



IDENTIFICATION AND PURIFICATION OF NOVEL CHLOROGENIC ACIDS IN  
*Artemisia annua L.*

Wenwen Zhao<sup>1</sup>, Weina Zhang<sup>1</sup>, Yuru Chen<sup>1,\*</sup>, Fengping Yang<sup>1</sup>, Qiming Cao<sup>2</sup>, Wenzhong Chen<sup>3</sup>,  
Junli Liu<sup>4</sup> and Kewei Dai<sup>5</sup>

<sup>1</sup>College of Life Science, Nanjing Normal University, Nanjing Engineering Research Center for Industrialization of Microbial Resources, Jiangsu Key Lab for Biodiversity and Biotechnology, Jiangsu Key Lab for Microbes and Functional Genomics, Nanjing 210023, P. R. China

<sup>2</sup>School of Resources and Environment, University of Electronic Science and Technology of China, Chengdu 610054, P.R. China

<sup>3</sup>Nanjing Luye Sike Pharmaceutical Co., Ltd. Nanjing, 210061, P. R. China

<sup>4</sup>Institute of Chemical Industry of Forest Products, CAF, Nanjing 210042, P. R. China

<sup>5</sup>College of Geography Science, Nanjing Normal University, Nanjing 210023, P. R. China

Received – August 28, 2015; Revision – September 14, 2015; Accepted – October 04, 2015

Available Online – October 20, 2015

DOI: [http://dx.doi.org/10.18006/2015.3\(5\).415.422](http://dx.doi.org/10.18006/2015.3(5).415.422)

KEYWORDS

*Artemisia annua L.*

Chlorogenic acids

Dicaffeoylquinic acids

LC/MS<sup>n</sup>

Identification

Purification

ABSTRACT

Present work has been carried out to study the identification and purification of chlorogenic acids in *Artemisia annua L.* Thirty-six chlorogenic acids were identified from this plant. Among these fifteen viz. two monocaffeoylquinic acids (Mr354), five dicaffeoylquinic acids (Mr516), one feruloylquinic acid (Mr368), three caffeoylferuloylquinic acids (Mr530), two ferulylquinic acids (Mr544), one dimethoxy-cinnamoylquinic acid (Mr382) and one p-coumaroylquinic acid (Mr338) were reported first time in present study by LC/MS<sup>n</sup>. Cis-isomers of these chlorogenic acids were also identified. Furthermore, column chromatography was used for the separation and purification of these chlorogenic acid; by the use of petroleum ether and ethyl acetate decolorization methods as mentioned in the literature, thus separation and purification process carried out at the same time. Polyamide and dextran were also used to purify Dicaffeoylquinic acid and purity level reached 85.7% with a yield of 53.4% after the secondary purification by Sephadex LH-20. Result of study revealed that *A. annua* can not only used for the production of artemisinin, but also yielding different kinds of chlorogenic acids, thus making comprehensive utilization of this plant.

\* Corresponding author

E-mail: [chenyuru@njnu.edu.cn](mailto:chenyuru@njnu.edu.cn) (Yuru Chen)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher ([www.my-vision.webs.com/horizon.html](http://www.my-vision.webs.com/horizon.html)).  
All rights reserved.

All the article published by (Journal of Experimental Biology and Agricultural Sciences) / CC BY-NC 4.0



## 1 Introduction

Recently, much attention has been paid by world research community for the exploitation of medicinal plants. *Artemisia annua* L. is a functional plant used as a natural resource of Chinese herbal medicine in the production of artemisinin. This plant is widely used for the treatment of malaria and fever from many centuries due to its important antimalarial ingredient artemisinin (Clifford, 2000). It is believed that medicinal effects of artemisinin can be enhanced to treat parasitic diseases and cancer if it simultaneously delivered with flavonoids analogs extracted from *A. annua* (Clifford et al., 2006). Furthermore, remarkable anti-HIV activity of *A. annua* plant was also reported by various researchers (Mueller et al., 2000; Clifford et al., 2003). In addition, *A. annua* leaves contain variety of chlorogenic acids (Willcox et al., 2011), known as potential and nontoxic HIV-1 Integrase Inhibitors (Ferreira et al., 2010; Lubbe et al., 2011). Therefore, chlorogenic acids may be potential candidates in anti-HIV treatment.

Chlorogenic acids belongs to a large family of esters condensed by quinic acid (such as shikimic acid, queenie methyl or butyl 4-deoxy-quinic acid) and trans-cinnamic acids (such as caffeic, ferulic, p-coumaric, sinapic and dimethoxycinnamic acid) (Robinson et al., 1996; Lai et al., 2007; Gu et al., 2007). In IUPAC system (-)-quinic acid is defined as 1L-1(OH), 3,4/5-tetrahydroxy-cyclohexane carboxylic acid and this same nomenclature is used throughout this paper (Han et al., 2008).

Crude acetone, water and methanol can be used to determined phenolic compositions of *A. annua* by HPLC-DAD-ESI/MS<sup>n</sup> (IUPAC, 1976; Carbonara et al., 2012; Gouveia & Castilho, 2013) but these compounds have not been fully studied so far. For this reason, it is necessary to develop a rapid and sensitive method to identify these components in *A. annua*. Therefore, present study has been conducted to develop a rapid and sensitive method for isolation and identification of chlorogenic acids from *A. annua*.

## 2 Materials and Methods

### 2.1 Chemicals and reagents

HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany). All aqueous solutions were prepared with pure water produced by Milli-Q system (Bedford, MA, USA). Other organic solvents and chemical reagents were analytical grade were purchased from Tingbest Co. (Nanjing, China). 5-caffeoylquinic acid, caffeic acid and 4,5-dicaffeoylquinic acid (>98% by HPLC for all) were obtained from Chengdo Biopurify Phytochemicals, Ltd China (Sichuan, China). Polyamide and dextran were purchased from Al Essex Bio-Technology Co., Ltd. Chengdu. *A. annua* and its residue (Chongqing, P. R. China) were smashed into 40 mesh by a pulverizer and then stored at room temperature.

### 2.2 Sample preparation

Extract of *A. annua* was obtained by dissolving 1 g of standard sample into 100 mL ethanol: water (70:30, v/v) under ultrasonication for 1 h at 50°C. After extraction, the sample was centrifuged at 6000 g for 3 min, and stored at -4 °C for analysis. Before HPLC analysis, the sample was filtered through 0.45 µm filter.

### 2.3 Analytical method

Ethanolic extracts of *A. annua* are used for isolating phenolic compounds while 0.05% (v/v) acetic acid was used as additives to adjust the pH value of the mobile phase.

#### 2.3.1 Liquid chromatography

The chromatography was performed on Ultimate AQ-C18 (250 mm×4.6 mm, 5 µm). The mobile phase consisted of 0.05% (v/v) acetic acid in water (A) and acetonitrile (B). The gradient program was used as follows with a total analysis time of 45 min: (88% A - 60% A. The column temperature was maintained at 30°C. The flow rate was 0.6 mL/min and the injection volume was 10 µL. UV-DAD detection was performed at λ=327 nm.

#### 2.3.2 Mass spectrometry

HPLC system interfaced with an Agilent 6460 triple quadrupole mass spectrometer (Agilent technologies, MA, USA) was used to carry out the HPLC/ DAD/ESI-MS/MS analysis, with the same column, elution program and flow rate of HPLC analysis. The conditions of ESI source were set as follows: source voltage: 3.5 kV; the flow rate of drying gas (N<sub>2</sub>) was 11.0 L/min; the drying gas temperature was 365°C and nebulizer pressure of 45 psi. The mass spectrometric data was acquired from m/z 50 to 800 in negative ion modes. As necessary, MS<sup>2</sup> fragment-targeted experiments were performed to focus only on compounds producing a parent ion at m/z 353, 515, 367, 529, 543 or 677. The first level of impact energy was 5 ev; the second level of impact energy was 5 ev, 10 ev, 15 ev, 20 ev.

### 2.4 Separation and purification of dicaffeoylquinic acid

Polyamide (for initial purification) and dextran (for secondary purification) were used to purify dicaffeoylquinic acid and then eluted with different concentrations of ethanol. Finally, the content of dicaffeoylquinic acid was determined by HPLC to calculate the purity and yield.

## 3 Results and Discussion

Total thirty six chlorogenic acids were isolated from the leaves of *A. annua*. HPLC results of all isolated chlorogenic acids from *A. annua* leaves and *A. annua* residue were represented in Figure 2 and Figure 3. The identification of each compound

was made based on the main fragment ions obtained in the MS<sup>n</sup> experiments. Table 1 showed the analytical data: retention time (R<sub>t</sub>), deprotonated molecular ions (MH<sup>-</sup>), and the most important MS<sup>n</sup> fragment ions for each peak. Thirty-six peaks

were separated and out of which fifteen were reported for the first time on the total ion chromatogram (TIC). The TIC of representative *A. annua* extracts was shown in Figure 1.

Table 1 Varieties of chlorogenic acids detected in *A. annua*

No.	R <sub>t</sub> (min)	(M-H) <sup>-</sup>	Fragment ions(intensity)	Identification
1*	10.2	353.0	MS <sup>2</sup> : 190.9(100), 353.0(11)	1-CQA
2	11.0	353.0	MS <sup>2</sup> : 191.0(78), 179.0(63), 352.9(100)	3-CQA
3	12.8	353.0	MS <sup>2</sup> : 191.0(100), 352.9(7)	5-CQA
4	13.4	353.0	MS <sup>2</sup> : 172.9(100), 179.0(76), 190.8(44), 353.0(88)	4-CQA
5	14.2	366.9	MS <sup>2</sup> : 193.0(100), 366.9(47)	3-FQA
6*	14.5	353.0	MS <sup>2</sup> : 191.0(100), 352.9(10)	cis-5-CQA
7*	14.8	515.0	MS <sup>2</sup> : 191.1(100), 179.0(89), 352.9(84)	1,3-diCQA
8	15.2	179.0	MS <sup>1</sup> : 179.0(100)	CA
9*	15.7	337.0	MS <sup>1</sup> : 336.9(100), 162.7(66)	3-pCoQA
10	16.7	366.9	MS <sup>2</sup> : 191.0(100), 366.9(34)	5-FQA
11	17.3	367.0	MS <sup>2</sup> : 173.0(100), 192.8(10), 367.0(33)	4-FQA
12*	17.9	367.0	MS <sup>2</sup> : 191.0(100), 367.0(25)	cis-5-FQA
13*	18.1	529.0	MS <sup>2</sup> : 366.5(15), 193.0(100), 173.0(5)	1C-3FQA
14*	19.6	381.0	MS <sup>1</sup> : 380.9(100)	DQA
15	20.8	515.0	MS <sup>2</sup> : 191.0(100), 352.7(5)	1,5-diCQA
16*	21.2	515.0	MS <sup>2</sup> : 190.8(100), 353.0(11)	cis-1,5-diCQA
17	21.7	515.0	MS <sup>2</sup> : 191.0(100), 178.9(81), 172.9(62), 334.7(18), 353(95)	3,5-diCQA
18	22.1	515.0	MS <sup>2</sup> : 178.9(100), 172.9(73), 190.9(87), 255.0(51), 352.8(83)	3,4-diCQA
19*	22.3	515.0	MS <sup>2</sup> : 190.9(100), 179.0(43), 172.9(27), 352.5(37)	cis-3,5-diCQA
20	22.9	515.0	MS <sup>2</sup> : 172.9(100), 179.0(66), 190.9(25), 352.9(93)	4,5-diCQA
21*	23.5	515.0	MS <sup>2</sup> : 173.1(100), 178.9(88), 190.7(42), 352.9(98)	cis-4,5-diCQA
22	24.2	529.0	MS <sup>2</sup> : 190.7(100), 172.8(43), 192.6(29)	1F-5CQA
23	24.6	529.0	MS <sup>2</sup> : 191.0(100), 173.0(7), 366.9(21)	1C-5FQA
24*	25.5	515.0	MS <sup>2</sup> : 172.8(100), 178.9(59), 191.0(51), 352.6(87)	cis-4,5-diCQA
25	25.9	529.0	MS <sup>2</sup> : 173.0(100), 192.7(24), 190.8(23), 367(32), 334.7(9)	3C-4FQA
26*	26.5	542.9	MS <sup>2</sup> : 542.9(100), 380.9(58), 160.7(51)	3D-5CQA
27	26.9	529.0	MS <sup>2</sup> : 173.0(100), 178.9(92), 190.9(67), 353.0(85)	4C-5FQA
28	27.4	529.0	MS <sup>2</sup> : 172.9(100), 192.7(45), 366.5(37)	4F-5CQA
29*	28.2	542.9	MS <sup>2</sup> : 380.8(100), 160.7(57), 179.0(25), 542.8(76)	3D-5CQA
30*	28.9	529.0	MS <sup>2</sup> : 172.9(100), 178.9(29), 191.0(18), 352.8(26), 367(17)	cis-4C-5FQA
31	29.1	677.0	MS <sup>2</sup> : 515.0(100), 353.0(40), 179.0(10)	3,4,5-triCQA
32*	29.3	543.0	MS <sup>2</sup> : 381.0(100), 178.8(52), 160.7(13)	4D-5CQA
33	29.7	543.0	MS <sup>2</sup> : 193.0(100), 172.9(25), 367.0(37), 348.9(16)	3,5-diFQA
34	30.1	543.0	MS <sup>2</sup> : 349.0(100), 172.6(72)	3,4-diFQA
35	31.1	543.0	MS <sup>2</sup> : 172.9(100), 192.6(53), 366.9(40), 349.1(29)	4,5-diFQA
36	36.9	173.0	MS <sup>1</sup> : 173.0(100), 126.6(12)	Quinic acid

\*- denotes those identified for the first time in *A. annua*.

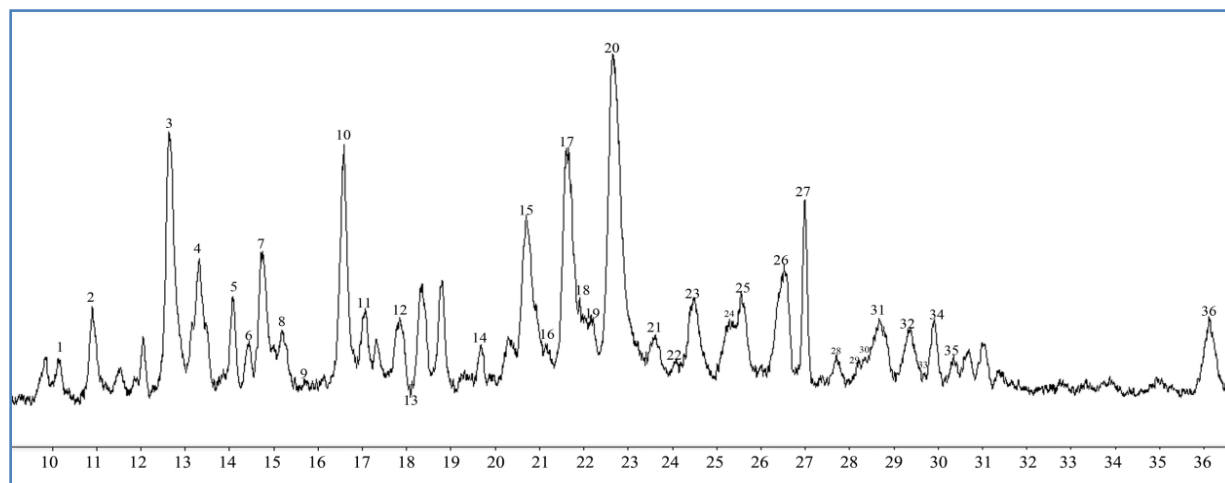


Figure 1 TIC chromatograms of chlorogenic acids extracted by ethyl alcohol in *A annua* extracts

### 3.1 Characterization of Caffeoylquinic (Mr 354), Dicafeoylquinic (Mr 516) and Tricafeoylquinic acid (Mr 678)

Compound 1, 2, 3, 4, 6 displayed a  $(MH^+)^-$  ion at  $m/z$  353.0 and four of them were assigned using the hierarchical keys previously developed as the well-known 1-CQA (1), 3-CQA (2), 4-CQA (3) and 5-CQA (4) (Jaiswal et al., 2010; Jaiswal et al., 2011). One more peak (6) presented as a minor component and displayed the  $m/z$  191.0 as the base peak in the  $MS^2$  spectrum, identical fragmentation patterns to 5-CQA, and it was suspected that it might be the cis-isomer of the corresponding 5-CQA: cis-5-CQA (Clifford et al., 2005). Compound 7, 15-21, 24 displayed a  $(MH^+)^-$  ion at  $m/z$  515.0 and showed similar fragmentation patterns as  $(M-H^+-162)^-$  ions at  $m/z$  353.0. The diCQA isomers were identified according to the base peaks and the relative intensities of secondary ions formed from  $MS^2$  fragmentation of their parent ions at  $m/z$  353.0 (Jaiswal et al., 2010; Jaiswal et al., 2011).

Compound 7 showed ( $m/z$  191 (100);  $m/z$  179 (89);  $m/z$  352.9 (84)) in the  $MS^2$  spectrum, so it was identified as 1,3-diCQA. Similarly, four diCQAs were assigned as 1,5-diCQA (15), 3,5-diCQA (17), 3,4-diCQA (18) and 4,5-diCQA (20) according to their characteristic fragmentation pattern as discussed previously (Clifford et al., 2006). Four other isomers were assigned as cis-1,5-diCQA (16), cis-3,5-diCQA (19), two cis-4,5-diCQA (21 and 24) based on their fragmentation pattern. However, it's impossible to distinguish the difference between 4-cis-5-trans-diCQA and 4-trans-5-cis-diCQA or cis-4,5-diCQA under these circumstance. The unequivocally identification of these compounds could only be established by NRM studies (Clifford et al., 2006).

Compound 31 displayed a  $(MH^+)^-$  ion at  $m/z$  677.0 and  $(M-H^+-162)^-$   $m/z$  515.0. In the  $MS^2$  spectrum, it displayed  $m/z$  353.0  $(M-H^+-324)^-$ ,  $m/z$  179.0 (caffeoyl acid- $H^+$ ) $^-$ . Based on the

information available in literature, this isolated compound was identified as 3,4,5-triCQA (Jaiswal et al., 2011).

### 3.2 Characterization of feruoylquinic acids (Mr 368), diferuoylquinic acids (Mr 544)

Compound 5, 10, 11, 12 displayed a  $(MH^+)^-$  ion at  $m/z$  367.0 and three of them were assigned 3-FQA (5), 5-FQA (10) and 4-FQA (11) name by using the hierarchical keys previously developed (Jaiswal et al., 2010).

Compound 12 displayed the  $m/z$  191.0 as the base peak in the  $MS^2$  spectrum, identical fragmentation patterns to 5-FQA, and it might be the cis-isomer of the corresponding 5-FQA: cis-5-FQA. The three isomers diferuloylquinic acid were identified as 3,5-diFQA (33), 3,4-diFQA (34) and 4,5-diFQA (35) based on the previous literature (Jaiswal et al., 2010; Jaiswal et al., 2011).

### 3.3 Characterization of dimethoxy-cinnamoylquinic acid (Mr 382), caffeoyl- dimeth-oxycinnamoylquinic acids (Mr 544)

Compound 14 displayed a  $(MH^+)^-$  ion at  $m/z$  381.0 in negative ion mode ESI-MS, suggesting one dimethoxy cinnamoylquinic acid according to previous studies conducted by Jaiswal et al. (2010). Compound 26, 29, 32 yielded molecular ion at  $m/z$  543.0 and they produced an  $MS^2$  base peak at  $m/z$  381.0, suggesting caffeoyl dimethoxycinnamoylquinic acids of 3D-4CQA (26), 3D-5CQA (29) and 4D-5CQA (32) (Jaiswal et al., 2010).

3.4 Characterization of caffeoyl-feruloylquinic acids (Mr 530)  
Compound 13, 22, 23, 25, 27, 28, 30 displayed a  $(MH^+)^-$  ion at  $m/z$  529.0. All of these seven compounds showed  $MS^2$  base peaks at either  $m/z$  353.0 (CQA- $H^+$ ) $^-$  or  $m/z$  367.0 ((FQA- $H^+$ ) $^-$ ).

According to Jaiswal et al. (2010), the peak 13 yielded an MS<sup>2</sup> base peak at m/z 366.5. From MS<sup>2</sup>, the base peak at m/z 193.0 indicated the presence of feruloyl residue at the 3-position (Clifford et al., 2006). The lower intensity of the MS<sup>2</sup> secondary peak at m/z 173.0 suggesting that isolated compound was 1C-3FQA. According to hierarchical key, they were identified as 1F-5CQA (22), 1C-5FQA (23), 3C-4FQA (25), 4C-5FQA (27), 4F-5CQA (28), Cis-4C-5FQA (30).

Compound 9 displayed a (MH<sup>+</sup>) ion at m/z 337.0 and produced ions at m/z 162.7. According to Jaiswal et al. (2010) this isolated compound was identified as 3-coumaroylquinic acid.

The simple phenolic acids were also detected in the extraction. Compound 8 displayed a (M-H<sup>+</sup>) ion at m/z 179.0. In comparison to chemical marker, it was identified as caffeic acid. Compound 36 displayed a (MH<sup>+</sup>-H<sub>2</sub>O)<sup>+</sup> ion at m/z 173.0 and produced ion at m/z 126.6. According to the observation made by Jaiswal et al. (2010) it was identified as Quinic acid.

A total of 18 chlorogenic acids compounds were identified or tentatively characterized in the methanol extract of *A. Annua* (Gouveia & Castilho, 2013). Teresa Carbonara characterized and quantified 16 chlorogenic acids compounds in *A.annua* tea

(IUPC, 1976). Furthermore, 40 hydrocinnamic acid derivatives and glycosylated flavonoids were isolated by the acetone extracts (Robinson et al., 1996). As chlorogenic acid has different solubility in different solvents, the numbers of peaks in methanol and acetone extracts were relatively less. Acetone found effective more for artemisinin, but it is ineffective for chlorogenic acids of *A. annua*. Optimized separation methods for extracting chlorogenic acids have been applied in this study. Ethanol : water (70:30, v/v) was chosen as the best solvent to improve the separation degree and solubility. This study found that the content of moncaffeoylquinic acids and dicaffeoylquinic acids in *A. annua* L. were much higher than other compounds. Therefore, the components which separated from the residue can be used for medicine, food and other purposes.

### 3.5 Purification by Polyamide and Sephadex LH-20

65.86 mg dicaffeoylquinic acid was obtained after concentration with 15g *A. annua*. residue and the extraction ratio is 43.90%. After first purification by polyamide, 44.02mg dicaffeoylquinic acid was obtained with a recovery rate of 66.84%. The purity was 45.7% after collecting dicaffeoylquinic acid liquid and then freeze drying it.

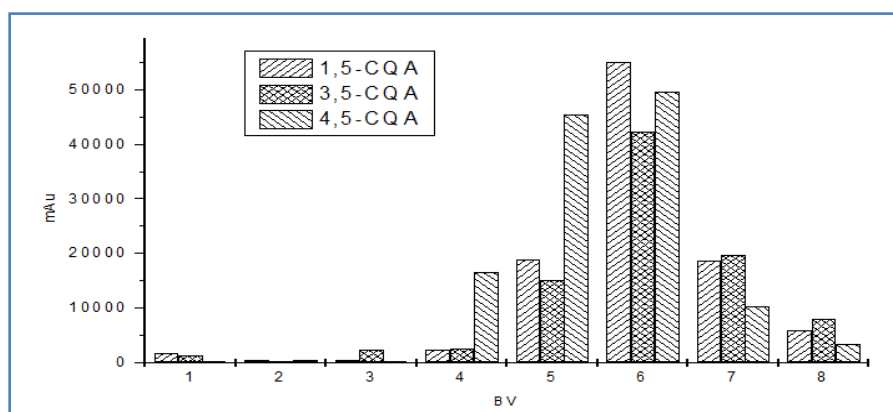


Figure 2 The desorption curve of polyamide

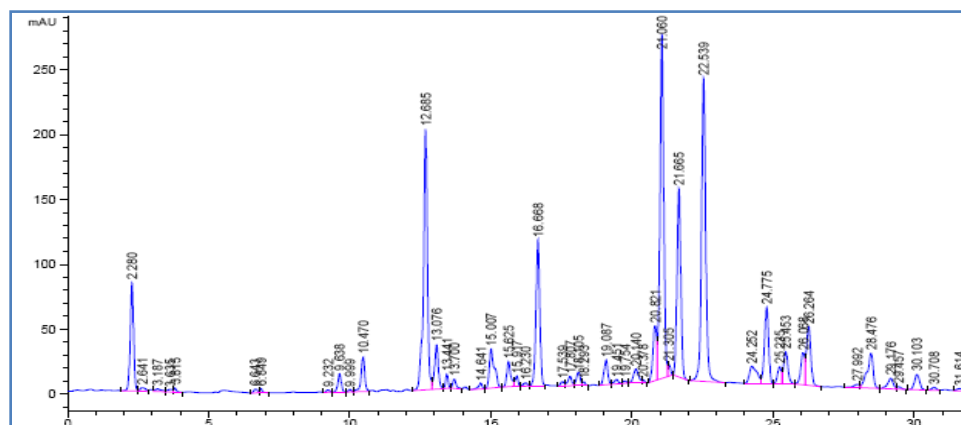


Figure 3 HPLC of chlorogenic acids extracted by ethyl alcohol in *A. annua* residue extracts

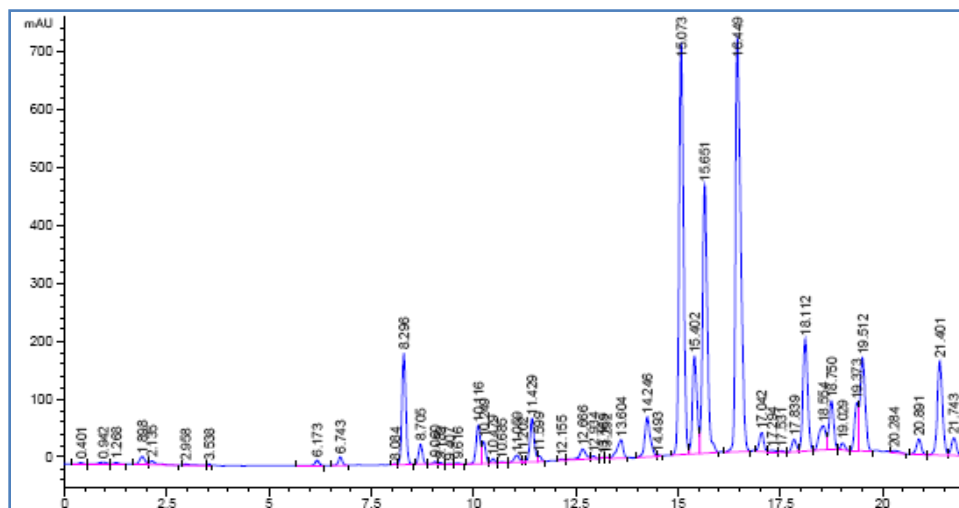


Figure 4 HPLC of polyamide desorption of CQA from *A. annua* residue extracts

Figure 3 shows that chlorogenic acids are rich in *A. annua* residue extracts, of which the single coffeoylquinic acid was mainly 5-coffeoylquinic acid, dicaffeoylquinic acid liquid was mainly 1,5- dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid.

The adsorption rate of polyamide to dicaffeoylquinic acid liquid is 89.4% with its desorption rate 99.5%. The desorption rate of polyamide to dicaffeoylquinic acid liquid is higher than the single coffeoylquinic acid (80.9%). So polyamide can be chosen as the most appropriate material to separate dicaffeoylquinic acid and single coffeoylquinic acid.

Figure 5 shows that SephadexLH-20 can purify dicaffeoylquinic acid effectively and the purity reached 85.7% with a yield of 53.4% after the secondary purification by Sephadex LH-20. On the basis of identification chlorogenic acids, dicaffeoylquinic acids isolated from *A. annua* residue, can produce artemisinin and which is new sources of raw materials for the production of dicaffeoylquinic acids. It was a new way to get two types of high value-added products from the same raw material, especially for the production of high purity dicaffeoylquinic acids. It has great practical significances for making full use of *A. annua* and provides broad prospects for development for *A. annua* L. industry.

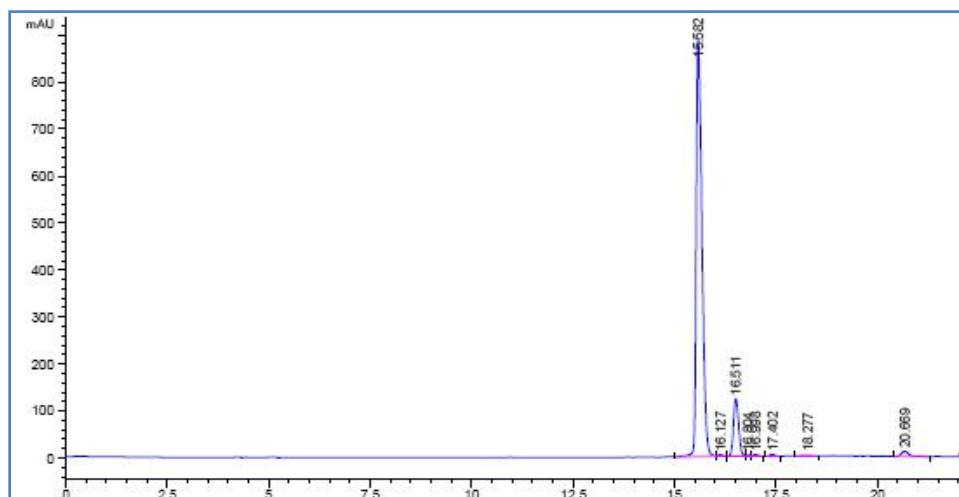


Figure 5 HPLC of CQA from extracts of *A. annua* residue purified by Sephadex LH-20

## Conclusions

In this study, a reliable and powerful method was established for the comprehensive and accurate identification of chlorogenic acids in *A. annua*. The LC/MS<sup>n</sup> system used in this work proved an excellent tool for identifying structures of different components, particularly for isomers. Thirty-six chlorogenic acids were successfully identified by MS spectra compared with standards or literature data. Fifteen were reported first time from *A. annua*. The purification method for dicaffeoylquinic acid provides effective use of chlorogenic acids, which were previously wasted in *A. annua* residue. Therefore, this work will promote the sustainable use of *A. annua* resources with great economic, social and environmental benefits.

## Acknowledgments

This work was supported in part by a research grant from Key Projects of Natural Science Research in Universities in Jiangsu Province and Project for Advantage Life Science of Jiangsu Province and Open Project of Jiangsu Key Laboratory for Biodiversity and Biotechnology.

## Conflict of Interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

## References

- Carbonara T, Pascale R, Argentieri MP, Papadia P, Fanizzi FP, Villanova L, Avato P (2012) Phytochemical analysis of a herbal tea from *Artemisia annua* L. *Journal of Pharmaceutical and Biomedical Analysis* 62:79-86. doi:10.1016/j.jpba.2012.01.015.
- Clifford MN, Johnston KL, Knight S, Kuhnert N (2003) Hierarchical Scheme for LC-MS<sup>n</sup> Identification of Chlorogenic Acids. *Journal of Agricultural and Food Chemistry* 51: 2900-2911. doi: 10.1021/jf026187q.
- Clifford MN, Knight S, Kuhnert N (2005) Discriminating between the Six Isomers of Dicaffeoylquinic Acid by LC-MS<sup>n</sup>. *Journal of Agricultural and Food Chemistry* 53: 3821-3832. doi: 10.1021/jf050046h.
- Clifford MN, Knight S, Surucu B, Kuhnert N (2006) Characterization by LC-MS<sup>n</sup> of Four New Classes of Chlorogenic Acids in Green Coffee Beans: Dimethoxycinnamoylquinic Acids, Diferuloylquinic Acids, Caffeoyl-dimethoxycinnamoylquinic Acids, and Feruloyl-dimethoxycinnamoylquinic Acids. *Journal of Agricultural and Food Chemistry* 54: 1957-1969. doi: 10.1021/jf0601665.
- Clifford MN, Marks S, Knight S, Kuhnert N (2006) Characterization by LC-MS<sup>(n)</sup> of four new classes of p-coumaric acid-containing diacyl chlorogenic acids in green coffee beans. *Journal of Agricultural and Food Chemistry* 54: 4095-4101. doi: 10.1021/jf060536p.
- Clifford MN (2000) Chlorogenic acids and other cinnamates – nature, occurrence, dietary burden, absorption and metabolism. *Journal of the Science of Food and Agriculture* 80: 1033-1043. doi: 10.1002/(SICI)1097-0010(20000515)80:7<1033::AID-JSFA595>3.0.CO;2-T.
- Ferreira JF, Luthria DL, Sasaki T, Heyerick A (2010) Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. *Molecules* 15: 3135-3170. doi: 10.3390/molecules15053135.
- Gouveia SC, Castilho PC (2013) *Artemisia annua* L.: Essential oil and acetone extract composition and antioxidant capacity. *Industrial Crops and Products* 45: 170-181. doi:10.1016/j.indcrop.2012.12.022.
- Gu R, Dou G, Wang J, Dong J, Meng Z (2007) Simultaneous determination of 1,5-dicaffeoylquinic acid and its active metabolites in human plasma by liquid chromatography-tandem mass spectrometry for pharmacokinetic studies. *Journal of Chromatography B* 852: 85-91. doi:10.1016/j.jchromb.2006.12.055.
- Han J, Ye M, Qiao X, Xu M, Wang BR, Guo DA (2008) Characterization of phenolic compounds in the Chinese herbal drug *Artemisia annua* by liquid chromatography coupled to electrospray ionization mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis* 47: 516-525. doi:10.1016/j.jpba.2008.02.013.
- IUPAC (1976) Nomenclature of cyclitols. *Biochemistry* 153: 23-31.
- Jaiswal R, Deshpande S, Kuhnert N (2011) Profiling the chlorogenic acids of *Rudbeckia hirta*, *Helianthus tuberosus*, *Carlina acaulis* and *Symphyotrichum novae-angliae* leaves by LC-MS<sup>n</sup>. *Phytochemical Analysis* 22: 432-441. doi: 10.1002/pca.1299.
- Jaiswal R, Sovdat T, Vivan F, Kuhnert N (2010) Profiling and characterization by LC-MS<sup>n</sup> of the chlorogenic acids and hydroxycinnamoylshikimate esters in maté (*Ilex paraguariensis*). *Journal of Agricultural and Food Chemistry* 58: 5471-5484. doi: 10.1021/jf904537z.
- Lai JP, Lim YH, Su J, Shen HM, Ong CN (2007) Identification and characterization of major flavonoids and caffeoylquinic acids in three Compositae plants by LC/DAD-APCI/MS. *Journal of Chromatography B* 848: 215-225. doi:10.1016/j.jchromb.2006.10.028.
- Lubbe A, Seibert I, Klimkait T, van der Kooy F (2012) Ethnopharmacology in overdrive: the remarkable anti-HIV

activity of *Artemisia annua*. Journal of Ethnopharmacology 141:854-859. doi:10.1016/j.jep.2012.03.024

Mueller MS, Karhagomba IB, Hirt HM, Wemakor E (2000) The potential of *Artemisia annua* L. as a locally produced remedy for malaria in the tropics: agricultural, chemical and clinical aspects. Journal of Ethnopharmacology 73:487-493. doi:10.1016/S0378-8741(00)00289-0.

Robinson WE, Reinecke MG, Abdel-Malek S, Jia Q, Chow SA (1996) Inhibitors of HIV-1 replication that inhibit HIV integrase. Proceedings of the National Academy of Sciences of the United States of America 93:6326-6331.

Willcox ML, Burton S, Oyweka R, Namyalo R, Challand S, Lindsey K (2011) Evaluation and pharmacovigilance of projects promoting cultivation and local use of *Artemisia annua* for malaria. Malaria Journal 10: 84. doi:10.1186/1475-2875-10-84.