2017

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Publication Details

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Abstract

The first phytochemical investigation of the twig extracts of *Maclura fruticosa* led to the isolation and identification of a new xanthone, maclurafruticosone (1), together with 14 known compounds (2-15). All compounds were elucidated using spectroscopic methods as well as through comparisons made with data reported in the literature. Some isolated compounds were evaluated for their antioxidant, α-glucosidase inhibitory and cytotoxic activities. Compound 4, 6 and 7 showed significant antioxidant activity against DPPH radicals with IC_{50} values ranging from 7.45-16.12 μM. Compound 4 also exhibited potent activity against $ABTS^{\bullet+}$ scavenging activity with an IC_{50} value of 0.55 ± 0.01 μM which was better than positive control (ascorbic acid, IC_{50} 2.35 ± 0.17 μM). Compound 12 showed significant α-glucosidase inhibitory activity with an IC_{50} value of 0.02 ± 0.37 mM. Compounds 5, 6 and 12 showed weak cytotoxic activities against a colon cancer cell line with IC_{50} values ranging from 22.35-47.62 μM.

Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

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Antioxidant, Cytotoxic and α-Glucosidase Inhibitory Activities of Compounds isolated from the Twig Extracts of *Maclura fruticosa*

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Received: May XX, 2017; Accepted: XX, 2017

The first phytochemical investigation of the twig extracts of *Maclura fruticosa* led to the isolation and identification of a new xanthone, maclurafruticosone (**1**), together with 14 known compounds (**2-15**). All compounds were elucidated using spectroscopic methods as well as through comparisons made with data reported in the literature. Some isolated compounds were evaluated for their antioxidant, α-glucosidase inhibitory and cytotoxic activities. Compound **4**, **6**, and **7** showed significant antioxidant activity against DPPH radicals with IC_{50} values ranging from 7.45–16.12 μM. Compound **4** also exhibited potent activity against $ABTS^{\bullet+}$ scavenging activity with an IC_{50} value of 0.55 ± 0.01 μM which was better than positive control (ascorbic acid, IC_{50} 2.35 ± 0.17 μM). Compound **12** showed significant α-glucosidase inhibitory activity with an IC_{50} value of 0.02 ± 0.37 mM. Compounds **5**, **6** and **12** showed weak cytotoxic activities against a colon cancer cell line with IC_{50} values ranging from 22.35–47.62 μM.

Keywords: *Maclura fruticosa*, Moraceae, xanthone, isoflavone, antioxidant activity, α-glucosidase inhibitory, cytotoxicity.

The *Maclura* genus belongs to the Moraceae family which is widely distributed in Asia, America, Africa, Australia, and Europe. Many species of this genus have been used as traditional medicines as well as food, pesticides, and dyes [1-4]. For examples, the resin of *M. tinctoria* is used to treat toothache and also used as colorant and its fruit is edible [3]. The decoction of the root of *M. pomifera* had been used for the treatment of sore eyes [4]; the bark had been used for uterine haemorrhage treatment [4] and the fruit is used as an insect repellant [1]. *M. aurantiaca* had been recorded to use for its use in the treatment of cardiovascular ailments [4]. Many types of compounds have been isolated from this genus including xanthones, triterpenes, stilbenes and flavonoids [2-11]. The latter compounds were founds as major phytochemicals in this genus and many of these compounds had broad spectrum biological activities such as anti-inflammatory [4] and anti-HIV activities [7], cholinesterase [5] and PDE5 inhibitory activities [8], and their antimicrobial [9], cytotoxic [10], and antioxidant [2] activities. In the course of our ongoing search for bioactive compounds from natural resources from the northern part of Thailand [12-16], we report here the first phytochemical investigation of the twig extracts of *M. fruticosa* (Roxb.) Corner (Figure 1) collected from Doi Tung, Chiang Rai Province, Thailand resulting in the isolation and identification of a new xanthone (**1**) together with 14 known compounds (**2-15**) (Figure 2). In addition, anti-oxidant, α-glucosidase inhibitory and cytotoxic activities of some of the isolated compounds were evaluated. To the best of our knowledge, this is the first report of the phytochemical investigation of this plant.

Figure 1: *Maclura fruticosa* (these photos were taken by Sarot Cheenpracha).

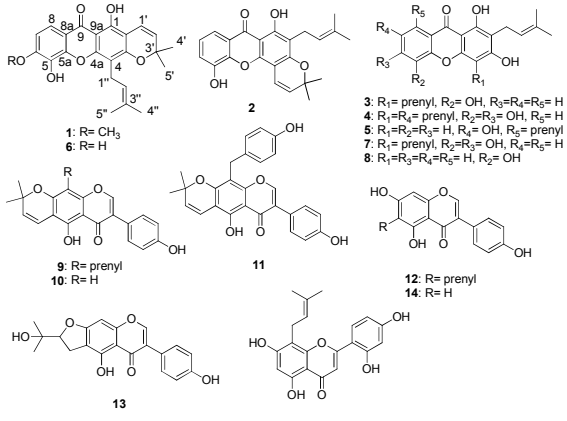

Figure 1: Chemical structures of isolated compounds from *M. fruticosa*.

The crude extraction of *M. fruticosa* (twigs) were separated by repeated silica gel column chromatography and Sephadex LH-20

column chromatography to obtain a new xanthone (**1**) together with 14 known compounds (**2-15**) were identified as ananixanthone (**2**) [17], 8-desoxygatanin (**3**) [18], parvifolixanthone A (**4**) [19], 1,3,7-trihydroxy-2,8-di-(3-methylbut-2-enyl)xanthone (**5**) [20], xanthone V_1 (**6**) [21], xanthone V_{1a} (**7**) [21], 1,3,5-trihydroxy-2-prenylxanthone (**8**) [22], scandenone (**9**) [23], alpinumisflavone (**10**) [24], cudraisoflavone G (**11**) [25], wighteone (**12**) [26], erypoegin C (**13**) [27], genistein (**14**) [28], and artonin Y (**15**) [29], from their NMR spectroscopic data as well as comparisons made with literature data.

Table 1: NMR Spectroscopic Data of Maclurafruticosone (**1**) in $CDCl_3$.

Position	δ_C,	δ_H [*J* in Hz]	HMBC
1	156.1		
2	104.6		
3	158.2		
4	107.7		
4a	154.3		
5	133.5		
5a	144.6		
6	151.1		
7	107.5	6.96 d (8.9)	C-5, C-6, C-8a
8	117.1	7.79 d (8.9)	C-5a, C-6, C-9
8a	115.3		
9	181.0		
9a	103.1		
1′	115.9	6.75 d (10.0)	C-3, C-3″
2′	127.3	5.60 d (10.0)	C-2, C-3″
3′	78.2		
4′	28.5	1.48 s	C-2″, C-3″, C-5″
5′	28.5	1.48 s	C-2″, C-3″, C-4″
1″	21.7	3.53 d (7.3)	C-3, C-4, C-4a, C-2′, C-3′
2″	122.5	5.30 m	C-5″
3″	131.7		
4″	25.9	1.69 d (1.0)	C-2′, C-3′, C-5′
5″	18.0	1.87 d (0.8)	C-2′, C-3′, C-4′
OH-1	-	13.20 s	C-1, C-2, C-9a
OCH_3-6	56.7	4.04 s	C-6

Maclurafruticosone (**1**), a yellow solid, had a 1,3,5,6-tetraoxygenated xanthone core structure which showed a $[M–H]^-$ ion at *m/z* 407.1480 (calcd, 407.1495) in HRESIMS, indicating a molecular formula of $C_{24}H_{24}O_6$. The UV spectrum showed the maxima absorptions at λ_{max} 286 and 337 nm while the IR spectrum showed absorption bands at 3198 cm^{-1} for hydroxy group and at 1652 cm^{-1} for a conjugated carbonyl group. The latter functional group was also supported by the ^{13}C-NMR resonance at δ_C 181.0 (C-9). This carbonyl group formed an H-bond with the hydroxy group at C-1 as evidenced by the resonance for an H-bonded hydroxy proton at δ_H 13.20 (1H, s, 1-OH) in the ^{1}H NMR spectrum (Table 1). The HMBC correlations of this H-bonded hydroxy proton (δ_H 13.20) with δ_C 156.1 (C-1), 104.6 (C-2) and 103.1 (C-9a) supported the structure of **1**. The *ortho*-coupled aromatic protons at δ_H 6.96 (1H, d, *J* = 8.9 Hz) and δ_H 7.79 (1H, d, *J* = 8.9 Hz) were assigned to protons H-7 and H-8, respectively, by the ^2J and ^3J HMBC correlations of H-7 (δ_H 6.96) to δ_C 133.5 (C-5), 151.1 (C-6), and 115.3 (C-8a); and H-8 (δ_H 7.79) to δ_C 144.6 (C-5a), 151.1 (C-6), and 181.0 (C-9). The ^{1}H and ^{13}C NMR spectra of **1** also displayed resonances for a 2,2-dimethylpyrano unit at δ_H 6.75 (1H, d, *J* = 10.0 Hz, H-1′)/δ_C 115.9 (C-1′), δ_H 5.60 (1H, d, *J* = 10.0 Hz, H-2′)/δ_C 127.3 (C-2′), δ_H 1.48 (6H, s, H-4′ and H-5′)/δ_C 28.5 (C-4′ and C-5′), and δ_C 78.2 (C-3′) and an prenyl unit at δ_H 5.30 (1H, m, H-2″)/δ_C 122.5 (C-2″), δ_H 3.53 (2H, d, *J* = 7.3 Hz, H-1″)/δ_C 21.7 (C-1″), δ_H 1.69 (3H, s, H-4″)/δ_C 25.9 (C-4″), 1.87 (3H, s, H-5″)/δ_C 18.0 (C-5″), and δ_C 131.7 (C-3″). The latter moiety was located at C-4 supported by the ^2J and ^3J HMBC correlations of H-1″ (δ_H 3.53) with C-3 (δ_C 158.2), C-4 (δ_C 107.7), and C-4a (δ_C 154.3); and H-1′ (δ_H 6.75) with C-3 (δ_C 158.2). The 2,2-dimethylpyrano moiety was placed at C-2/C-3 supported with the following HMBC correlations: H-1′ (δ_H 6.75) with C-3 (δ_C 158.2); H-2′ (δ_H 5.60) with C-2 (δ_C 104.6); and OH-1 (δ_H 13.20) with C-2 (δ_C 104.6). The remaining methoxy group with a resonance at δ_H 4.04 (3H, s,) was located at C-6 from the ^3J HMBC correlations of its methoxy protons (δ_H 4.04) and H-8 (δ_H 7.79) with C-6 (δ_C 151.1) as well as the cross peak between H-7 (δ_H 6.96) and the methoxy protons (δ_H 4.04) in the NOESY experiment. Thus, maclurafruticosone was assigned the structure **1**.

Most of isolated compounds were evaluated for their antioxidant activities against DPPH radical and $ABTS^{\bullet+}$ (Table 2). The EtOAc extract of the twigs of *M. fruticosa* showed significant antioxidant activities against DPPH radical and $ABTS^{\bullet+}$ with high % inhibition of 95.14 % (at 500 µg/mL) and 99.36 % (at 200 µg/mL), respectively. Compounds **4**, **6**, and **7** showed significant anti-oxidant activity against the DPPH radical with IC_{50} values ranging from 7.45–16.12 µM, with compound **6** having the highest activity. Only compound **4** exhibited potent scavenging activity against $ABTS^{\bullet+}$ with an IC_{50} value of 0.55 ± 0.01 µM, which was better than the positive control ascorbic acid (IC_{50} 2.35 ± 0.17 µM). It is interesting to note that all tested xanthones were found to have antioxidant activities against both of DPPH radical and $ABTS^{\bullet+}$ better than those of isoflavones.

The EtOAc extract of the twig of *M. fruticosa* showed moderate α-glucosidase inhibitory activity with 73.97 % inhibition at 200 µg/mL. Compounds **3**, **5**, **6**, **9**-**12**, **14** and **15** were evaluated for their α-glucosidase inhibitory activities. Only compound **12** exhibited significant α-glucosidase inhibitory activity with an IC_{50} value of 0.02 ± 0.37 mM which was better than acarbose (IC_{50} 1.55 ± 0.39 mM). Compounds **5**, **6**, **12** and **14** were evaluated for their cytotoxicity against a colon cancer cell line and all of them, except compound **14**, were found to have weak cytotoxicity, with IC_{50} values ranging from 22.35–47.62 µM (positive control, doxorubicin, IC_{50} 9.74 µM). Compound **14** was inactive.

Table 2: Antioxidant Activities of Compounds **3**–**15**.

Compounds	DPPH assay		ABTS assay	
	%Inhibition (500 µg/mL)	IC_{50} (µM)	%Inhibition (200 µg/mL)	IC_{50} (µM)
Crude extract	95.14 ± 0.52	–	99.36 ± 1.01	–
3	84.19 ± 1.26	181.74 ± 4.78	81.72 ± 1.48	53.12 ± 3.00
4	97.47 ± 0.32	16.12 ± 0.70	96.37 ± 0.31	0.55 ± 0.01
5	80.24 ± 2.37	208.21 ± 5.07	28.66 ± 0.95	–
6	95.89 ± 0.63	7.45 ± 0.32	30.06 ± 0.81	–
7	88.46 ± 0.79	10.43 ± 0.44	93.69 ± 1.22	not tested*
8	32.67 ± 1.26	–	64.46 ± 1.80	–
9	24.13 ± 0.32	–	54.08 ± 5.00	–
10	25.40 ± 0.95	–	84.40 ± 0.50	116.92 ± 2.73
11	44.52 ± 3.00	–	94.71 ± 0.50	64.05 ± 5.62
12	30.61 ± 0.16	–	89.30 ± 3.53	79.61 ± 4.79
13	38.04 ± 1.26	–	67.07 ± 1.89	–
14	50.68 ± 3.48	–	95.92 ± 0.95	168.18 ± 0.30
15	86.72 ± 1.26	423.06 ± 8.70	97.32 ± 0.87	not tested*
Ascorbic acid	–	2.01 ± 0.22	–	2.35 ± 0.17

*Inadequate sample available. All determinations were carried out in triplicate, and results were expressed as mean ± standard deviation (SD).

In summary, we have isolated eight xanthones and seven flavonoids from the twig extract of *M. fruticosa*. These results are in agreement of previous research on the *Maclura* species [2-10].The difference between *M. fruticosa* and the other species of *Maclura* studied is that both xanthones and flavonoids were major compounds of *M. fruticosa* while in the other species of *Maclura* studied only flavonoids or xanthones were isolated as major compounds. Therefore, the flavonoids and xanthones from this plant might be a useful as a chemotaxonomic marker to differentiate *M. fruticosa* from other species of *Maclura*. In the present work, compounds **4** and **12** exhibited potent antioxidant activity against $ABTS^{\bullet+}$ and α-glucosidase inhibitory activity, respectively, showing better activity than those of the standard controls. This preliminary result indicated that compound **12** might be a good candidate for further study and development as an anti-diabetic agent and compound **4** might be a potential lead compound for antioxidant agent development.

Experimental

General Experimental Procedures: Melting point was determined using a Gallenkamp melting point apparatus. UV-vis spectra were recorded with BMG LABTECH/SPECTROstar Nano spectrometer or Agilent 8453 UV-visible spectrophotometer. The IR spectra were recorded using a PerkinElmer FTS FT-IR spectrometer or SHIMADSU spectrophotometer. The NMR spectra were recorded using a 400 MHz Bruker spectrometer or a 400 MHz Bruker AM400 spectrometer. HRESIMS mass spectra carried out on a Bruker micro TOF mass spectrometer. Unless otherwise indicated, all quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 H (5-40 μm, SiliCycle® Inc.) and silica gel 100 (63-200 μm, SiliCycle® Inc.), respectively. Sephadex LH-20, when indicated, was also used for CC. Precoated TLC plates of silica gel 60 F_{254} were used for analytical purposes.

Plant Material: The twigs of *M. fruticosa* were collected from Doi Tung, Chiang Rai Province, Thailand in August 2015. The plant was identified by Mr. Martin Van de Bult, and the voucher specimen (MFU-NPR0111) was deposited at the Natural Products Research Laboratory of Mae Fah Luang University.

Extraction and Isolation: Air-dried twigs of *M. fruticosa* (5.4 kg) were macerated in EtOAc (3 × 10 L) at room temperature. The extract was filtered and concentrated under reduced pressure to give the crude EtOAc extract (108.3 g). This extract was subjected to QCC over silica gel, eluting with a gradient system of acetone/hexanes (100% hexanes to 100% acetone) to give 16 fractions (1A-1P). Fraction 1J (3.4 g) was further separated by QCC (100% hexanes to 100% acetone) to give six subfractions (2JA-2JF). Compound **1** (1.2 mg) was obtained from subfraction 2JB (126.0 mg) by CC over Sephadex LH-20 (100% MeOH) and then by CC (4:1 CH_2Cl_2/hexanes). Subfraction 3JB (20.1 mg) was purified by CC (1:49 acetone/hexanes) to yield compound **2** (2.2 mg). Compound **9** (25.2 mg) was obtained from subfraction 2JC (820.4 mg) by CC over Sephadex LH-20 (100% MeOH) and then by CC (1:4 EtOAc/hexanes). Fraction 1K (4.6 g) was further separated by QCC (100% hexanes to 100% acetone) to give eight subfractions (2KA-2KH). Compounds **3** (11.1 mg) and **4** (11.3) were obtained from subfraction 2KC (319.1 mg) by repeated CC (1:9 CH_2Cl_2/hexanes). Purification of subfraction 4KC (90.8 mg) by CC over Sephadex LH-20 (100% MeOH) and then by CC (1:49 EtOAc/CH_2Cl_2) afforded compound **10** (16.3 mg). Subfraction 4KD (31.9 mg) was purified by CC over Sephadex LH-20 (100% MeOH) and then by CC (1:49 acetone/hexanes) to yield compound **11** (6.9 mg). Compound **5** (37.3 mg) was obtained from subfraction 2KE (1.2 mg) by CC (100% CH_2Cl_2), followed by repeated CC (1:49 EtOAc/CH_2Cl_2). Separation of subfraction 3KC (944.3 mg) by repeated CC (1:49 acetone/CH_2Cl_2) afforded compound **6** (51.8 mg). Fraction 1L (4.8 g) was further separated by CC over Sephadex LH-20 (100% MeOH) to give six subfractions (2LA-2LF). Compound **7** (4.3 mg) was obtained from subfraction 2LC (360.9 mg) by CC (1:49 MeOH/CH_2Cl_2) and then by repeated CC (1:49 acetone/hexanes). Separation of subfraction 2LD (728.1 mg) by CC (1:49 MeOH/CH_2Cl_2) and further CC (1:24 EtOAc/CH_2Cl_2) yielded compound **8** (4.7 mg). Fraction 1M (6.6 g) was further separated by CC (100% hexanes to 100% acetone) to give nine subfractions (2MA-2MI). Compound **12** (204.5 mg) was obtained from subfraction 2MC (1.2 g) by CC (1:4 EtOAc/hexanes) and further CC (1:49 acetone/CH_2Cl_2). Subfraction 2MD (1.2 g) was subjected to CC over Sephadex LH-20 (100% MeOH) and then by CC (3:7 EtOAc/CH_2Cl_2) to afford compound **13** (8.7 mg). Separation of subfraction 3ME (189.2 mg) by CC over Sephadex LH-20 (100% MeOH) and then by CC (3:7 EtOAc/hexanes) gave compound **14** (21.1 mg). Compound **15** (12.4 mg) was obtained from subfraction 2MF (332.7 mg) by CC over Sephadex LH-20 (100% MeOH) and further CC (1:49 MeOH/CH_2Cl_2). The isolation flowchart of compounds **1**-**15** is shown in Figure S8.

Maclurafruticosone (**1**)
Yellow solid.
MP: 206°C (decomposed).
IR (neat) V_{max}: 3198, 2920, 1652, 1576, 1436, 1286, 798 cm^{-1}.
UV (MeOH) λ_{max} (log ε): 286.0 (4.55), 337.0 (4.39) nm.
^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz): Table 1.
HRESIMS *m/z* 407.1480 $[M–H]^-$ (calcd for $C_{24}H_{23}O_6$, 407.1495).

DPPH Radical Scavenging Assay: The DPPH scavenging activity assay was modified from a previous paper [14]. Briefly, 6×10^{-5} M DPPH was prepared in absolute EtOH, and then 100 μL of this solution was mixed with 100 μL of sample solution in EtOH in a 96-well microplate. After 30 min of reaction, in the absence of light at room temperature, the absorbance was measured using a microplate reader (SPECTROstar Nano) at 517 nm. The DPPH radical scavenging capacity was calculated using the following equation. % inhibition = $[(A_B – A_S)/ A_B]$ x 100, where A_B and A_S are the absorbance of the blank sample and sample, respectively. All experiments were performed in triplicate, with ascorbic acid used as the positive control (r^2 = 0.9829, IC_{50} 2.01 ± 0.22 μM.). The half maximal inhibitory concentration (IC_{50}) of DPPH scavenging activity was calculated by plotting inhibition percentages against concentrations of the sample. The inhibition values are reported as means ± SD.

$ABTS^{•+}$ Scavenging Assay: The determination of $ABTS^{•+}$ scavenging activity was carried out using a modified literature procedure [30]. $ABTS^{•+}$ were produced by reacting a 7 mM stock solution of ABTS in DI water with 2.45 mM potassium persulfate ($K_2S_2O_8$) and allowing the mixture to stand in the dark at room temperature for 16 h before use. The $ABTS^{•+}$ solution was diluted with water to an absorbance of 0.7 ± 0.02 at 734 nm. Different concentrations of test samples in EtOH (100 μL) and $ABTS^{•+}$ solution (150 μL) were added to each well of the 96-well microplate. The absorbance at 750 nm was determined after 5 min of mixing. The percentage of ABTS free radical scavenging activity was calculated using the same formula as for the DPPH assay. The measurements were performed in triplicate and the positive control was ascorbic acid (r^2 = 0.9777, IC_{50} 2.35 ± 0.17 μM).

α-Glucosidase Inhibitory Assay: Briefly, the reaction mixture contained 50 μL of sample in DMSO was pre-incubated with α-glucosidase enzyme (0.35 U/mL, 100 μL) at 37°C for 10 min. The reaction was started when 100 μL of *p*-nitrophenyl glucopyranoside (*p*-NPG) substrate was added in phosphate buffer. The reaction was incubated at 37°C for 20 min and stopped by the addition of 1 mL 1 M of Na_2CO_3 solution. The α-glucosidase activity was determined by measuring the amount of *p*-nitrophenol released from *p*-NPG at 405 nm using a microplate reader (SPECTROstar Nano) [16]. Blanks were prepared by adding solvent instead of samples. Acarbose was used as a positive control (r^2 = 0.9897, IC_{50} 1.55 ± 0.39 mM). All assays were carried out in triplicate.

Cytotoxicity Assay: Colon cancer cells (HCT116) (1×10^4 cells/well) were cultured in 96 well plate and allowed to adhere for 24 h at 37 ºC. The cells were treated with compounds (10 μM or μg/mL) in DMEM medium for 24 h. Then the medium were removed and fresh of DMEM containing 0.5 mg/mL of MTT solution were added to each well for 2 h. After that, the medium were discarded by aspirator. The violet formazan crystals in the viable cells were dissolved in 100 μL of DMSO. The absorbance of

each well was then read at a wavelength of 570 nm using a microplate reader. Doxorubicin was used as a positive control with an IC_{50} value of 9.74 µM.

% Cell viability is expressed as: $\frac{\text{Absorbance of treated well}}{\text{Absorbance of control well}} \times 100$

% Cytotoxicity = 100 – % cell viability

Supplementary data: ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC, HMBC and HRESIMS of **1**.

Acknowledgments – This work was supported by the Thailand Research Fund through the Direct Basic Research Grant (DBG5980001) and Mae Fah Luang University through the graduate student research grant. Mae Fah Luang University and the University of Wollongong were also acknowledged for laboratory facilities. We would like to thank Mr. Matin Van de Bult, Doi Tung Development Project, Chiang Rai, Thailand for plant collection and identification. S. Laphookhieo thanks the Australian Government via the Endeavour Award 2016 for a research fellowship.

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