescence-time curve of samples, and ∫ SA represents the integrated area under the curve of the control. By referring to the regression equation of concentration and CAA value, the EC_{50} values and quercetin equivalents were calculated.

2.9. Cytotoxicity and antiproliferative activity tests

The cytotoxicity and antiproliferative activity assay of moracin N, resveratrol, and quercetin were measured against HepG2 cells. The cytotoxicity test was performed by using MTT staining method as described previously (Wen et al., 2014). Briefly, growth medium that contained the cells were seeded on a 96-well plate at a density of 5×10^3 cells/well. After being incubated for 24 h at 37 °C, the growth medium was removed, and the wells were washed with 100 μL of PBS. After that, the growth medium containing compound at various concentrations was added, and those wells adding the medium without tested compounds served as control. After incubation for another 24 h, 10 μL of MTT solution (5 mg/mL) were added to the plates, and the plates were then stained for 4 h at 37 °C. The staining solution was removed from each well, and the formazan were dissolved in DMSO before measuring the absorbance at 570 nm.

The absorbance values of samples were compared to that of control, and the results were expressed as 50% inhibitory concentration (IC_{50}), which was defined as the sample concentration that inhibited 50% cell viability of control. The cytotoxicity of each sample against the cancer cells was calculated as follows:

$$Cell\ viability\ (\%) = \left(\frac{As}{Ac}\right) \times 100\%$$

In this equation, As means the absorbance of the well that contains the sample, and Ac means the absorbance of the well in control group.

For the antiproliferation activity test against cancer cells, the experiment was conducted following our previous protocol (Wen et al., 2014). In brief, HepG2 cells were seeded at a density of 5×10^3 cells/well in 96-well plate. The growth medium was removed after the cells were attached to the wells, and then the wells were washed with PBS. Growth medium with different concentrations of analyte was added. Those wells added with medium containing no samples served as control. The cells were incubated for 72 h at 37 °C before staining. The following steps were the same as described in the cytotoxicity test. Each sample was measured for at least three times, and the antiproliferative activity was calculated as follows:

$$Cell\ viability\ (\%) = \left(\frac{As}{Ac}\right) \times 100\%$$

In this equation, As is the absorbance of the well added with samples, and Ac represents that of control.

2.10. Quantum chemical analysis

The quantum chemical properties of moracin N and resveratrol were analyzed. The bond-dissociation energy of O–H, ΔE between LUMO orbital and HOMO orbital, and log*P* (the octanol/water partition coefficient) were determined by using the software Hyperchem 8.0.10. In order to simulating molecular conformation of tested compounds, MM+ (Molecular mechanics +) algorithm and AM1 semi-empirical algorithm were used.

2.11. Statistical analyses

Triplicated assays were performed for the measurement of all parameters. The results were expressed as mean ± standard deviation and subjected to variance analysis by SPSS statistical analysis software, version 22 (IBM, New York, USA). The least significant difference was set as $P < 0.05$.

3. Results and discussion

3.1. Structure identification

The compound 8-12-3 was purified as described above. It appeared as brown amorphous powder. ESI-MS spectra showed a parent ion $[M-H]^-$ at *m/z* 309.1. There were two absorbance peaks at 217 and 318 nm, respectively. ^{1}H-NMR (acetone-*d6*) data: δ7.24 (1H, s, H-4), 6.97 (1H, s, H-3), 6.95 (1H, s, H-7), 6.81 (1H, s, H-2′) 6.80 (H, s, H-6′), 6.32 (1H, t, *J* = 4.2, 2.1 Hz, H-4′), 5.36 (1H, t, *J* = 2.8, 1.4 Hz, H-2″), 3.35 (2H, m, *J* = 7.2 Hz, H-1″), 1.70 (6H, s, H-4″, H-5″); ^{13}C-NMR (acetone-*d6*): δ159.9 (C-3′, 5′), 155.4 (C-2), 155.1 (C-7a), 154.4 (C-6), 133.6 (C-1′), 132.4 (C-3″), 125.9 (C-5), 124.2 (C-2″), 122.6 (C-3a), 121.5 (C-4), 103.8 (C-2′, 6′), 103.5 (C-4’), 102.4 (C-3), 98.1 (C-7), 30.7 (C-1″), 26.0 (C-5″), 17.9 (C-4”).

In the ^{1}H NMR spectra, a singlet at δ_H 1.70 was assigned to two overlapped methyls (H-4″, H-5″). An olefinic triplet at δ_H 5.36 (H-2″) was the characteristic signal of a linear prenyl. The cross peaks of H-2″ with C-1″, C-4″ and C-5″ in HMBC spectra indicated the presence of prenyl group. The H-1″ of prenyl was assigned at 3.35. A 2,5,7-trisubstituted benzofuran moiety was observed by H-3 (6.97), H-4 (7.24) and H-7 (6.95). H-2’ (6.81), H-4’ (6.32) and H-6’ (6.80) revealed the presence of a trisubstituted phenyl. The long distance correlation of H-2′ and H-6′ with C-2 indicated the linkage between benzofuran and prenyl, which confirmed the skeleton of stilbenoid. The cross peak of H-1″ in prenyl and C-5 of benzofuran suggested that the prenyl location on stilbenoid. Based on the analyses of NMR and MS data, this compound was identified to be moracin N. The chemical structure and UPLC chromatogram are shown in Fig. 1.

3.2. Antioxidant activity

3.2.1. Oxygen radical absorption capacity

To compare the *in vitro* antioxidant activities of moracin N and resveratrol, the ORAC values of moracin N and resveratrol were measured. This assay was based on thermal decomposition of ABAP into alkoxy radicals and subsequent oxidation of fluorescent substance. By adding antioxidant, decay of fluorescence can be inhibited to different extents. The calculated results are showed in Table 1 in the form of Trolox equivalents. The positive control, quercetin, showed a better peroxyl radical scavenging activity when compared to moracin N and resveratrol. The possible mechanisms were explained as below. Firstly, there are more hydroxyl groups on quercetin molecules, which enhanced hydrogen/electron donating ability. Secondly, the 3-OH and 3′,4′-catechol structure of quercetin can stabilize the flavonoid radical generated after the radical reaction (Heim et al., 2002; Rice-Evans et al., 1997). However, moracin N and resveratrol both showed comparatively low oxygen radical scavenging capacities, and the difference of their ORAC values was insignificant ($p > 0.05$).

3.2.2. DPPH radical scavenging activity

Moracin N, quercetin and resveratrol were detected for DPPH radical scavenging activity. The former two were effective on eliminating DPPH radicals with a dose-dependent behaviour. When these three compounds were at the same level, the activity of resveratrol was lower than the other two. The IC_{50} values are shown in Table 1. The IC_{50} value of resveratrol was 5-fold of other compounds. The IC_{50} value of moracin N was the lowest, slightly lower than that of quercetin. Therefore, the DPPH radical scavenging activities of these compounds were in a decreasing order, moracin N and quercetin > resveratrol.

The classic hydrogen atom transfer mechanism explains how the peroxyl radicals get scavenged in the ORAC assay (Ou et al., 2001). It measures the capability of an antioxidant to quench free radicals (usually peroxyl radicals) by H-atom donation. The reaction is $ROO\cdot + AH/ArOH \rightarrow ROOH + A\cdot/ArO\cdot$. Most of *in vitro* antioxidant activity assay are based on a mixed mechanism of electron transfer

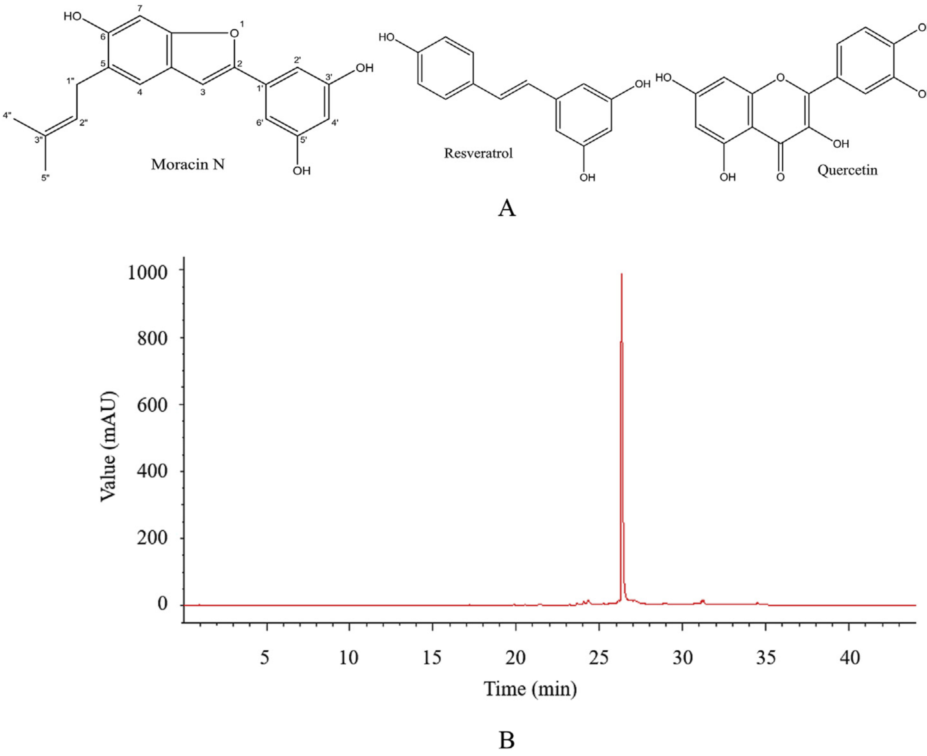


Fig. 1. Chemical structures and UPLC chromatogram of moracin N. A, chemical structure of moracin N, resveratrol and quercetin; B, UPLC chromatogram of moracin N.

Table 1
ORAC values and DPPH radical scavenging activities of moracin N, resveratrol and quercetin. The values in a column having no letters in common are significantly different ($p < 0.05$).

Compounds	ORAC values (μmol TE/μmol)	IC_{50} values against DPPH (μM)
Moracin N	1.70 ± 0.05a	40.00 ± 1.09a
Resveratrol	1.55 ± 0.79a	285.54 ± 27.11b
Quercetin	10.86 ± 0.81b	47.60 ± 0.54a

mechanism and hydrogen atom transfer mechanism, including Trolox-equivalent antioxidant capacity and DPPH assays (Apak et al., 2016). The DPPH reaction is DPPH· + AH/ArOH→ DPPH-H + A·/ArO·. Electron transfer reaction is fast and not diffusion-controlled, while hydrogen atom transfer reaction is relatively slow and diffusion-controlled. Phenolics are recognized as good antioxidant candidates due to the presence of phenolic hydroxyl. The number and position of hydroxyl groups, and *ortho*-dihydroxy groups were important structure features influencing the antioxidant activities for a phenolic compound (Yang et al., 2012). The improved hydrophilicity and steric conformation are responsible for the antioxidation behaviour. The DPPH radical scavenging activity of moracin N in this experiment was obviously higher than that of resveratrol, which was different to the ORAC assay. This difference indicated that moracin N could donate electron more readily than resveratrol.

3.2.3. *Cellular antioxidant activity*

As shown in Fig. 2, the fluorescence was in proportion to the degree of DCFH oxidation induced by alkoxy radicals, and the cellular antioxidant activities of analytes were reflected by how much the fluorescence was inhibited. Fig. 2A, C, and 2E show the results with PBS wash protocol, and Fig. 2B, D, and 2F show the results obtained from no PBS-wash protocol. All three analytes inhibited the fluorescence of DCF formation in a dose-dependent manner. Table 2 lists the CAA values of moracin N and resveratrol. In the protocol without PBS wash, moracin N showed a significantly higher activity than resveratrol. The EC_{50} value of moracin N was 24.92 μM, while resveratrol had a value of 84.16 μM. When presented as quercetin equivalent, moracin N had a cellular antioxidant activity of 22.51 μmol QE/100 μmol, which was 3.37-fold of resveratrol. In the PBS wash protocol, the EC_{50} value of moracin N was 21.82 μM, which was insignificantly ($p > 0.05$) different to that in the no PBS wash protocol. It suggested that moracin N was uptaken effectively in HepG2 cells. Quercetin has been documented to be an efficient compound with 100% uptake in $HepG_2$ cells (Zhao et al., 2015). On the contrary, resveratrol showed a very poor activity in the PBS wash protocol, which suggested a poor uptake to cells. These results suggested that moracin N had a better antioxidant activity and a better absorption than resveratrol in cell.

CAA assay is a biological antioxidant assay that reflects the effect of an antioxidant at the cell level. The physiological conditions involved in this assay are more biologically representative, which enables bioaccumulation, uptake, distribution and metabolism of the analytes contribute to the antioxidant behaviour. There were PBS wash protocol and no wash protocol in this assay, and the different results between these two protocols revealed the cellular uptake of tested compound (Wen et al., 2017). In this work, moracin N showed a better cellular uptake than resveratrol. The prenyl group of moracin N might improve membrane attachment as it increases the hydrophobicity.

3.3. *Cytotoxicity and antiproliferative activity against cancer cells*

HepG2 cell line has been commonly used in cytotoxicity and antiproliferative activity assays for their liver-specific suppression responses towards phytochemicals. As shown in Fig. 3A, moracin N and resveratrol showed no significant effects when comparing to control, suggesting that moracin N and resveratrol had no toxicities when the concentration was less than 100 μM. It also indicated that the reduced fluorescence in CAA assay was not from cytotoxicity.

The antiproliferative activity assay was also conducted against HepG2 cells. As shown in Fig. 3B, both moracin N and resveratrol

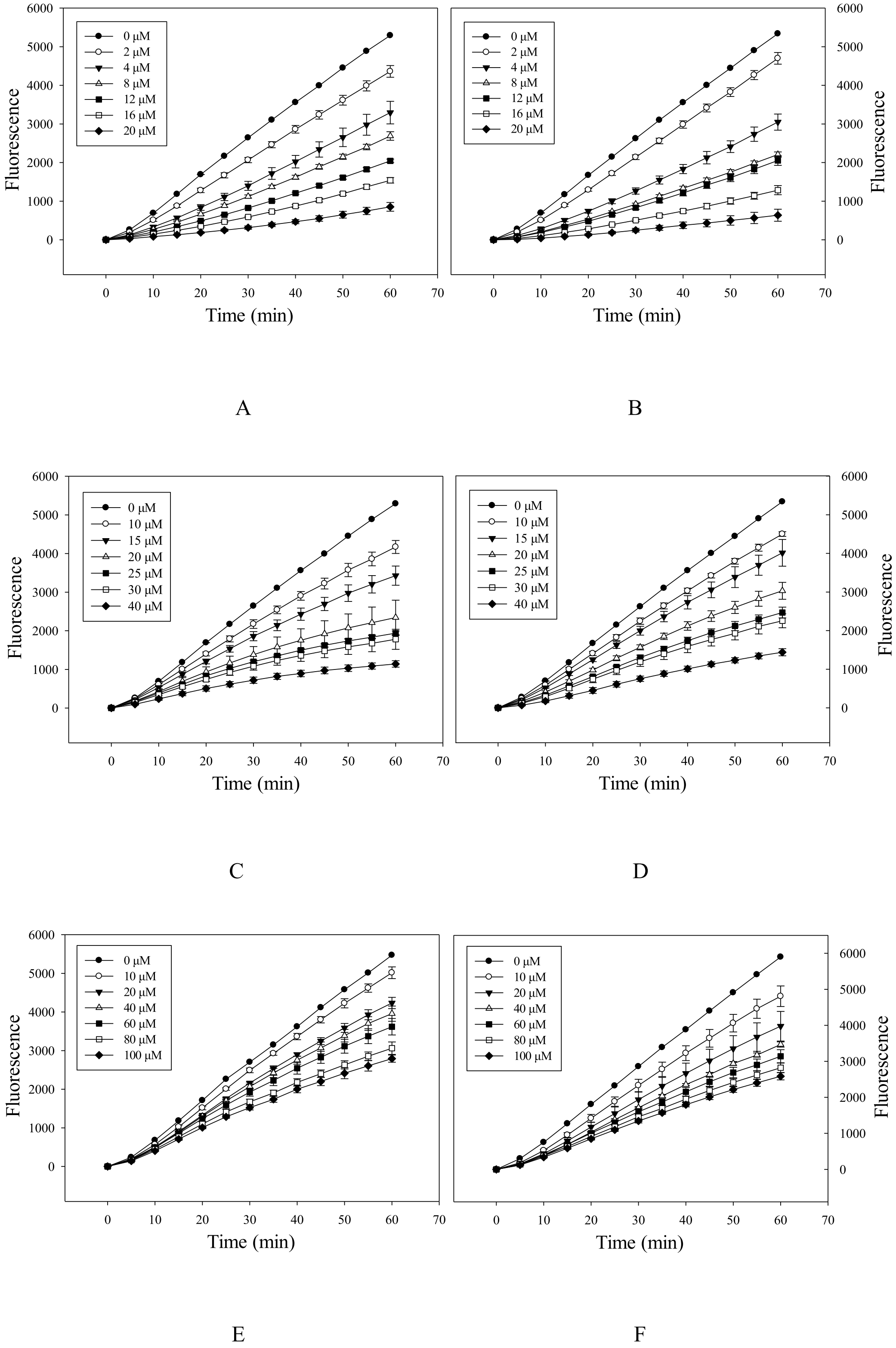


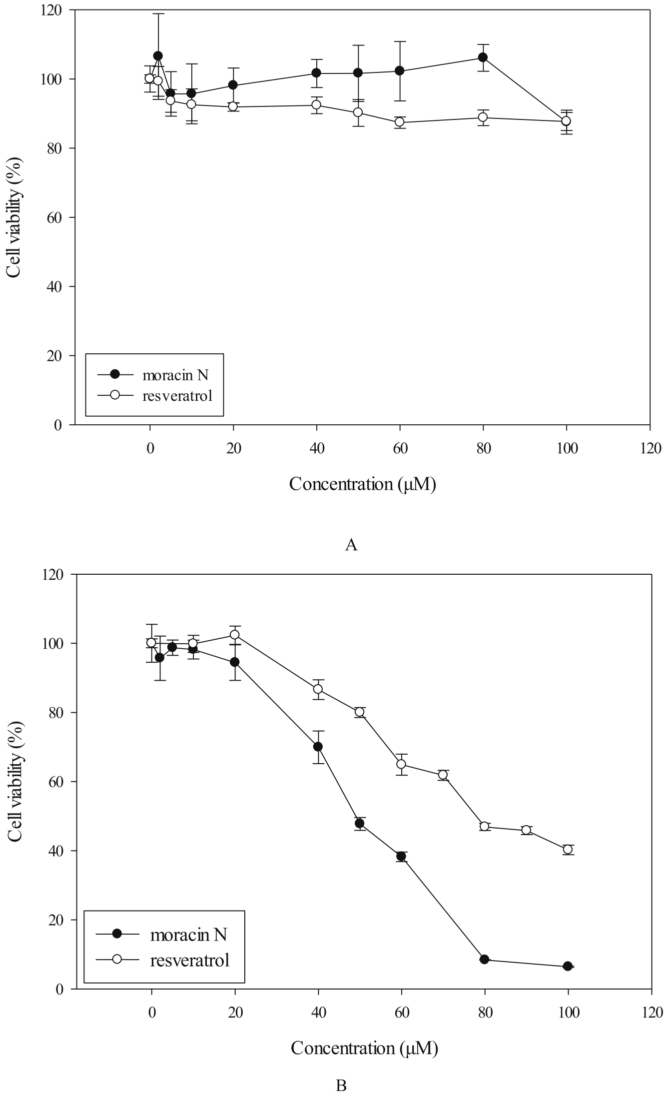
Fig. 2. Cellular antioxidant activities of quercetin (A, B), moracin N (C, D), and resveratrol (E, F). A, C, E were from PBS wash protocol. B, D, F were from the protocol without PBS wash.

showed dose-dependent toxicities against HepG2 cells after 72 h incubation, which suggested antiproliferative activity of them. In addition, moracin N showed a better activity than resveratrol. The IC_{50} value of resveratrol was calculated to be 80 µM, much higher than that of moracin N, which was 50 µM.

Resveratrol has been recognized as a phytoestrogen. It shows anti-inflammatory activity, neuroprotective effect and inhibitory effect against breast cancer cell proliferation (Yang et al., 2019). Positive effects against colon cancer, colorectal cancer and gastrointestinal cancer have been documented for resveratrol (Varoni et al., 2016). The

Table 2
The EC_{50} values and cellular antioxidant activities of moracin N, resveratrol, and quercetin. The values in a column having no letters in common are significantly different ($p < 0.05$).

Compounds	EC_{50} value (μM)		Cellular antioxidant activity (μmol of QE/100 μmol)	
	No PBS wash	PBS wash	No PBS wash	PBS wash
Moracin N	24.92 ± 2.58b	21.82 ± 1.64b	22.51 ± 2.23b	27.81 ± 2.06
Resveratrol	84.16 ± 9.89a	> 100a	6.68 ± 0.83a	–



A

B

Fig. 3. Effects of moracin N and resveratrol on the viabilities of HepG2 cells after 24- and 72-h incubation. A, moracin N and resveratrol for 24 h; B, moracin N and resveratrol for 72 h. Bars with no letters in common were significantly different ($p < 0.05$).

resveratrol derivative has been found to act as anti-breast cancer agent in an ERβ-dependent fashion by inducing ERβ expression and inhibiting ERα expression (Ronghe et al., 2016). Moracin N, as reported, could possibly induce G1 phase arrest and detachment-induced cell death or apoptosis by induction of Bax (Bcl-2-associated X protein) and suppression of estradiol induction of Bcl-2 (B-cell lymphoma 2, a regulation protein family involved in apoptosis) (Katsanou et al., 2007). The prenyl substitution can improve the bioaccumulation and uptake in cell. This should be responsible for the enhanced anti-proliferative effect against HepG2 cells in this work.

Table 3
Molecular descriptors for moracin and trans-resveratrol. ΔHOF-1, ΔHOF-2 and ΔHOF-3 represents dissociation enthalpy of 6-OH, 3′-OH and 5′-OH, respectively.

Molecules	$\Delta E_{LUMO-HOMO}$(eV)	ΔHOF-1 (kcal/mol)	ΔHOF-2 (kcal/mol)	ΔHOF-3 (kcal/mol)	log*P*
Resveratrol	7.99	- 51.47	- 23.06	- 23.04	- 0.34
Moracin N	7.73	- 69.25	- 40.24	- 40.05	- 1.20

3.4. Quantitative structure and activity relationship

The $\Delta E_{LUMO-HOMO}$, ΔHOF, and log*P* of moracin and resveratrol are listed in Table 3. $\Delta E_{LUMO-HOMO}$ represents the energy of a molecule required from basic state to excited state. A lower $\Delta E_{LUMO-HOMO}$ suggests an easier access to chemical reactions, and a better hydrogen/electron donation ability. The results in Table 3 showed that $\Delta E_{LUMO-HOMO}$ of moracin N was lower than resveratrol, and this result was consistent with the results in DPPH scavenging assay and CAA assay.

ΔHOF is the O–H bond dissociation enthalpy, which can be calculated by the enthalpy difference between a molecule and its corresponding radical. A low ΔHOF indicates that the chemical is easy to form a radical, or is stable in the radical form. Hydroxyls having the same topological relationship with the conjugated structure were grouped as ΔHOF-1, ΔHOF-2 and ΔHOF-3 in Table 3. The results showed that all the hydroxyls of moracin N have lower ΔHOF values than the corresponding ones of resveratrol, which were consistent with previous results.

log*P* represents the partitioning coefficient of a compound in *n*-octanol/water system. A higher log*P* defines a higher ratio in *n*-octanol, which indicates a higher hydrophobicity of the compound. Log*P* of moracin N was −1.20, while that of resveratrol was −0.34, suggesting that moracin N had a higher hydrophilicity than resveratrol. The structure difference between moracin N and resveratrol was the furan ring, which offered moracin N a more planar structure, which was helpful for electron dislocation and phenoxy radical stabilization.

4. Conclusions

Moracin N was identified as a bioactive chemical in mulberry leaf. This compound had a similar structure to resveratrol. Extracellular and intracellular antioxidant assays suggested that moracin N was good antioxidant, and acted better than resveratrol. In addition, moracin N showed good safety. The prenyl group contributed to membrane attachment and uptake. It is worthy to analyse *in vivo* antioxidant activity and mechanism of moracin N in the future work.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors appreciate the financial support from National Natural Science Foundation of China (31871851 and 31671906), National Key Research and Development Program of China (2017YFD0401301), and Science and Technology Planning Project of Guangdong Province (2018A050506062) and Ten-Thousand Talents Program of China.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.110730.

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