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**HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY FOR  
STANDARDIZATION OF CHLOROGENIC ACID IN TRADITIONAL CHINESE  
MEDICINE: A SYSTEMATIC REVIEW**

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**ABSTRACT**

Traditional Chinese Medicine (TCM) is currently used as an alternative treatment because it is considered cheaper and does not cause any side effects. Chlorogenic acid is a marker compound as a standard in a number of TCM preparations. HPLC are required not only for authentication of herbal formulations by fingerprint analysis but also for quality assurance a marker. Therefore, the aim of this literature review is to resume the method of HPLC that has been used to standardize in TCM with chlorogenic acid as a marker. This literature review focused on the type of detector for the HPLC method used in the analysis of chlorogenic acid as a marker. The results of the study showed the method HPLC-UV/Vis, HPLC-DAD, HPLC-MS/MS, HPLC-DAD-ESI/MS, and HPLC-ELSD had been used to analyze chlorogenic acid and standardized TCM. The HPLC-DAD-ESI/MS is the best method because it can perform analyses on a wider range of LOD and LOQ ranges.

**Keywords: Chlorogenic acid, Detector, HPLC, Standardization, TCM**

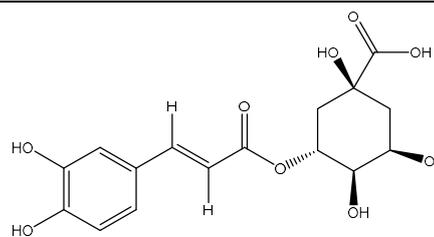
**INTRODUCTION**

Chlorogenic acid (**Figure 1**) is a phenolic compound derived from a class of hydroxycinnamic acid (a derivative of cinnamates acid) [1]. The chemical

structure of this compound is composed of a caffeine group and a quinic acid group, and chlorogenic acid also referred to as 5-caffeoylquinic acid (5-CQA). Chlorogenic

acid is a compound that belongs to a group of esters, quinic acid and some trans-cinnamic acid, where generally caffeine, coumarin, and ferulic acid are dispersed in a wide variety of plants and are the fraction of the phenol compounds most consumed by humans [2]. Chlorogenic acid can protect plants from microorganisms, insects, and UV radiation [3]. Besides, chlorogenic acid also provides many other benefits that are good for human health, namely as antioxidants, antifungal, and anti-inflammatory [4]. However, some also mention as 3-CQA, the difference between the nomenclature is caused by the history of chlorogenic acid [5].

Nowadays, chlorogenic acid is widely studied because the compound is scattered in various plants. Chlorogenic acid shows the activity of cardiovascular protection, antioxidant, antiviral, antibacterial, liver protection, lipid-lowering, and hypoglycemic [6]. Chlorogenic acid can be found in foods and spices such as apples, artichokes, betel nut, carrot, coffee beans, eggplant, grape, kiwi, pear, potato, tea, tomato, and tobacco leaf [7]. Most of the research conducted on chlorogenic acid is associated with its benefits to disorders related to metabolic syndrome, that is related to metabolism and biochemistry such as type 2 diabetes and cardiovascular disease [8].



**Figure 1: Chemical structure of chlorogenic acid (5-caffeoylquinic) [9]**

Traditional Chinese Medicine (TCM) has been used and believed to cure various diseases for thousands of years. The community uses this treatment as an alternative treatment of medical treatment because the community considers medical treatment is more expensive, sometimes causes side effects, and makes the patient's body weaker than ever so that the quality-of-life decreases. The Area of TCM is very wide, including acupuncture, herbal Medicine, and energy healing (Qi Gong) [10]. The National Pharmacopoeia of China establishes chlorogenic acid as a marker in the standardization and identification of Herbs [11]. In addition, the determination of chlorogenic acid as marker is also included in the European Pharmacopoeia and USP 35-NF30 is used for its identification, purity, and content [12]. The analysis of the compound marker has also been introduced and applied by the World Health Organization (WHO) and the Food and Drug Administration (FDA) as a quality assessment strategy for herbal medicine [13].

In conventional methods, chlorogenic acid is isolated from the dried plant by using a

solvent extraction and followed by filtration [14]. The method of HPLC to determinate chlorogenic acid in various herbs can be used as a standardized method of herbal plants so that the herbs that are later used for the treatment is standardized, either the compounds contained and the content or amount of the compounds contained [15]. Reversed-phase high-performance liquid chromatography (RP-HPLC) with mass spectrometry detector (HPLC-MS/MS) has been successfully and extensively applied to the structure of phenolic compounds in a wide variety of samples, having high sensitivity advantages, speed, and low sample consumption. Besides, liquid chromatography with tandem mass spectrometry techniques (LC-MS/MS) is useful for explaining the structure of an active compound (e.g., a non-volatile

phenolic compound) and discriminating the compound based on its molecular weights [11].

The purpose of this literature review is to review the research that has been conducted related to the determination of chlorogenic acid as a marker compound in TCM using the method of HPLC.

## METHOD

The journal source used for this study was journals from PubMed sources using the keywords namely Traditional Chinese Medicine, HPLC, chlorogenic acid, and standardization; published from 1996 to 2020. They are grouped by UV-Vis, MS/MS, DAD, DAD-ESI/MS, and ELSD detectors. The focus of this literature review is the type of detector used in the analysis of chlorogenic acid by using HPLC (Table 1).

**Table 1: HPLC detector attributes in quality assurance and fingerprint analysis of chlorogenic acid as a marker in Traditional Chinese Medicine (TCM)**

Detector	Key Attributes	Application	Reference
UV-Vis	Most widely used and accepted.	Qualitative, Quantitative, and Fingerprint.	[16]*, [17-19*], [24-26], [29], [34]*, [41], [53], [55], [57], [58]*, and [59]*.
Photodiode Array Detectors (PDA) and Diode Array Detectors (DAD)	PDA or DAD peak purity = homogeneity; reliable; and easy to use.	Qualitative, Quantitative, and Fingerprint.	[13]*, [18], [20-21], [23], [27], [37-38], [43], [45]*, [49], [52]*, and [57]
Evaporative Light Scattering Detector (ELSD)	Detects most nonvolatile analytes.	Qualitative and Quantitative.	[22] and [57]
Mass Spectrometry	Identify the component using m/z and retention time (t(R)), exclusion and high selectivity,	Qualitative, Quantitative, and Fingerprint.	[1], [17], [18], [21], [28], [30-33], [35-40], [42], [44, 45*- 51*], [54]*, [56], and [57].

\*- tandem fingerprint analysis

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## RESULT AND DISCUSSION

### Traditional Chinese Medicine (TCM)

Traditional Chinese Medicine (TCM) has been used for the treatment and prevention of various diseases for more than 1000 years [60]. In China, plant processing has been developed for a long time and is an important aspect of the use of herbal plants. Traditional Chinese Medicine (TCM) has used for reducing toxicity, improving efficacy, and stabilizing the active ingredient [26]. The chemical compounds in TCM is influenced by geographic origin of species, cultivation practices, harvest time, storage conditions, and processing methods. It makes complexity and difficulties for the quality assessment of TCM [61]. China, as the country of origin and implementation of TCM, has a unique TCM theoretical system and effective methods of treatment. By research and improvement for many years, a great breakthroughs and extraordinary achievements have been made for standardization TCM, but there are still many problems related to standardization TCM [57]. The World Health Organization (WHO) reported that TCM has grown into an international industry. However, due to the lack of standardization of TCM, there is a demand for the development of standardization methods in the modern era and globalism as it is today [62]. Many methods of standardization and evaluation

of TCM, both single and simultaneous, have been applied [63]. Biological tests are also used for standardization TCM but they have certain limitations in operation, high cost, and low in precision [64].

At this time, many herbs are used as ingredients for TCM, among others, *Gardeniae fructus* and *Helianthus tuberosus* L. *Gardeniae fructus* is TCM commonly called *Zhizi* in Chinese. It was derived from the dried fruit of *Gardenia jasminoides* of family madder. The *Gardeniae fructus* has been investigated to contain a wide range of phytochemical content, and the results show that in the *Gardeniae fructus* there are genial compounds, chlorogenic acid, crocin-1, and rutin as its main components [65]. This herb is used for the treatment of cholagogue effects (Gallstone shredders), to reduce fever, and diuretics [60]. *LepidoGrammitisdrymoglossum* is used in the treatment of tuberculosis, pulmonary, inflammatory, diarrhea, and fever by the Chinese ethnic community Miao, Yao, and Tong. This herb is a member of the Family Polypodiaceae, which is widespread in western and southern China, especially in Guangxi, Guizhou, and Guangdong provinces. In some reports of chlorogenic acid contained in the plant due to the resemblance of its activity, the common chlorogenic acid is used as a biomarker of herbal plants in China [4].

*Helianthus tuberosus* L. (Jerusalem artichoke), the family of Asteraceae is an herbal medicine originating from the north of America. As a source of inulin, the tuber has been used as a traditional remedy for the treatment of rheumatism and diabetes with a variety of pharmacological activities, such as cholagogue, gastric, aperient, spermatogenic, diuretic, tonic, and antioxidants. The main phenolic acids in *H. tuberosus* leaves are chlorogenic acid [11]. *Artemisiae argy folium* is a widespread herbal plant in the region of East Asia. This herb is commonly used in TCM prescriptions. The pharmacological activity of this plant is to treat bleeding, insomnia, asthma, and osteoarthritis [6]. Epimedium is a traditional herb used to strengthen and nourish the kidneys in China. Epimedium brevicorne Maxim has been listed in the Chinese Pharmacopeia [31]. Currently, pharmacological studies have shown that Epimedium has activities as tumor inhibitors, antioxidants, and anti-inflammatory [66-70].

Many compounds can be isolated from Epimedium, such as flavonoids glycosides, phenylpropanoid, alkaloids, polysaccharides, lignin and sesquiterpenes [71].

### High-Performance Liquid Chromatography (HPLC)

High-Performance liquid chromatography (HPLC) (Figure 2) was developed from

classical column chromatography; the sample solution is injected into the porous material column (stationary phase) and solvent (mobile phase) by using a high-pressure pump. The separation of the samples is based on differing migration rates of components through the columns which arising from different partitions of the components into the stationary phase and the mobile phase. This difference depends on the partitions and the timing of the elution of various components [72].

The development and validation methods play an important role in the discovery, development and manufacturing of drug. This method is used to ensure identity, potency, purity, performance of medicinal preparations. In developing of a method, initially by collected the information about the physicochemical properties of analytes (pKa, logP, and solubility) for determine which mode of detection will be suitable for analysis [73]. The purpose of the HPLC method is to isolate and quantify the main active compounds, other compounds, and impurities.

The UV-Visible absorption detector is the most commonly used in HPLC method because many of the compounds are absorbed in the UV and Visible region (190 – 600 nm). The concentration of the samples was proportional to the absorbance, determined by the light fraction transmitted

through the detector cell by the law of Lambert-Beer.

### 1. HPLC-UV/Vis

The UV-Visible detector is the most commonly used in HPLC for compounds that absorb in the UV (or Visible) regions (from 190 – 600 nm). The concentration of samples is comparable to its absorbance. The HPLC with the UV-Vis detector is distinguished into a fixed and variable-wavelength detector, where by the fixed wavelength detector can only read at a certain wavelength, usually with a 254 nm from a low-pressure mercury lamp. While the variable wavelength detectors can be adjusted to operate at maximum absorption of analytes or at wavelengths that provide more selectivity [73].

The HPLC-UV/Vis method is used to analyze chlorogenic acid as a marker in herbal plants as a TCM raw material. From the results of studies that have been conducted, the method of HPLC-UV/V is valid is used to analyze the compound of chlorogenic acid. Lepido Grammitis drymoglossum have contained chlorogenic acid by using the HPLC-UV/Vis. The HPLC conditions were carried out a Phenomenex Luna C<sub>18</sub> (250 × 4.6 mm i.d.; 5 μm particle size; 100 Å pore size) column; the mixture of acetonitrile and 0.5% aqueous phosphoric acid (11.5:88.5, v/v) as mobile phase; the flow rate of 1.0 mL/min and wavelength at 327 nm with

injection volume of 10 μL (Figure 3). The LOD and LQD values of CGA standard were 0.049 and 0.132 mg/L, respectively [4].

### 2. HPLC-PDA

The Photodiode Array Detectors (PDA) and Diode Array Detector (DAD) is a detector that has the same absorption as the UV-Vis detector of 190 – 600 nm. This detector has an optical pathway similar to a variable-wavelength detector except the light passes through the flow cell before crashing the grid, allowing it to diffuse the spectrum across an array of diodes [73]. The detector is generated both absorbance and spectral data and it can be used for identification, quantitation, and peak purity.

Analysis of chlorogenic acid using HPLC-PDA on the sample of *Gardeniae fructus* was done simultaneously with the content of rutin compounds, geniposide, and crocin-1 [65]. Detection wavelengths for rutin, geniposide, chlorogenic acid, and crocin1 were set at 254 nm, 240 nm, 330 nm, and 440 nm, respectively. An Agilent Zorbax Extend C<sub>18</sub> (250 mm × 4.6 mm, 5 μm) was used at a flow rate of 1.0 mL/min. A mixture of (A) aqueous phosphoric acid and (B) acetonitrile (0.4%, v/v) as mobile phase and operated in a gradient elution of 0~45 min, 5%~30% B; 45~60 min, and 30%~50% B. Typical chromatograms of the contents (%) of chlorogenic acid and

reference compounds in *Gardeniae fructus* (Figure 5 and Table 6).

### 3. HPLC-ELSD

The HPLC with evaporative light scattering detector (ELSD) is commonly used for analysis of compounds not absorb UV radiation, such as sugars, antibiotics, antivirals, lipids, fatty acids, oils, polymers, phospholipids, terpenoids, surfactants, and triglycerides. The detector has a high sensitivity of up to 10 ng and compatibility with the gradient elution.

The HPLC-ELSD method has been used to determinate the compounds contained in the *Sargentodoxa cuneata* simultaneously. It is known that the plant contains 3,4-dihydroxyphenylethyl alcohol glycosides, chlorogenic acid, salidroside, and liriiodendrin. A Hypersil ODS (C<sub>18</sub>) column (250 mm × 4.6 mm id, 5 μm) was operated at 35°C. A mixture of acetonitrile (A) and 0.2% formic acid aqueous solution (B) was used as the mobile phase and performed using a stepwise gradient elution [22]. Typical chromatograms of reference compounds and the contents (%) of chlorogenic acid in *Gardeniae fructus* (Figure 5). This method was validated for limits of detection and quantification, precision, and reproducibility. The calibration curves of this method showed good linearity. The range of recoveries were 96.39%–104.64%. The relative

standard deviations of precision for intraday and interday were less than 2.90% and 3.30%, respectively.

### 4. HPLC-MS/MS

HPLC-MS/MS provides an effective means of analyzing complex samples both quantitative analysis and qualitative identification [21].

Each mass spectrometer consists of three main components, which are sources of ionization, analyzers, and detectors. Ions are obtained by ionization of electrospray and the ionization of the most widely used atmospheric pressure.

HPLC-MS/MS has been used for the determination of phenolic compounds and flavonoids in *Artemisiae argyifolium*. Twenty-two components including 5 phenolic acids and 17 flavonoids in *Folium Artemisiae argyi* samples were quantified by the developed LC-MS/MS method. Chromatographic separation was achieved on a reversed-phase chromatography (Shiseido, Capcell Pak C<sub>18</sub> column) (150×3.0mm I.D., 3μm) at 35°C, and the eluent was acetonitrile (A)- aqueous formic acid (100:0.1, v/v) (B) and performed using a gradient elution [6].

### 5. HPLC-DAD-ESI/MS

The high-performance liquid chromatography–diode array detector coupled to an electrospray tandem mass spectrometer (HPLC-DAD-ESI/MS) is an HPLC with photodiode detector and mass

ionization electrospray spectrometry and commonly used for the identification of a compound. Both detectors (DAD and MS) if used together can produce three-dimensional data i.e., retention time, UV/Vis spectrum, and ion mass analysis. When coupled with the proper chromatography method, a three-dimensional analysis will provide scientific and accurate data to identify and measure the detected compounds [57].

The HPLC–DAD–ESI/MS method has been used to standardize the herb plant of *Gardenia fructus* and chemometric analysis by using principal component analysis (PCA) and cluster analysis (CA) (Figure 7-8). The eight compounds identified in the *Gardeniae fructus* and chlorogenic acid are the main components.

Chromatographic separations were performed on an Agilent ZORBAX SB-C<sub>18</sub> analytical column and a mixture of aqueous solution of 0.1% formic acid (A) and acetonitrile (B) as mobile phase [21]. The results indicated that the contents of the 8 major bioactive constituents in *G. fructus* (34 batches samples).

## 6. Final consideration

The HPLC-UV/Vis method is the most widely used detector, and the advantages of this HPLC-UV/Vis method is that it is generally easy to use, it can be used by all samples, more cost-effective, qualitative and quantitative analysis.

But the disadvantages of this method are that the analyzed compounds should have a chromophore group, the solvent used should be colorless, only to detect at one wavelength and not be good if used for identification and test purity [73]. From the review of the literature, LOD and LOQ values with UV-Vis detector are greater than other detectors. The high values of LOD and LOQ indicate less sensitive UV-Vis detectors if the analytical compounds are in very small quantities.

The HPLC with DAD detector is better than the UV-Vis detector because the UV/Vis detector can detect a single wavelength while the DAD can detect at variable wavelength (190-800 nm). DAD is a UV/Vis detector but has some photodiodes, then scans all the components that can respond in wavelength range of 190-800 nm which no need for any adjustments. In the development of the method, the DAD is a better choice because the UV/Vis detector is very time-consuming. HPLC-DAD can collect peak data that issued for identification, evaluation of peak homogeneities and purity [73]. The parameter that becomes the advantage of the HPLC-DAD method is the small value of RSD repeatability of 1.01%. It shows high accuracy and repeatability but still not as good as the HPLC-MS/MS detector.

HPLC-MS/MS is a better method if compared to the above two methods. This is

because HPLC-MS/MS will give an overview of the compound structure. This method can be both qualitative analysis and more accurate identification to identify compounds that are not yet known structure [39] the HPLC-MS/MS method has the lowest LOD and LOQ, the most sensitive MS detector in analyzing the analytes as it can detect up tonanograms (ng).HPLC-DAD-ESI/MS is a combination of photodiode detector and mass spectroscopy; it certainly gives better results as it can provide more analytical data because the DAD detector serves to quantify while the MS detector for identification can be used for purity testing. The advantage of this method is that it can analyze with a wide range of wavelengths than can be known the information of structure and mass of a compound. Based on the parameters in this review of the literature, HPLC-DAD-

ESI/MS has a good level of precision compared to other methods.

HPLC-ELSD is typically used for non-UV compounds, ELSD is the main choice because the detection principle does not rely on the optical properties of the dissolved substances. From the results of the literature review HPLC-ELSD has good repeatability value over other methods.

Of the five methods that have been mentioned, HPLC-DAD-ESI/MS is the best method for the determination of chlorogenic acid in the standardization of TCM, because to determine because to determine the standard required comprehensive parameter data. The HPLC-DAD-ESI/MS method is considered comprehensive because it uses two detectors that can perform quantification and identification analysis.

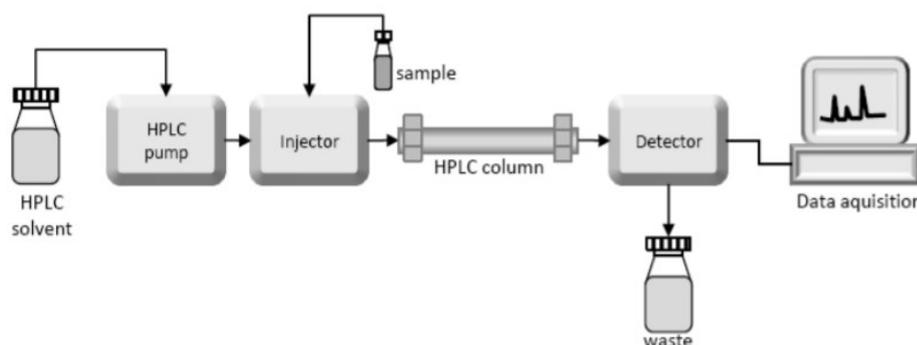


Figure 2: High Performance liquid chromatography (HPLC) schematic diagram [73]

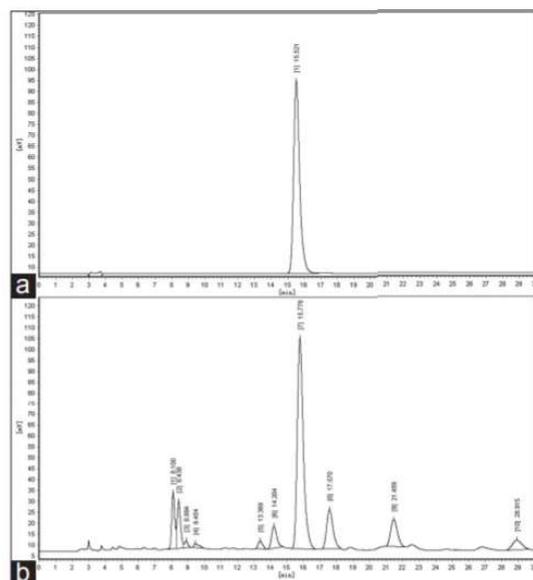


Figure 3: Typical chromatograms of (a) CGA standard and (b) *L. drymoglossoides* extracts with the retention time of analyte is 15.521 min of (a) and 15.778 min of (b).[4]

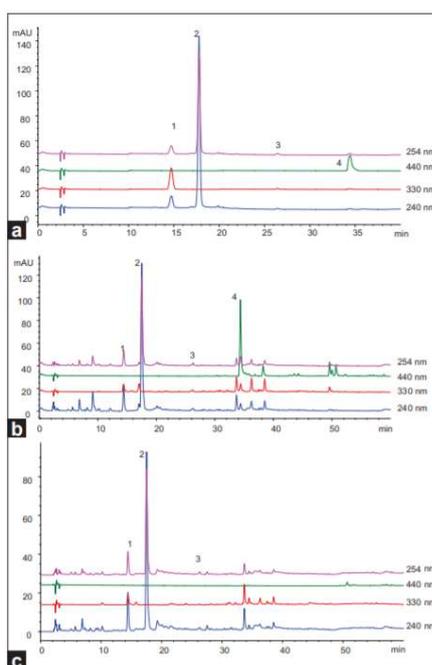


Figure 4: Typical chromatograms of components in *Gardeniae fructus* [65]

Table 2: The contents (%) of chlorogenic acid in *Gardeniaefructus* [65]

Sample	Suppliers	Rutin	Chlorogenic acid	Geniposide	Crocin-1
Crude	Hunan	0.207	0.398	3.897	0.417
	Jiangxi	0.251	0.407	3.699	0.837
	Zhejiang	0.238	0.162	4.242	0.625
CZP	Hunan	-	0.273	2.594	-
	Jiangxi	0.116	0.286	2.492	-
	Zhejiang	-	0.189	2.658	-

'-' not detected

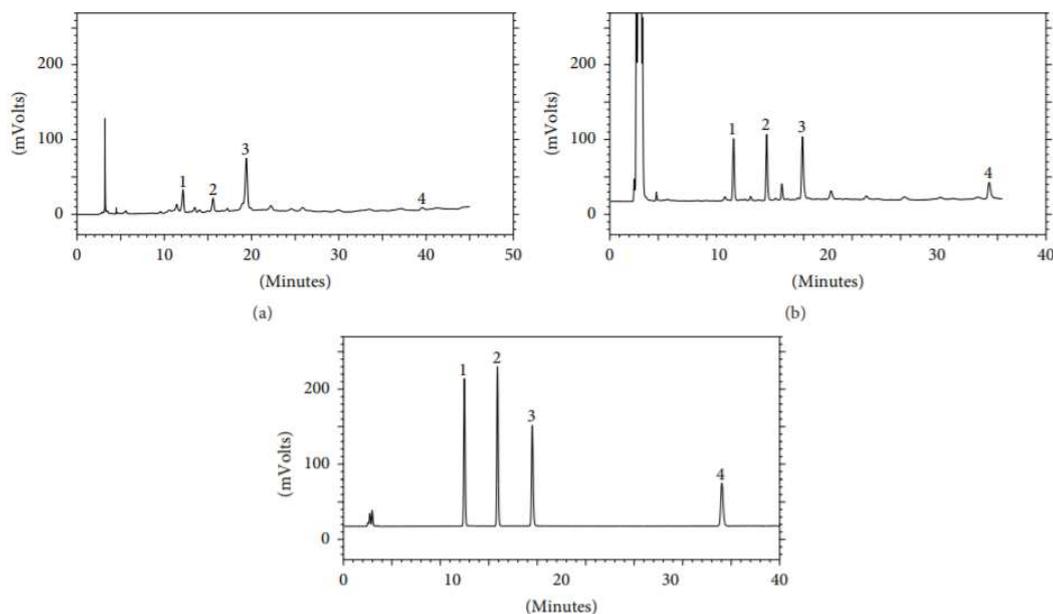


Figure 5: Typical chromatogram of reference compounds and the contents (%) of chlorogenic acid in *Gardeniae fructus*. Samples at wavelengths 271 nm (a); Samples with ELSD (b); Standard solution of ELSD (c). Peak: (1) 3, 4 - 3,4-dihydroxyphenylethyl alcohol glycosides; (2) Salidroside; (3) chlorogenic acid; and (4) Liriodendrin [22]

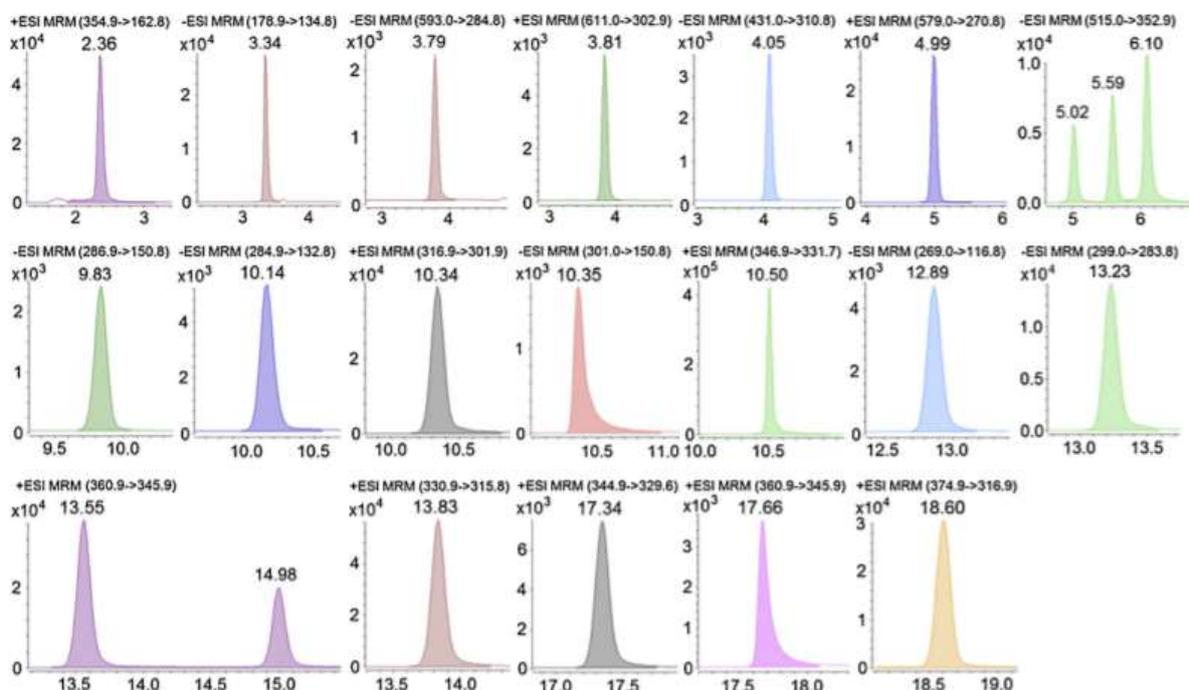


Figure 6: Typical HPLC-MS/MS chromatogram of 22 constituents in *Folium Artemisiae argyi* extract [6]

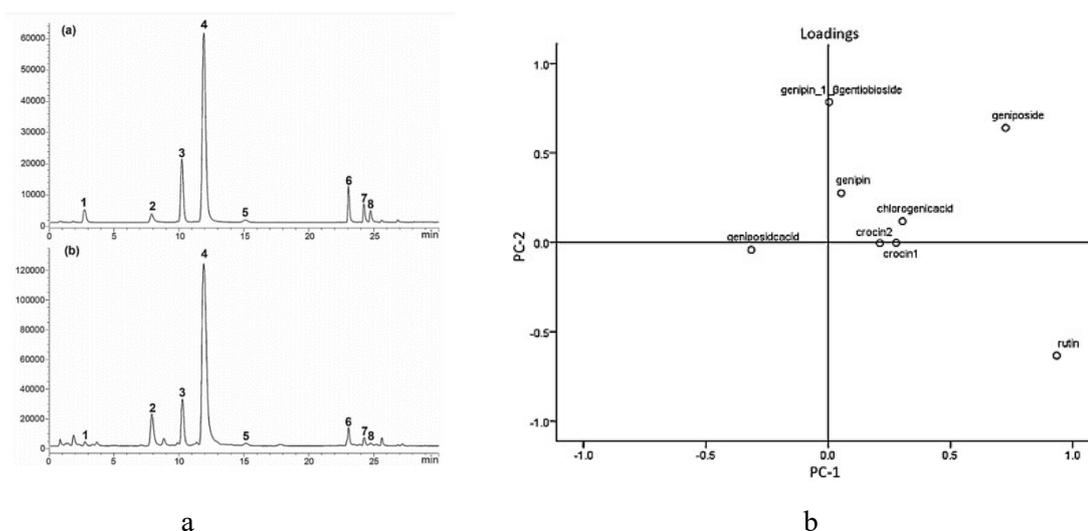


Figure 7: Typical chromatograms in negative-ion ESI/MS mode (a) and score plot (b) for major bioactive constituents in *G. fructus* [21]

## CONCLUSION

The HPLC-UV-Vis, HPLC-DAD, HPLC-MS/MS, HPLC-DAD-ESI/MS, and HPLC-ELSD methods are valid to be used as standardized of chlorogenic acid content in Traditional Chinese Medicine (TCM). The HPLC method with the DAD-ESI/MS detector is the best method because it can perform qualitative, quantitative, high sensitivity analysis, and can be used for compound identification and purity test.

The results of the literature review show that the HPLC-DAD-ESI/MS method can be used for the analysis of chlorogenic acid in herbal preparations of TCM with good sensitivity. Therefore, this method can be developed to detect chlorogenic acid in the Indonesian natural product.

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