



## Isolation and Identification of Components in the Dihydroquercetin-Rich Extract from Larch Wood (*Larix olgensis* Henry)

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### Authors' contributions

This work was carried out in collaboration between all authors. Author SYH carried out the isolation and identification of components from the extract and wrote the first draft of the manuscript. Authors CBD, YWH and BC prepared and provided the extracts. Author YNZ group determined DHQ content in the extracts by HPLC method. Author LX as the correspondence author, designed this study, managed data analysis and corrected the manuscript. All authors read and approved the final manuscript.

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### ABSTRACT

**Background:** The finding that antioxidant dihydroquercetin (DHQ) present in high content in the wood of Dahurian larch (*Larix gmelinii* Rupr.) which distributes mainly in Khingan Mountains led to the development and manufacture of a DHQ-rich extract, Lavitol (a trade name). Whether the composition of DHQ-rich extract from *L. olgensis* Henry, a great resource of Larch species distributed in Changbai Mountain in China, is same or similar with trade DHQ product becomes an interesting question.

**Aims:** To isolate and identify the components in the DHQ-rich extract from larch wood (*L. olgensis*).

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**Methodology:** Compounds were isolated from a DHQ-rich extract (91% purity) of *L. olgensis* through polyamide and Sephadex LH-20 column chromatography, and their structures were elucidated based on <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, MS and CD data analysis. Thin layer chromatography (TLC) was applied to quickly identify the components in the extract.

**Results:** Five compounds were isolated from the extract, the main one was (2*R*,3*R*)-dihydroquercetin (**1**), four minor components were identified as (2*R*,3*R*)-aromadendrin (**2**), quercetin (**3**), 3,5,7-trihydroxychromone (**4**) and (2*R*,3*R*)-3'-O-methyl-taxifolin (**5**). Polyamide and silica gel TLC were developed to identify these components in the extract, and the results indicated that three batches of DHQ-rich extracts contained the same components.

**Conclusion:** Except for the presence of trace impurities **4** and **5**, DHQ-rich extracts from *L. olgensis* contained (2*R*,3*R*)-DHQ (**1**) and two minor impurities **2** and **3**, which were similar with the composition of trade DHQ-rich extract from Dahurian larch (*L. gmelinii*). Further quantification of these impurities in DHQ-rich extract from *L. olgensis* by HPLC analysis need to be done in the future.

**Keywords:** Dihydroquercetin-rich extract; larch wood (*Larix olgensis* Henry); components; isolation; identification.

## 1. INTRODUCTION

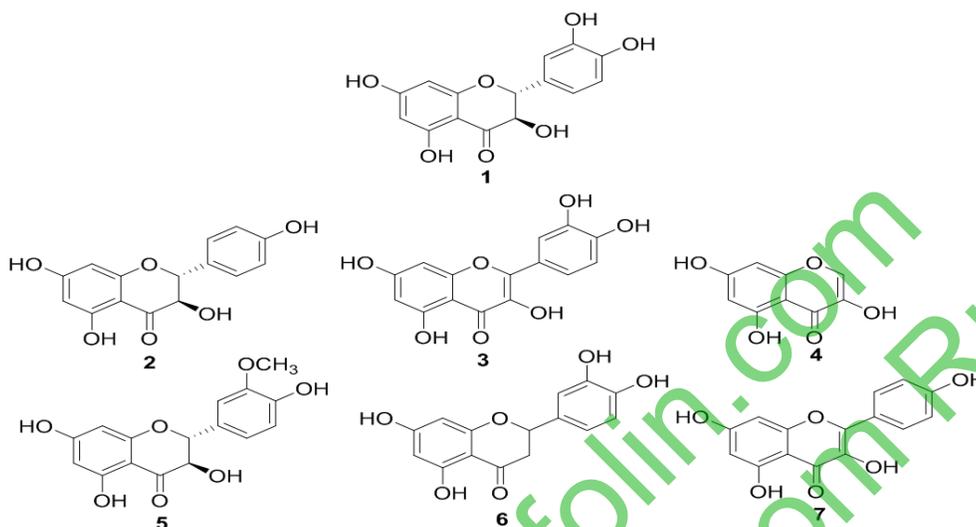
Dihydroquercetin ((2*R*,3*R*)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-2,3-dihydrochromen-4-one, DHQ, **1** in Fig.1), also known as taxifolin, is a naturally occurring flavonoid of many plants consumed by humans. It is found in abundance in onion, fruits (apple, berry, grapes), nuts (peanut, pine seeds), grains, and many other foods, and the estimated individual DHQ exposure based on daily consumption of commonly consumed foods in the United States is about 426.24 mg/d [1,2]. DHQ is classified as a potent antioxidant owing to its free radical scavenging activity and several assessments provide the supports for the safety of DHQ consumption as a food additive and food supplement [1,2]. Moreover, experimental evidence indicated that DHQ displayed other health beneficial effects including hepatoprotective [3], cardioprotective [4-5], neuroprotective [6-7], and radioprotective [1] activities.

The finding that DHQ present in high content of 3% in the wood of Dahurian larch (*L. gmelinii*) led to the development and manufacture of a DHQ-rich water-ethanol extract, Lavitol (a trade name), which contains a minimum of 90% DHQ with (2*R*,3*R*)-configuration [1,2]. This extract is used as antioxidant in the food additive and dietary supplement in Russia, Switzerland, United States and Canada, and over a period of more than 10 years secured regulatory approval for use as a food ingredient in Russia [1]. For example, DHQ-rich extract is added in beverages, dairy products and oil products. The daily recommended amount of DHQ-rich extract as a dietary

supplement is 100 mg for the general population with an age of 14 years and above [2].

Since DHQ is rich in larch tree, *Larix* species becomes the important source for the preparation of DHQ-rich extract [8-9]. *L. gmelinii* is distributed widely in Khingan Mountains in China, however, another great resource of Larch species widely distributed in Changbai Mountain in Northeast China is *L. olgensis* (also known as Olga Bay larch). Moreover, it is widely distributed in North Korea and eastern Russia, and is a common temperate, coniferous tree species in East Asia. It is one of the most industrially important coniferous species in the plantation forests in northeastern China because its wood products have anti-corrosive properties. China holds the largest total plantation area of *L. olgensis* for conifers [10]. Using the same extraction method, the outward appearances of straw-colored powder of DHQ-rich extracts from these two larch trees are same, however, whether the composition of DHQ-rich extract from *L. olgensis* is same or similar with the trade extract from *L. gmelinii* becomes an interesting question.

HPLC analysis showed that DHQ content in the trade products derived from *L. gmelinii* is generally around 90 - 93%. The minor components are other flavonoids which are chemically similar to DHQ in their structures, including aromadendrin (ranging from 2.5% - 3.5%, **2** in Fig. 1), quercetin (0.3% - 0.5%, **3** in Fig. 1), naringenin (0.2% - 0.3%), eriodictyol (**6** in Fig. 1) (0.1% - 0.3%), kaempferol (**7** in Fig. 1) (0.01% - 0.1%), pinocembrin (0.05% - 0.12%), and other unidentified flavonoids (1% - 3%) [1,2].



**Fig. 1. Compounds 1-5 isolated from DHQ-rich extract of larch wood (*L. olgensis*) and two other standards 6 and 7 for TLC identification**

Determination of DHQ content in extract from *L. olgensis* was performed by HPLC method [9] in our previous study, and DHQ contents in the present three batches were 91%, 92% and 93% of dry weight, respectively. It is fit for the quality requirement that the extract contains a minimum of 90% DHQ. However, some minor components were not very similar with the trade products from *L. gmelinii*. Further HPLC-MS/MS analysis was performed in our previous study to identify the chemically similar impurities to DHQ, however, the exact structures were difficult to be determined, including the location of hydroxyl or methoxyl groups and the absolute configuration of dihydroflavonols. Therefore, no standards can be available for some unknown impurities in HPLC analysis.

In this paper, isolation and structural elucidation of the components from DHQ-rich extract of *L. olgensis* were performed to resolve above problems. At the same time, to ensure the manufacturing process produce a consistent product within the product specifications, quick TLC identification method was developed for in-process control of the extract.

## 2. MATERIALS AND METHODS

### 2.1 Instruments and Reagents

Optical rotations were measured with a Gyromat-Hp digital automatic polarimeter (Kernchen Co., Germany).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on Bruker Avance DRX-600

spectrometers with TMS as an internal standard. HR-ESI-MS spectra were measured with a 1200RRLC-6520 Accurate-Mass Q-TOF MS spectrometer (Agilent Technologies Co., USA). Polyamide (60 - 100 mesh, Taizhou Luqiao Siqing Biochemical Plastics Factory, China) and Sephadex LH-20 (Pharmacia Fine Chemicals, USA) were used for column chromatography. TLC was carried out on pre-coated silica gel GF<sub>254</sub> plates (Qingdao Marine Chemical Industry, Qingdao, China) or polyamide film (Taizhou Luqiao Siqing Biochemical Plastics Factory, China). Analytical grade solvents were provided by Tianjin Fuyu Fine Chemical Factory. Kaempferol (MUST-13121112, Hengyuan Qitian Chemical Technology Research Institute, Beijing), and eriodictyol (BCBM7471V, Sigma-Aldrich) were used as reference substance.

### 2.2 Plant Material and Extracts

Three batches (No. 1-3) of DHQ-rich extracts were obtained from the wood of *Larix olgensis*, DHQ content in three batches was 91%, 92% and 93% respectively, as determined by HPLC analysis. This plant (wood) was authenticated by Prof. Yi-Nan Zheng in College of Chinese Medicinal Materials, Jilin Agricultural University.

### 2.3 Isolation

DHQ-rich extract (91% purity, 2 g) was subjected on polyamide column chromatography (160 g)

and eluted with gradients of ethyl acetate-methanol-formic acid (10: 0: 0.1–6: 4: 0.1, v/v) to yield 59 fractions (Fr. 1-59). Fr. 3-5 (42.8 mg) was purified by Sephadex LH-20 column chromatography eluted with MeOH to get sub-fractions Fr. L1-20. Fr. L14 and Fr. L15 were purified by recrystallisation to yield **4** (0.8 mg) and **5** (1.2 mg). Fr. 6-8 (35.3 mg) was purified by Sephadex LH-20 column chromatography eluted with MeOH to get sub-fractions Fr. L'1-100, Fr. L'48-52 was purified by recrystallisation to yield **2** (4.1 mg). Fr. 14-16 (37.2 mg) was purified by Sephadex LH-20 column chromatography eluted with MeOH to get sub-fractions Fr. C1-100, Fr. C69-72 was purified by recrystallisation to yield **1** (25.4 mg). Fr. 33-38 (125 mg) was purified by Sephadex LH-20 column chromatography eluted with MeOH to get sub-fractions Fr. LH1-140, Fr. LH121-126 was purified by recrystallization to yield **3** (2.6 mg).

## 2.4 Qualitative Identification by TLC Method

### 2.4.1 Preparation of reference solution

(2*R*,3*R*)-dihydroquercetin (DHQ, **1**), (2*R*,3*R*)-aromadendrin (**2**), quercetin (**3**), 3,5,7-trihydroxychromone (**4**), (2*R*,3*R*)-3'-O-methyl-taxifolin (**5**), eriodictyol (**6**) and kaempferol (**7**) were used as the reference standards for TLC analysis. These standards were weighed precisely and dissolved in methanol to get each solution at 1 mg/mL. Quercetin solution was then diluted to 0.02 mg/mL with methanol.

### 2.4.2 Preparation of extract solution

Three batches of DHQ-rich extracts (No. 1-3) from *L. olgensis* were weighed precisely and dissolved in methanol to yield extract solution at 10 mg/mL.

### 2.4.3 Polyamide TLC

Extract solutions and reference solutions were applied (5  $\mu$ L) on polyamide film, developed by toluene-ethyl acetate-methanol-formic acid (10: 4: 1: 2) or ethyl acetate-formic acid-water (8: 1: 1), and sprayed with FeCl<sub>3</sub> reagent.

### 2.4.4 Silica gel TLC

Extract solutions and reference solutions were applied (5  $\mu$ L) on silica gel GF<sub>254</sub> plate, developed by toluene-ethyl acetate-methanol-formic acid (10: 4: 1: 2), and sprayed with FeCl<sub>3</sub> reagent.

## 3. RESULTS AND DISCUSSION

### 3.1 Structural Elucidation

Compound **1**: Yellow powder;  $[\alpha]_D^{25} +20.0$  (c 0.1, MeOH); <sup>1</sup>H-NMR (600 MHz, CD<sub>3</sub>OD)  $\delta$ : 4.50 (1H, d, J = 11.5 Hz, H-3), 4.91 (1H, overlapped, J = 11.5 Hz, H-2), 5.87 (1H, d, J = 2.1 Hz, H-8), 5.91 (1H, d, J = 2.1 Hz, H-6), 6.79 (1H, d, J = 8.1 Hz, H-5'), 6.84 (1H, dd, J = 2.0, 8.1 Hz, H-6'), 6.95 (1H, d, J = 2.0 Hz, H-2'); <sup>13</sup>C-NMR (150 MHz, CD<sub>3</sub>OD)  $\delta$ : 72.2 (C-3), 83.7 (C-2), 94.8 (C-8), 95.8 (C-6), 100.4 (C-10), 114.4 (C-2'), 114.6 (C-5'), 119.5 (C-6'), 128.4 (C-1'), 144.9 (C-4'), 145.7 (C-3'), 163.1 (C-9), 163.9 (C-7), 167.3 (C-5), 97.0 (C-4). These data were same with those of dihydroquercetin [11]. In addition, according to the coupling constant of H<sub>2,3</sub> (11.5 Hz), as well as negative Cotton effect at 295 nm and positive Cotton effect at 329 nm in CD spectrum [12-14], compound **1** was confirmed to be in 2*R*,3*R*-configuration. Therefore, compound **1** was identified as (2*R*,3*R*)-dihydroquercetin.

Compound **2**: White pinnate crystallization;  $[\alpha]_D^{25} +18.9$  (c 0.1, MeOH); <sup>1</sup>H-NMR (600 MHz, CD<sub>3</sub>OD)  $\delta$ : 4.52 (1H, d, J = 11.6 Hz, H-2), 4.96 (1H, d, J = 11.6 Hz, H-3), 5.84 (1H, br. s, H-8), 5.88 (1H, br. s, H-6), 6.82 (2H, d, J = 8.3 Hz, H-3', 5'), 7.34 (2H, d, J = 8.3 Hz, H-2', 6'); <sup>13</sup>C-NMR (150 MHz, CD<sub>3</sub>OD)  $\delta$ : 72.2 (C-3), 83.5 (C-2), 95.2 (C-8), 96.2 (C-6), 100.1 (C-10), 114.7 (C-3', 5'), 127.9 (C-1'), 128.9 (C-2', 6'), 157.8 (C-4'), 163.1 (C-9), 163.9 (C-7), 168.5 (C-5), 196.7 (C-4). These data were same with those of aromadendrin [15]. In addition, according to the coupling constant of H<sub>2,3</sub> (11.6 Hz), as well as negative Cotton effect at 291 nm and positive Cotton effect at 328 nm in CD spectrum [12-14], compound **2** was confirmed to be in 2*R*,3*R*-configuration. Therefore, compound **2** was identified as (2*R*,3*R*)-aromadendrin.

Compound **3**: Yellow powder; <sup>1</sup>H-NMR (600 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 6.18 (1H, br. s, H-6), 6.40 (1H, br. s, H-8), 6.89 (1H, d, J = 8.4 Hz, H-5'), 7.54 (1H, br. d, J = 8.4 Hz, H-6'), 7.67 (1H, br. s, H-2'), 12.50 (1H, s, 5-OH); <sup>13</sup>C-NMR (150 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 93.7 (C-8), 98.7 (C-6), 103.3 (C-10), 115.4 (C-2'), 116.2 (C-5'), 120.5 (C-6'), 122.5 (C-1'), 136.2 (C-3), 145.6 (C-3'), 147.2 (C-2), 148.1 (C-4'), 156.6 (C-9), 161.3 (C-5), 164.5 (C-7), 176.4 (C-4). These data were same with those of quercetin [16] and compound **3** was therefore identified as quercetin.

Compound **4**: Yellowish needle crystal; HR-ESI-MS  $m/z$ : 193.0144 [M-H]<sup>-</sup> (calcd 193.0137 for C<sub>9</sub>H<sub>5</sub>O<sub>5</sub>). <sup>1</sup>H-NMR (600 MHz, DMSO-d<sub>6</sub>) δ: 6.18 (1H, d, J = 1.3 Hz, H-6), 6.32 (1H, d, J = 1.3 Hz, H-8), 8.08 (1H, s, H-2), 9.20 (1H, s, 3-OH), 10.78 (1H, s, 7-OH), 12.50 (1H, s, 5-OH); <sup>13</sup>C-NMR (150 MHz, DMSO-d<sub>6</sub>) δ: 94.0 (C-8), 98.8 (C-6), 104.8 (C-10), 140.3 (C-3), 141.6 (C-2), 157.8 (C-9), 161.7 (C-5), 164.3 (C-7), 177.1 (C-4). The above data was in accordance with those of 3,5,7-trihydroxychromone [17] and compound **4** was therefore identified as 3,5,7-trihydroxychromone.

Compound **5**: White cluster crystal; [α]<sub>D</sub><sup>25</sup> +10.2 (c 0.1, MeOH); HR-ESI-MS  $m/z$ : 317.0665 [M-H]<sup>-</sup> (calcd 317.0661 for C<sub>16</sub>H<sub>13</sub>O<sub>7</sub>). <sup>1</sup>H-NMR (600 MHz, DMSO-d<sub>6</sub>) δ: 3.78 (3H, s, 3'-OMe), 4.66 (1H, dd, J = 11.4, 6.0 Hz, H-3), 5.04 (1H, d, J = 11.4 Hz, H-2), 5.74 (1H, d, J = 6.0 Hz, 3-OH), 5.86 (1H, d, J = 1.8 Hz, H-6), 5.91 (1H, d, J = 1.8 Hz, H-8), 6.79 (1H, d, J = 7.8 Hz, H-5'), 6.90 (1H, dd, J = 7.8, 1.8 Hz, H-6'), 7.10 (1H, d, J = 1.8 Hz, H-2'), 9.11 (1H, s, 4'-OH), 10.83 (1H, s, 7-OH), 11.92 (1H, s, 5-OH); <sup>13</sup>C-NMR (150 MHz, DMSO-d<sub>6</sub>) δ: 56.2 (3'-OMe), 71.8 (C-3), 83.6 (C-2), 95.5 (C-8), 96.5 (C-6), 100.9 (C-10), 112.7 (C-2'), 115.4 (C-5'), 121.6 (C-6'), 128.5 (C-1'), 147.5 (C-3'), 147.8 (C-4'), 163.0 (C-9), 163.8 (C-5), 167.3 (C-7), 198.4 (C-4). These data were same with those of 3'-O-methyl-taxifolin [18]. In addition, according to the coupling constant of H<sub>2,3</sub> (11.4 Hz) as well as negative Cotton effect at 290 nm and positive Cotton effect at 330 nm in CD spectrum [12-14], compound **5** was confirmed to be in (2*R*,3*R*)-configuration. Therefore, compound **5** was identified as (2*R*,3*R*)-3'-O-methyl-taxifolin.

In this research, 5 compounds were isolated from a DHQ-rich extract (91% purity) of *L. olgensis* through polyamide and Sephadex LH-20 column chromatography, and their structures were identified through NMR, MS and CD analysis (Supplementary Fig. S1-S15). The main component in DHQ-rich extract from *L. olgensis* was (2*R*,3*R*)-dihydroquercetin (**1**), and two minor impurities were (2*R*,3*R*)-aromadendrin (**2**) and quercetin (**3**), which were same with the trade extract from *L. gmelinii*. Two trace impurities 3,5,7-trihydroxychromone (**4**) and (2*R*,3*R*)-3'-O-methyl-taxifolin (**5**) were firstly isolated from the extract of *L. olgensis*, which was slightly different from the trade DHQ-rich extract of *L. gmelinii*.

### 3.2 TLC Results

The results of polyamide TLC and silica gel TLC were displayed in Figs. 2-5.

### 3.3 Discussion

Compounds **1-5**, eriodictyol (**6**) and kaempferol (**7**) were used as reference standards for TLC detection (**1-7**, Fig. 1). According to the spots numbers, separation performance and tailing situation in the TLC, the adsorbents and solvent system were adjusted, and three TLC systems were established for quickly qualitative identification of components in DHQ-rich extract from *L. olgensis*. In polyamide TLC, the solvent system was toluene-ethyl acetate-methanol-formic acid (10: 4: 1: 2) (Figs. 2-3) or ethyl acetate-formic acid-water (8: 1: 1) (Fig. 4). In silica gel TLC, the solvent system was toluene-ethyl acetate-methanol-formic acid (10: 4: 1: 2) (Fig. 5). FeCl<sub>3</sub> was sprayed for the visualization of the spots.

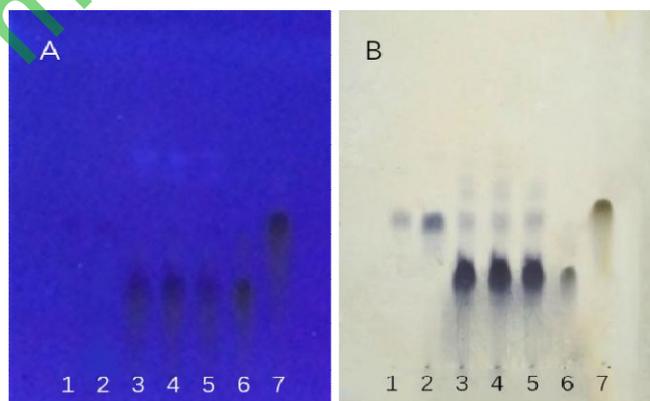


Fig. 2. Polyamide TLC, solvent system was toluene-ethyl acetate-methanol-formic acid (10: 4: 1: 2); 1-7 were aromadendrin, eriodictyol, DHQ-rich extracts from *L. olgensis* (No. 1-3), quercetin and kaempferol, respectively; (A) under UV 365 nm; (B) sprayed with FeCl<sub>3</sub> reagent

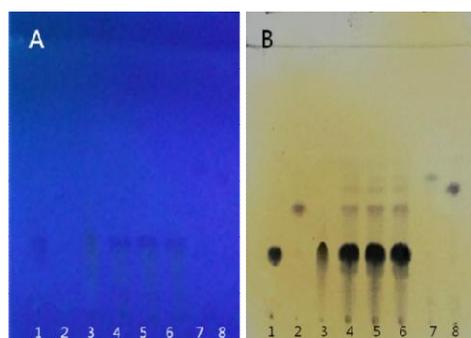


Fig. 3. Polyamide TLC, solvent system was toluene-ethyl acetate-methanol-formic acid (10: 4: 1: 2); 1-8 were DHQ, aromadendrin, quercetin, DHQ-rich extracts from *L. olgensis* (No. 1-3), 3,5,7-trihydroxychromone, 3'-O-methyl-taxifolin, respectively; (A) under UV 365 nm; (B) sprayed with  $\text{FeCl}_3$  reagent

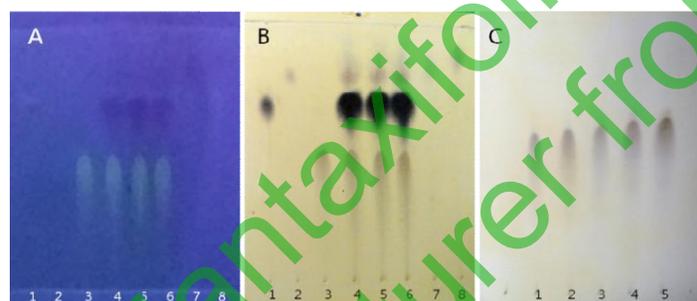


Fig. 4. Polyamide TLC, solvent system was ethyl acetate-formic acid-water (8: 1: 1); 1-8 were DHQ, aromadendrin, quercetin, DHQ-rich extracts from *L. olgensis* (No. 1-3), 3,5,7-trihydroxychromone, 3'-O-methyl-taxifolin, respectively; (A) under UV 365 nm; (B) sprayed with  $\text{FeCl}_3$  reagent; (C) 1-5 were 1-5  $\mu\text{L}$  of quercetin solution, sequentially, sprayed with  $\text{FeCl}_3$  reagent

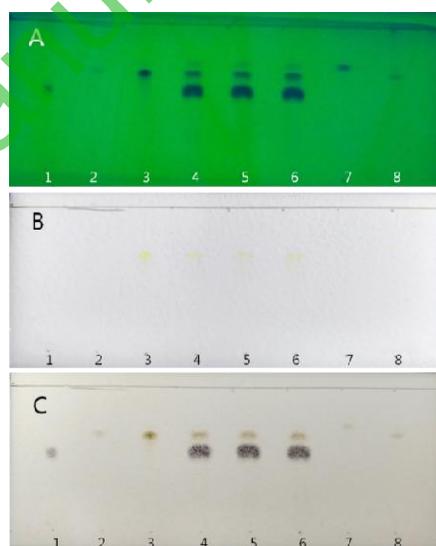


Fig. 5. Silica gel  $\text{GF}_{254}$  TLC, solvent system was toluene-ethyl acetate-methanol-formic acid (10: 4: 1: 2); 1-8 were DHQ, aromadendrin, quercetin, DHQ-rich extracts from *L. olgensis* (No. 1-3), 3,5,7-trihydroxychromone, 3'-O-methyl-taxifolin, respectively; (A) under UV 254 nm, (B) under sunshine, (C) sprayed with  $\text{FeCl}_3$  reagent

Fig. 2 and Fig. 3 showed that three batches of DHQ-rich extracts from *L. olgensis* all contained three impurities aromadendrin (**2**), 3,5,7-trihydroxychromone (**4**) and 3'-O-methyl-taxifolin (**5**), which had larger R<sub>f</sub> values than DHQ. Among them, **2** and **5** showed purple color and **4** showed blue color when sprayed with FeCl<sub>3</sub>. Eriodictyol (**6**) and kaempferol (**7**) had the similar R<sub>f</sub> value with **2** in polyamide TLC; however, **6** and **7** displaying dark blue and yellow-green colour were naturally differentiated with **2** and were not identified in three batches of DHQ-rich extracts from *L. olgensis*. This solvent system was suitable for the qualitative identification of impurities **2**, **4** and **5**, but it was not fit for the identification of minor component quercetin (**3**), since **3** and DHQ partially overlapped under this condition.

Fig. 4A and 4B confirmed that three batches of DHQ-rich extracts of from *L. olgensis* all contained minor components aromadendrin (**2**) and quercetin (**3**), which displayed purple and light brown-blue color when sprayed with FeCl<sub>3</sub> reagent. DHQ presented an evident dark-blue spot when sprayed with FeCl<sub>3</sub> reagent, and it had a higher R<sub>f</sub> value than **3** and was easily distinguished with **3**. Although this polyamide TLC system was suitable for identification of impurity **3**, it should be noted that the concentration of reference standard could not be too higher; otherwise, it will influence the tailing degree and R<sub>f</sub> value, resulting in interference with accurate identification of **3**. As displayed in Fig. 4C, the R<sub>f</sub> value of **3** showed an increasing trend along with the increase in the quantity of **3**.

Since quercetin (**3**) and DHQ partially overlapped (Figs. 2-3) and tailing of quercetin (**3**) was visible (Fig. 4) in polyamide TLC, silica gel TLC was used to confirm the existence of quercetin (**3**). Fig. 5 showed that in addition to the major DHQ, the extract also contained 2 minor components quercetin (**3**) and 3,5,7-trihydroxychromone (**4**). Among them, **3** present a dark spot under UV 254 nm, a yellow spot under sunshine, and yellow-green when sprayed with FeCl<sub>3</sub>, while **4** showed a shady spot under UV 254 nm, and light brown when sprayed with FeCl<sub>3</sub>. Because there was no tailing of the places, silica gel TLC was more suitable for the qualitative detection of quercetin (**3**) in the extract than polyamide TLC. However, since aromadendrin (**2**) and 3'-O-methyl-taxifolin (**5**) had the same R<sub>f</sub> value with **3**, meanwhile, they showed no color under sunshine and light brown spot when sprayed with FeCl<sub>3</sub>, it's hard to confirm the presence of

impurities **2** and **5** in the extract under this silica gel TLC system.

#### 4. CONCLUSION

Concerning the advantage and disadvantage of methods mentioned above, three different TLC systems should be combined to identify the components in the extracts. The results showed that three batches of extracts (91%, 92% and 93% DHQ) from *L. olgensis* contained the same minor impurities. Except for the presence of trace impurities 3,5,7-trihydroxychromone (**4**) and 3'-O-methyl-taxifolin (**5**), DHQ-rich extracts from *L. olgensis* all contained the significant component DHQ (**1**) and two main impurities aromadendrin (**2**) and quercetin (**3**), which were similar with the composition of trade DHQ-rich extract from *L. gmelinii*. The traditional TLC systems provided a rapid, simple qualitative identification method for in-process control of DHQ-rich extract, further quantification of these impurities in DHQ-rich extract from *L. olgensis* by HPLC analysis need to be done in the future.

#### SUPPLEMENTARY INFORMATION

Fig. S1-S15 were NMR, MS and CD spectra of compounds 1-5.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. Schauss AG, Tselyico SS, Kuznetsova VA, Yegorova I. Toxicological and genotoxicity assessment of a dihydroquercetin-rich Dahurian larch tree (*Larix gmelinii* Rupr) extract (Lavitol). Int J Toxicol. 2015;34(2): 162-181.
2. Turck D, Bresson J, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, et al. Scientific opinion on taxifolin-rich extract from Dahurian larch (*Larix gmelinii*). EFSA J. 2017;15(2):4682.

3. Chen J, Sun X, Xia T, Mao Q, Zhong L. Pretreatment with dihydroquercetin, a dietary flavonoid, protected against concanavalin A-induced immunological hepatic injury in mice and TNF- $\alpha$ /ActD-induced apoptosis in HepG2 cells. *Food Funct.* 2008;9(4):2341-2352.
4. Plotnikov MB, Aliev OI, Sidekhmenova AV, Shamanaev AY, Anishchenko AM, Nosarev AV, et al. Modes of hypotensive action of dihydroquercetin in arterial hypertension. *Bull Exp Biol Med.* 2017; 162(3):353-356.
5. Wang QH, Kuang HX, Wu L, Li Y, Wang ZB. Protective effect of dihydroquercetin on acute myocardial ischemia induced by isoproterenol in rats. *Chin J Exper Trad Med Form.* 2011;7(17):177-180.
6. Wang YY, Wang QW, Bao XM, Ding YF, Shen-Tu JY, Cui W, et al. Taxifolin prevents  $\beta$ -amyloid-induced impairments of synaptic formation and deficits of memory via the inhibition of cytosolic phospholipase A<sub>2</sub>/prostaglandin E<sub>2</sub> content. *Metab Brain Dis;* 2018. (In press).
7. Mahdavimehr M, Meratan AA, Ghobeh M, Ghasemi A, Saboury AA, Nemat-Gorgani M. Inhibition of HEWL fibril formation by taxifolin: Mechanism of action. *Plos One.* 2017;12(11):e0187841.
8. Du XQ, Chen FL, Hou KX, Li SY, Li XR, Liu JL. Distribution of taxifolin in *Larix gmelinii* tree trunk. *Bot Res.* 2016;5(1):1-5.
9. Zhang WP, Liu W, Jing-Hui FU, Chai J, Liu WC, Zheng YN. Structural identification and quantitative analysis of taxifolin in *Larix olgensis* Henry var. *koreana* Nakai. *Food Sci.* 2013;34(16):293-296.
10. Hu XQ, Yang JL, Li CH. Transcriptomic response to nitric oxide treatment in *Larix olgensis* Henry. *Int J Mol Sci.* 2015;16(12): 28582-28597.
11. Han XH, Hong SS, Hwang JS, Lee MK, Hwang BY, Ro JS. Monoamine oxidase inhibitory components from *Cayratia japonica*. *Arch Pharm Res.* 2007;30(1):13-17.
12. Kim NC, Graf TN, Sparacino CM, Wani MC, Wall ME. Complete isolation and characterization of silybins and isosilybins from milk thistle (*Silybum marianum*). *Org Biomol Chem.* 2003;1(10):1684-1689.
13. Chin YW, Chai HB, Keller WJ, Kinghorn AD. Lignans and other constituents of the fruits of *Euterpe oleracea* (Acai) with antioxidant and cytoprotective activities. *J Agr Food Chem.* 2008;56(17):7759-7764.
14. Cho JG, Song NY, Nam TG, Shrestha S, Park HJ, Lyu HN, et al. Flavonoids from the grains of c1/r-s transgenic rice, the transgenic *Oryza sativa* spp. *japonica*, and their radical scavenging activities. *J Agr Food Chem.* 2013;61(43):10354-10359.
15. Lee EH, Kim HJ, Song YS, Jin C, Lee KT, Cho J, et al. Constituents of the stems and fruits of *Opuntia ficus-indica* var. *saboten*. *Arch Pharm Res.* 2003;26(12):1018-1023.
16. Miyazawa M, Hisama M. Antimutagenic activity of flavonoids from *Chrysanthemum morifolium*. *Biosci Biotech Bioch.* 2003; 67(10):2091-2099.
17. Liu YZ, Lee YW. Standardization and Identification of minor components of Silymarin (MK-001). *Chin Herb Med.* 2012; 4(3):237-244.
18. Wang X, Zhou H, Zeng S. Identification and assay of 3'-O-methyltaxifolin by UPLC-MS in rat plasma. *J Chromatogr B.* 2012;911:34-42.

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