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Research Article

Study on the Dynamic Change of Mangiferin and Genkwanin in *Aquilaria sinensis* Leaves: A New Food Raw Material

[1]Yang Maoxun, [2]Zha Qianhui, [2]Huang Yongfang, [3]Liang Yaoguang, [1]Zhao Bin, [1]Xie Chaoliang and [3]Lv Qiaoli
[1]Zhongshan Torch Polytechnic, Zhongshan 528436,
[2]College of Forestry, South China Agricultural University, Guangzhou 510642,
[3]UNO Scientific Co., Ltd., Zhongshan 528400, P.R. China

Abstract: Contents of mangiferin and genkwanin were detected by High Performance Liquid Chromatography (HPLC) to explore the dynamic changes of active chemical components from different growing year and different sampling season in *Aquilaria sinensis* leaves and determine the appropriate growing year and season for sampling. It is disclosed that, growing year was an important influence on content of mangiferin, the appropriate growing year for mangiferin was two to three year and the highest content, 2.31% of mangiferin was in two-year *A. sinensis* leaves, the appropriate growing year for genkwanin was one to two year and the highest content, 0.23% of genkwanin was in one-year *A. sinensis* leaves; while the appropriate sampling season for mangiferin was Autumn and the appropriate sampling season for genkwanin was Spring.

Keywords: *Aquilaria sinensis* leaves, dynamic changes, genkwanin, mangiferin, new food raw material

INTRODUCTION

Aquilaria sinensis (Lour.) Gilg (Thymelaeaceae), distributed mainly in south China, is the the plant origin of expensive agilawood, which has been widely used in relieving pain, warming innards, stoping vomit and preventing asthma for hundreds of years in China, Indonesia, Vietnam and so on (Liu *et al*., 2013). The leaves of *A. sinensis*, an abundant new food raw material, exhibited notable biological activities such as anti-nociceptive, anti-inflammatory, antioxidative, purgation, hemostasis and α-glucosidase inhibitory (Wang, 2008; Chen *et al*., 2013; Feng *et al*., 2011; Qi *et al*., 2009; Wang *et al*., 2008; Zhou *et al*., 2008).

Mangiferin and genkwanin are two active chemical components of *A. sinensis* leaves. Mangiferin was reported to have anti-diabetic, anti-cancer, antiviral and immunoregulation activities (Li *et al*., 2013; Ren *et al*., 2011); Genkwanin was reported to have anti-tussive, anti-inflammation, anti-cancer, anti-oxidant, expectorant and immunoregulation activities (Li *et al*., 2010; Wang *et al*., 2008).

Considering their various biological activities, a large quantity of mangiferin and genkwanin are needed for further scientific studies and industrial applications. Dynamic changes of these two active chemical components from different growing year and different sampling season in *A. sinensis* leaves to determine the appropriate growing year and season for sampling were carried out in this study, to establish the scientific basis for further development of *A. sinensis* leaves and improve economic benefit of *A. sinensis* planting industry.

MATERIALS AND METHODS

Instruments and materials: An LC-10Avp liquid chromatography (HPLC) system used was equipped with a CTO-10ASvp column oven, a manual sample injection valve (model 7725) with a 20-μL loop and an SPD-10Avp ultraviolet detector (Shimadzu, Kyoto, Japan); YMC-Pack ODS-A columns (5 μm, 250×4.6 mm I.D.).

High performance liquid chromatography (HPLC) grade methanol (MeOH) was from Merck Chemical Co. (Darmstadt, Germany); Analytic grade acetic acid (AcOH) and ethanol (MeOH) were purchased from Guangzhou Chemical Reagent Co. (Guangzhou, China). Chemical reference substances of mangiferin and genkwanin were self-made with the purity greater than 98%.

The leaves from of *A. sinensis* trees cultivated for half year, 1~8 year were collected in different seasons from Zhongshan Agarwood Huang Agroforestry Development *Co*., *Ltd*, Zhongshan City, Guangdong Province, China. The plant material was botanically authenticated by Prof. Zhijian Feng in College of Forestry, South China Agricultural University.

Corresponding Author: Huang Yongfang, College of Forestry, South China Agricultural University, Guangzhou 510642, P.R. China

Preparation of samples for HPLC test: 1.0 g oven-dried leaves of different growing years and seasons of *A. sinensis* leaves were extracted with 79.5% ethanol 15.0 mL at 80°C for 1 h under heating reflux method. After filtration 0.5 mL filtered extract was diluted with 79.5% ethanol to 10 mL to give the samples for HPLC test.

HPLC profiles for contents of mangiferin: Column: Welch Ultimate C18 (250×4.6 mm i.d., 5 μm); Detection wavelength: 258 nm and 338 nm; Flow velocity: 1 mL/min; Column temperature: 28°C; Injection Volume: 20 μL.

Elution mode: Gradient; eluant (*V/V*): MeOH(A): 2% AcOH(B), 0.01~10.0 min 30% (A)→10.01~25.0 min 30~100% (A) →25.01~30.0 min 100% (A) →30.01~31.0 min 100~30% (A) →31.01~35.0 min 100% (A).

HPLC profiles for contents of genkwanin: Column: Welch Ultimate C18 (250×4.6 mm i.d., 5 μm); Detection wavelength: 280 nm and 360 nm; Flow velocity: 1 mL/min; Column temperature: 28°C; Injection Volume: 20 μL.

Elution mode: Isocratic; eluant (*V/V*): MeOH: 2% AcOH in water = 70: 30.

RESULTS AND DISCUSSION

HPLC detection result: Using the above-mentioned HPLC profiles for contents of mangiferin or genkwanin, all sample of different growing years

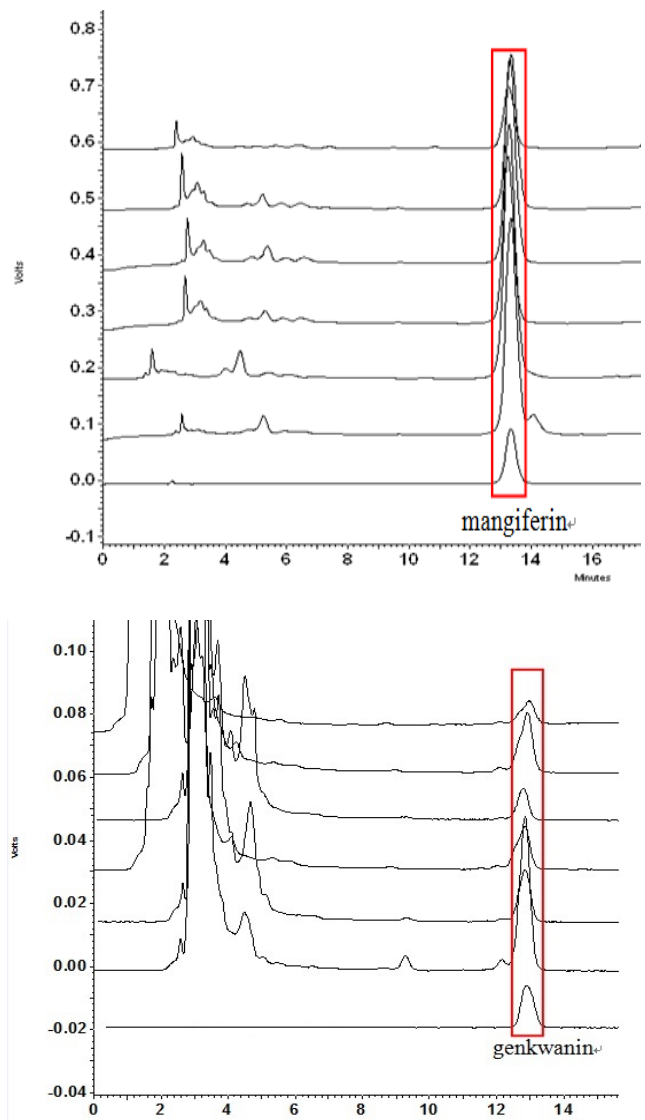

Fig. 1: HPLC chromatogram of mangiferin and genkwanin from different growing years; The nethermost chromatogram belong to chemical reference substances of mangiferin or genkwanin and all the rest chromatogram belong to samples from different growing years

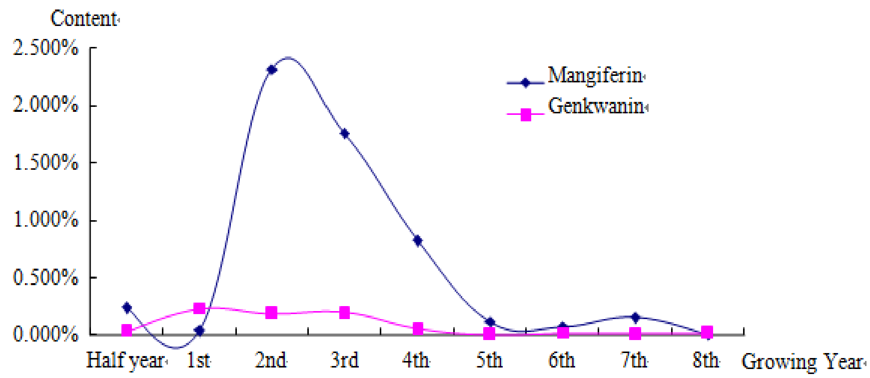

Fig. 2: Content change of mangiferin and genkwanin from samples of different growing years

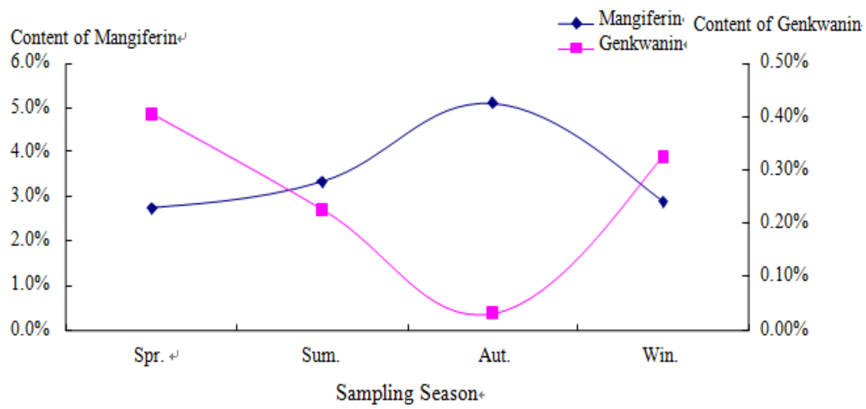

Fig. 3: Content change of mangiferin and genkwanin from samples of different sampling seasons

collected in different seasons were successfully detected by HPLC, the HPLC chromatogram of some representative samples of mangiferin and genkwanin from different growing years can be seen in Fig. 1.

Dynamic change of mangiferin and genkwanin in *aquilaria sinensis* leaves: The content detection result of mangiferin and genkwanin from samples of different growing years and different sampling seasons can be seen in Fig. 2 and 3.

From the above data analysis, It is disclosed that, growing year was an important influence on content of mangiferin, the appropriate growing year for mangiferin was two to three year and the highest content, 2.31% of mangiferin was in two-year *A. sinensis* leaves, while the appropriate growing year for genkwanin was one to two year and the highest content, 0.23% of genkwanin was in one-year *A. sinensis* leaves.

Sampling season also affected content of mangiferin and genkwanin, the appropriate sampling season for mangiferin was Autumn and the appropriate sampling season for genkwanin was Spring.

In recent agilawood industry, *A. sinensis* leaves usually be seen as non-medicinal part and discarded after agilawood collection. Such discarded leaves can be used as the raw material to produce active chemical components like mangiferin and genkwanin and the research findings of dynamic change of mangiferin and genkwanin in *A. Sinensis* leaves in this study can be applied to further greatly reduce the production cost.

CONCLUSION

According to the experimental result of this study, all sample of different growing years collected in different seasons were successfully detected by HPLC. Growing year was an important influence on content of mangiferin, the appropriate growing year for mangiferin and genkwanin in *A. sinensis* leaves were determined.

Sampling season also affected content of mangiferin and genkwanin, the appropriate sampling season for mangiferin and genkwanin in *A. sinensis* leaves were determined.

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