Simultaneous determination three phytosterol compounds, campesterol, stigmasterol and daucosterol in *Artemisia apiacea* by high performance liquid chromatography-diode array ultraviolet/visible detector

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Submitted: 23-04-2014 Revised: 29-04-2014 Published: 12-03-2015

ABSTRACT

Background: *Artemisia apiacea* is a traditional herbal medicine using treatment of eczema and jaundice in Eastern Asia, including China, Korea, and Japan. **Objective:** An accurate and sensitive analysis method using high performance liquid chromatography-diode array ultraviolet/visible detector and liquid chromatography–mass spectrometry for the simultaneous determination of three phytosterol compounds, campesterol, stigmasterol and daucosterol in *A. apiacea* was established. **Materials and Methods:** The analytes were separated on a Shiseido C_{18} column (5 μm, 4.6 mm I.D. ×250 mm) with gradient elution of 0.1% trifluoroacetic acid and acetonitrile. The flow rate was 1 mL/min and detection wavelengths were set at 205 and 254 nm. **Results:** Validation of the method was performed to demonstrate its linearity, precision and accuracy. The calibration curves showed good linearity (R^2 > 0.9994). The limits of detection and limits of quantification were within the ranges 0.55–7.07 μg/mL and 1.67–21.44 μg/mL, respectively. And, the relative standard deviations of intra- and inter-day precision were <2.93%. The recoveries were found to be in the range of 90.03–104.91%. **Conclusion:** The developed method has been successfully applied to the analysis for quality control of campesterol, stigmasterol and daucosterol in *A. apiacea.*

Key words: *Artemisia apiacea,* high performance liquid chromatography-diode array ultraviolet/visible detector, liquid chromatography–mass spectrometry, simultaneous determination

Access this article online

Website: www.phcog.com

DOI: 10.4103/0973-1296.153082

Quick Response Code:

INTRODUCTION

For 1,000s of years, herbal product is used for prevention and treatment of various diseases in many countries. These herbal medicines have lower toxicity with high compliance and as single components, these exhibit therapeutic effects for multiple diseases.[1] Therefore, herbal products have gained increasing popularity and have become a popular form of healthcare.[2,3]

Artemisia species are genus of the family *Compositae* consisting of more than 350 species. *Artemisia apiacea* is widely distributed at wasteland and river beaches of Korea, China and Japan. *A. apiacea* traditionally used for treatment of dermatomycosis, jaundice, eczema, decubitus and alopecia.[4,5] The recent studies about the isolated compounds from *A. apiacea* show the presence of campesterol, stigmasterol, β-sitosterol, daucosterol, artemisterol, 7-methoxycoumarin, 7,8-dimethoxycoumarin, daphnetin, 7-hydroxy-8-methoxycoumarin, arteminin, artemisinin, scopoletin, protocatechualdehyde, and volatile constituents, including apicin, α-pinene and *Artemisia* ketone.[5-14] Recent studies about *Artemisia* species showed various biological activities including antimalarial, antiviral, antitumor, antipyretic, antihemorrhagic, antioxidant, antihepatitis and anticomplementary activities.[15,16] Biological activity of *A. apiacea* was reported that it has hair-growth activity.[17] *A. apiacea* was found to possess the antioxidant activity and protective property in CCl_4-intoxicated rats.[18] Furthermore, *A. apiacea* showed antiinflammation activity via nuclear factor-κB inactivation.[19]

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The phytosterol derived from vegetable oils or wood pulp has various bioactivities.[20] Phytosterols, including stigmasterol, campesterol and daucosterol were detected in *A. apiacea*. Stigmasterol has antiosteoarthritic, neutralization of viper and cobra venom, thyroid hormone and glucose regulatory activities.[21-23] In recent study, it also exhibited cognitive ameliorative effects against scopolamine-induced memory impairments in mice.[24] Campesterol have antiangiogenic activity.[25] Daucosterol exhibits immunoregulatory activity and promotion activity for the proliferation of neural stem cells.[26,27]

The natural products contained various chemical compounds such as terpenoid, flavonoid, alkaloid, saponin and phenol etc. Chemical composition of compounds was varied depending on several factors, such as plant origins, geographic area, harvest time and even storage method.[28] This variability can result in significant differences in pharmacological activity. Therefore, the establishing reliable and accurate analytical quality control method for natural products is necessary for evaluation of safety and efficacy.[2] In many approaches, high performance liquid chromatography (HPLC) is a simple and popular method for the analysis of natural products. Due to its easy operation, side suitability and high accuracy, HPLC method extensively applied to analysis of natural product over the past decades.

In this study, a simple and reliable HPLC-diode array ultraviolet/visible detector (UV/VIS) (DAD) and liquid chromatography–mass spectrometry (LC-MS) method has been established for simultaneous determination of three phytosterol compounds, campesterol, stigmasterol and daucosterol in *A. apiacea* [Figure 1].

MATERIALS AND METHODS

Plant materials

Artemisia apiacea samples were purchased from Kyung-Dong Market in Seoul (Korea) and were authenticated by Dr. Young Bae Seo, a professor of the College of Oriental Medicine, Daejeon University (Korea). A voucher specimen (no. CJ064M) was deposited at the Kangwon National University in Chuncheon (Korea).

Reagents

Campesterol, stigmasterol and daucosterol used for standard compounds were isolated from *A. apiacea* by silica gel column chromatography. Structures of isolated three compounds were determined by spectroscopic methods, including nuclear magnetic resonance spectrum and compared with spectroscopic data of the literatures.

High performance liquid chromatography-grade acetonitrile (ACN) and water were purchased from J. T. Baker (USA). Trifluoroacetic acid (TFA) was purchased from DAE JUNG (Korea). Methanol and dimethyl sulfoxide (DMSO) was purchased from DAE JUNG (Korea).

Preparation of standard and sample solutions

Standard stock solution of campesterol (500 μg/mL), stigmasterol (620 μg/mL) and daucosterol (640 μg/mL) were prepared in 2% DMSO in MeOH, respectively and stored below 4ºC. The working standard solutions were prepared by appropriate dilution of stock solutions with MeOH. These diluted working solutions were used for establishment of calibration curves.

The herb of *A. apiacea* sample was extracted by ultrasonication in 80% MeOH. The solvent was removed by vacuum evaporator and the residue was freeze-dried. The dried sample was dissolved in 5 mL 2% DMSO in MeOH. All sample solutions were filtered through a 0.45 μm membrane filter before HPLC analysis.

High performance liquid chromatography-diode array ultraviolet/visible detector analysis condition

The HPLC equipment used was Dionex system (Dionex, Germany) composed of a pump (LPG 3X00), an auto sampler (ACC-3000), a column oven (TCC-3000SD) and DAD-3000(RS). System control and data analyses were

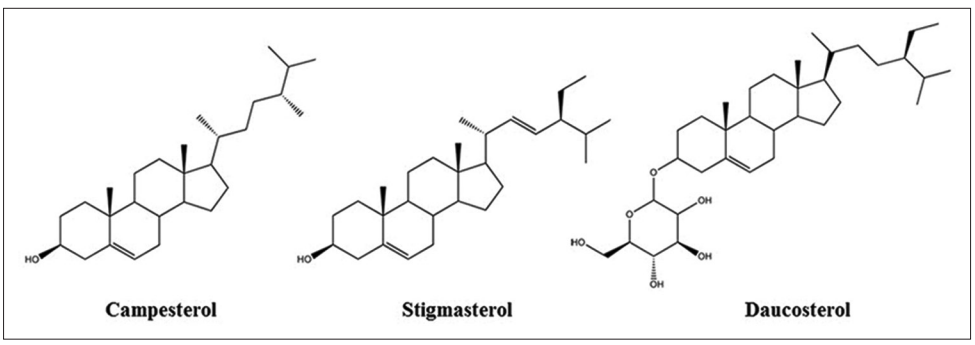

Figure 1: Chemical structure of three standard compounds of *Artemisia apiacea*

executed by Dionex Chromelon™ Chromatography Data System software (Dionex, Germany). HPLC analysis was conducted on Shiseido C_{18} column (4.6 mm I.D. × 250 mm, 5 μm pore size).

The mobile phase was composed of 0.1% TFA aqueous solution (a) and ACN (b) at a flow rate of 1.0 mL/min. The HPLC gradient profile was as follows: 10% b at 0–5 min, 10–90% b at 5–45 min, 100% b at 45–65 min. The sample injection volume was 20 μL. Four different ultraviolet (UV) spectra (205, 254, 280 and 330 nm) were selected to determination of each standard compounds and each chromatographic peaks of compounds were confirmed by comparing their retention time and UV patterns.

Liquid chromatography–mass spectrometry analysis condition

Liquid chromatography–mass spectrometry analysis was performed on TSQ Quantum Ultra Triple Stage Quadrupole Mass Spectrometer (Thermo Fisher Scientific, Germany) equipped with electrospray ionization (ESI) ion source in positive ion mode. The chromatographic separation was achieved on Shiseido C_{18} column (4.6 mm I.D. × 250 mm, 5 μm pore size) with the same elution program of HPLC-DAD analysis. The MS operating condition (positive ESI ion source) were as follows: Ion spray voltage at 4,000 V, the vaporizer temperature at 100°C, capillary temperature at 350°C, sheath gas pressure at 60 psi and aux gas pressure at 30 psi. Mass spectra were recorded in the range of m/z 250–650.

Validation of the high performance liquid chromatography method

The established HPLC method was validated according to the International Conference on Harmonization guidelines. Validation was performed in terms of linearity, precision and accuracy.[29,30]

The standard stock solution containing three marker compounds was diluted to a series of appropriate concentrations with MeOH for the construction of calibration curves. Each diluted standard solutions were analyzed in triplicate. The calibration curves were constructed by plotting the peak areas versus the concentrations of analytes and obtained regression equations. The correlation of coefficient (R^2) was used as measure of linearity. The limit of detection and limits of quantification (LOQ) values were determined at signal-to-noise (S/N) ratios of 3 and 10 times, respectively. The precision of developed method was estimated by inter- and intra-day variations. The relative standard deviation (RSD) (%) was considered as a measure of precision. Accuracy of the method was evaluated using a spike recovery test. The accurate amounts of mixed standard solution were added to *A. apiacea* sample, and then analyzed three different concentrations in triplicate, respectively. The spike recoveries were calculated by the equation;

Spike recovery (%) = (amount found − original amount)/(amount spiked) × 100 (%).

Quantification of *Artemisia apiacea* samples

Twelve *A. apiacea* samples (A1–A12) were separated by established method for quality control and each sample was analyzed in three times. A1–A6 samples were collected from Korea and A7–A12 samples were collected from China. The content of three standard compounds in *A. apiacea* samples was calculated from calibration curves of standard compounds.

RESULTS AND DISCUSSION

Pharmacological effects of *A. apiaceae* have been attributed to the bioactivity compounds. Stigmasterol, campesterol and daucosterol were important phytosterols of *A. apiaceae* and considered to be responsible for therapeutic effect, such as antiosteoarthritic, cognitive ameliorative effect, antiangiogenic activity and immunoregulatory activity.

Quality control of herbal medicine could identify and quantitate variation of compounds by cultivation environment. Quantitative analysis method of *A. apiaceae* has not yet reported. Thus, efficient analysis method of *A. apiacea* need for quality control. We applied HPLC coupled to DAD technique to establish analysis method and simultaneously determined three compounds, stigmasterol, campesterol and daucosterol.

Optimization of high performance liquid chromatography-diode array ultraviolet/visible detector condition

To development of optimal analytic condition, different HPLC parameters were tested including column type, mobile phase, elution system and detection wavelength. The analytical conditions were optimized considering with resolution, baseline and elution time. In mobile phase, TFA (0.1% in water) was added to obtain the inhibition of peak tailing and improvement in peak shape. Due to differentiation in highest detection wavelength of each standard compounds, the detection wavelength was optimized at 205 nm (daucosterol) and 254 nm (campesterol and stigmasterol) [Figure 2]. Injection volume was 20 μL. All peaks of each compound were separated successfully within 65 min. HPLC chromatogram of the three standards is shown in Figure 3a. The identification of the each compound's peaks was performed by comparing the retention time and UV spectrum. The retention time of campesterol, stigmasterol and daucosterol were 30.61, 57.62 and 60.12 min, respectively.

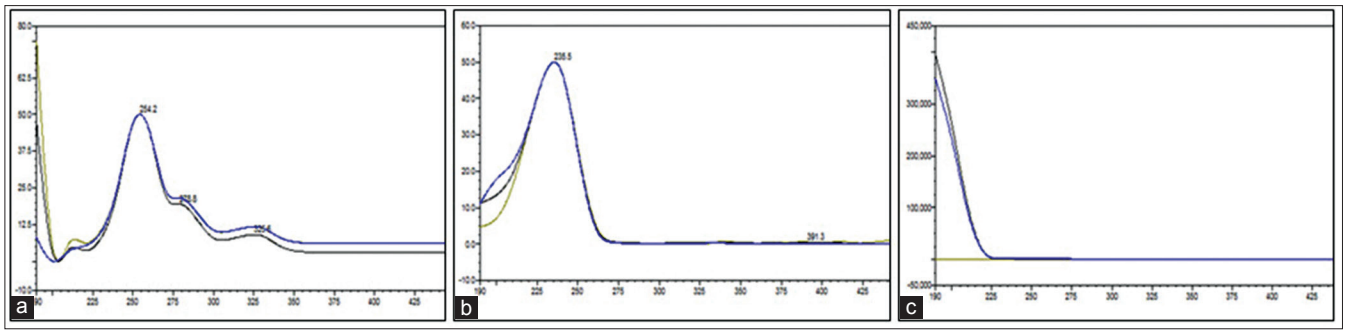

Figure 2: Ultraviolet absorption spectra of three standard compounds in *Artemisia apiacea*. Campesterol (a), stigmasterol (b) and daucosterol (c)

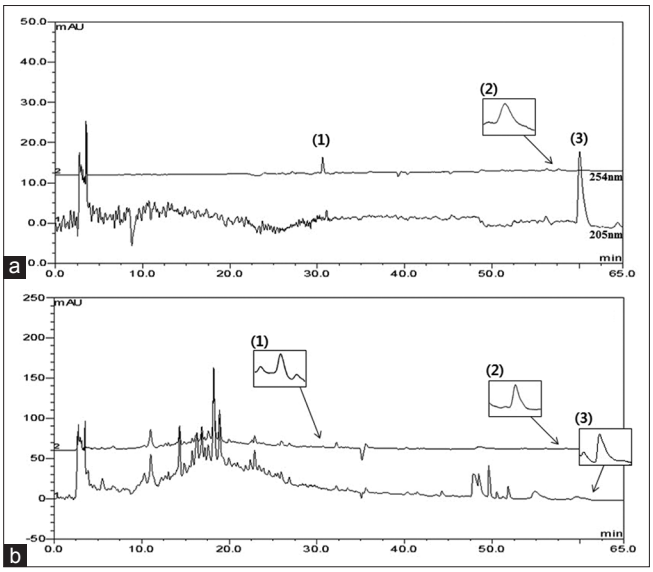

Figure 3: The high performance liquid chromatography (HPLC) chromatogram of standard compounds mixture (a) and *Artemisia apiacea* sample (b). HPLC chromatogram is detected at 205 and 254 nm. Campesterol (1), stigmasterol (2) and daucosterol (3)

Identification of standard compounds

Liquid chromatography-electrospray ionization-mass spectrometry was used to identify peaks of campesterol, stigmasterol and daucosterol obtained by HPLC-DAD analysis. MS spectra of campesterol, stigmasterol and daucosterol in positive ion mode were shown in Figure 4. In MS spectra, the fragments of three compounds exhibited at m/s 424 [M + Na] + for campesterol, m/z 413 [M + H] + for stigmatsterol and m/z 608 [M + Na + 9H] + for daucosterol.

Linearity, limits of detection and limits of quantification

Calibration curves were plotted for each standard compounds and relative regression coefficients (R^2) were calculated to validate their linearity. The calibration data of the three standard compounds showed good linearity ($R^2 > 0.9994$) in a relatively wide concentration range. The limits of detection and LOQ values of all standard compounds were in the range 0.55–7.07 μg/mL and 1.67–21.44 μg/mL, respectively [Table 1]. These results indicate that established HPLC-DAD method has good sensitivity.

Table 1: The regression data, LOD and LOQs of three compounds in *Artemisia apiacea*

Compounds	Linear range (μg/mL)	Regression equation	R^2	LOD (μg/mL)	LOQ (μg/mL)
Campesterol	20.84-500.00	y=0.0054 x−0.0431	0.9998	0.55	1.67
Stigmasterol	25.84-620.00	y=0.0008 x+0.0009[a]	0.9999	2.18	6.61
Daucosterol	26.68-640.00	y=0.0511 x−0.2023	0.9994	7.07	21.44

[a]y: Peak area; x: Amount (μg). LOD: Limits of detection; LOQ: Limits of quantification

Precision and accuracy

The precision of developed method was evaluated by repetitive intra- and inter-day test. Mixed standard solutions of three different concentrations were prepared and analyzed by developed HPLC method. The intra-day test was determined by analyzing each mixed solution five times within 1-day. For the inter-day test, the same mixed solutions were analyzed five times within each three successive days. The result of detected amount of each compound was calculated using the corresponding calibration curve. The Precision was expressed by the RSD values. As a result, the RSD values of the intra- and inter-day test were found to be within the ranges 0.41–2.85% and 0.91–2.93%, respectively. Accuracy of intra- and inter-day assay was ranged 96.60–109.57% and 97.24–107.24%, respectively. The results of the intra- and inter-day tests are shown in Table 2.

To assess the accuracy of the method, the recovery test of three standard compounds was performed. The recovery of the selected standard compounds ranged from 90.16% to 104.91%, and their RSD values were < 2.59% [Table 3]. These results showed that the established method has

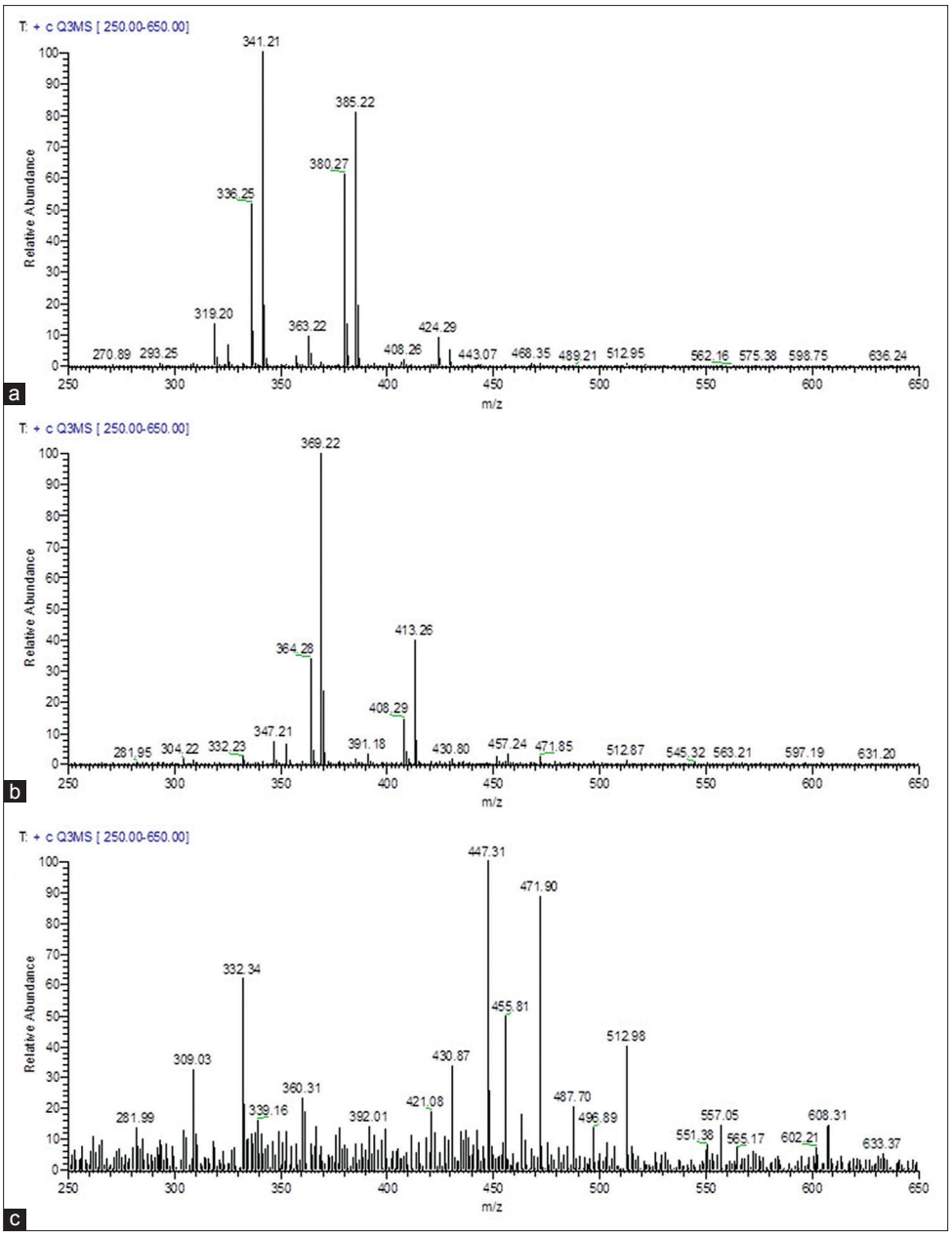

Figure 4: Mass spectrometry spectra of ion fragment of campesterol (a), stigmasterol (b) and daucosterol (c) in positive electrospray ionization

Table 2: Intra- and inter-day precision of three compounds in *Artemisia apiacea*

Compounds	Concentration (μg/mL)	Intra-day (*n*=5)			Inter-day (*n*=5)		
		Mean±SD (μg/mL)	RSD[a] (%)	Accuracy (%)	Mean±SD (μg/mL)	RSD (%)	Accuracy (%)
Campesterol	166.67	160.55±0.66	0.41	96.33	163.86±1.50	0.91	98.32
	83.34	83.40±0.40	0.48	100.07	83.79±1.18	1.41	100.54
	41.67	40.25±0.37	0.93	96.60	41.00±0.40	0.98	98.39
Stigmasterol	206.67	226.45±2.51	1.11	109.57	221.63±5.45	2.46	107.24
	103.34	112.00±2.48	2.22	108.38	108.93±1.83	1.68	105.40
	51.67	53.13±1.31	2.46	102.82	54.26±1.59	2.93	105.01
Daucosterol	213.33	208.68±5.24	2.51	97.82	207.44±1.97	0.95	97.24
	106.67	106.26±1.71	1.61	99.62	105.62±1.36	1.28	99.02
	53.36	55.36±1.58	2.85	103.76	54.10±1.47	2.72	101.38

[a]RSD. SD: Standard deviation; RSD: Relative standard deviations

a suitable precision and accuracy for the simultaneous determination of *A. apiacea*.

Artemisia apiacea sample quantitative analysis and cluster analysis

Quantitative analysis of campesterol, stigmasterol and daucosterol in twelve *A. apiacea* samples was performed under the optimized HPLC condition. HPLC-DAD chromatogram of *A. apiacea* sample is shown in Figure 3b. The content (μg/mg) was tabulated in Table 4. Table 4 shows that campesterol was in the range of 16.74–19.53 μg/mg and was highest content among three compounds. The content ranges of stigmasterol and daucosterl were 3.49–4.74 μg/mg and 2.05–2.40 μg/mg. the content of campesterol in A1 was higher than other samples. Stigmastrol and daucosterol was abundant in A6 and A1, respectively. Contents of campesterol, stigmasterol and daucosterol are different between Korea and China.

Table 3: Recovery of the 3 compounds in *Artemisia apiacea*

Compounds	Spiked amount (μg/mL)	Measured amount (μg/mL)	Recovery[a] (%)	RSD (%)
Campesterol	83.34	80.68±0.25	96.81	0.31
	41.67	41.67±0.53	100.01	1.27
	20.84	21.86±0.57	104.91	2.59
Stigmasterol	103.34	93.04±0.94	90.03	1.01
	51.67	46.96±0.56	90.88	1.20
	25.84	23.54±0.40	91.11	1.71
Daucosterol	106.67	110.61±1.89	103.69	1.71
	53.36	48.11±0.48	90.16	0.99
	26.68	25.59±0.49	95.90	1.93

[a]Recovery (%): (amount found-original amount)/amount spiked ×100 (%).
RSD: Relative standard deviations

Table 4: Contents of 3 compounds in *Artemisia apiacea* sample

Sample	Content (μg/mg)		
	Campesterol	Stigmasterol	Daucosterol
A1	19.53±0.44	4.22±0.28	2.34±0.04
A2	18.92±0.40	4.11±0.61	2.05±0.04
A3	18.06±0.41	4.14±0.54	2.40±0.04
A4	19.08±0.45	4.04±0.25	2.06±0.01
A5	19.22±0.55	4.13±0.31	2.00±0.01
A6	18.46±0.36	4.74±0.34	2.08±0.08
A7	17.85±0.50	4.32±0.25	2.24±0.05
A8	17.40±0.48	3.90±0.44	2.22±0.02
A9	17.87±0.39	3.49±0.14	2.10±0.01
A10	16.74±0.24	4.20±0.25	2.11±0.02
A11	17.11±0.65	4.17±0.41	2.10±0.01
A12	17.77±0.34	4.12±0.21	2.29±0.10

Hierarchical cluster analysis was performed to confirm homogeneous clusters using IBM SPSS Statistics (IBM, USA) 21. Cluster difference from twelve *A. apiacea* was exhibited by dendrogram [Figure 5]. We found that there are three pair samples (Cluster I, II, III). Cluster I (A2, A4 and A6) was samples collected from Korea. Two of pairs, cluster II (A7, A12, A3 and A8) and III (A9, A10 and A11) were samples collected from China exclude A3 sample. The result showed that contents of compounds in *A. apiacea* samples are different by cultivation environment such as collection region.

CONCLUSION

In this study, a reliable and accurate HPLC-DAD and LC-DAD method for the simultaneous determination of three phytosterol compounds (campesterol, stigmasterol and daucosterol) in *A. apiacea* was established. Three compounds, campesterol, stigmasterol and daucosterol confirmed by UV wavelength pattern and MS spectra. The developed method showed good linearity, precision and recovery. This developed method successfully applied to quantitative analysis of campesterol, stigmasterol and daucosterol in twelve *A. apiacea* samples. Thus, this established method can provide improvement quality control of *A. apiacea*.

ACKNOWLEDGMENTS

This research was supported by a Basic Science Research Program grant from the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0005149).

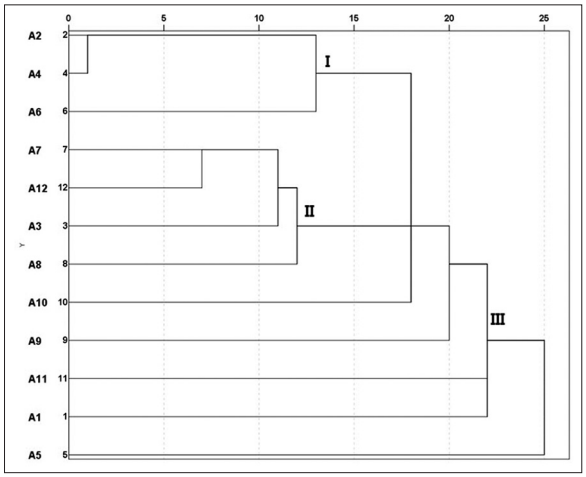

Figure 5: Dendrogram of cluster analysis for twelve *Artemisia apiacea* samples

REFERENCES

1. Jiang WY. Therapeutic wisdom in traditional Chinese medicine: A perspective from modern science. Trends Pharmacol Sci 2005;26:558-63.
2. Bent S. Herbal medicine in the United States: Review of efficacy, safety, and regulation: Grand rounds at University of California, San Francisco medical center. J Gen Intern Med 2008;23:854-9.
3. Firenzuoli F, Gori L. Herbal medicine today: Clinical and research issues. Evid Based Complement Alternat Med 2007;4:37-40.
4. Yook CS. Coloured Medicinal Plants of Korea. Seoul: Academy Press; 1989. p. 522.
5. Kim OC, Jang HJ. Volatile components of *Artemisia apiacea* herba. Agric Chem Biotechnol 1994;37:37-42.
6. Shimomura H, Sashida Y, Ohshima Y. Coumarins from *Artemisia apiacea*. Phytochemistry 1979;18:1761-2.
7. Shimomura H, Sashida Y, Ohshima Y. The chemical components of *Artemisia apiacea* Hance. More coumarins from the flower heads. Chem Pharm Bull 1980a;28:347-8.
8. Shimomura H, Sashida Y, Ohshima Y, Azuma T, Saitoh M. The chemical components of *Artemisia apiacea* Hance, components of stems and leaves. Yakugaku Zasshi 1980b;100:1164-6.
9. Yano K. Mono-and sesqui-terpenes of the essential oils from *Artemisia japonica* and *Artemisia apiacea.* Flavour Ind 1970;1:328-30.
10. Lee SJ, Kim HM, Lee S, Kim HY, Um BH, Ahn YH. Apicin, a new flavonoid from *Artemisia apiacea.* Bull Korean Chem Soc 2006;27:1225-6.
11. Kim KS, Lee S, Shin JS, Shim SH, Kim BK. Arteminin, a new coumarin from *Artemisia apiacea*. Fitoterapia 2002;73:266-8.
12. Lee S, Kim KS, Jang JM, Park Y, Kim YB, Kim BK. Phytochemical constituents from the herba of *Artemisia apiacea*. Arch Pharm Res 2002;25:285-8.
13. Lee S, Kim KS, Shim SH, Park YM, Kim BK. Constituents from the non-polar fraction of *Artemisia apiacea*. Arch Pharm Res 2003;26:902-5.
14. Lee SJ, Kim HM, Lee JM, Park HS, Lee S. Artemisterol, a new steryl ester from the whole plant of *Artemisia apiacea*. J Asian Nat Prod Res 2008;10:313-6.
15. Tan RX, Zheng WF, Tang HQ. Biologically active substances from the genus Artemisia. Planta Med 1998;64:295-302.
16. Hsu E. Reflections on the 'discovery' of the antimalarial qinghao. Br J Clin Pharmacol 2006;61:666-70.
17. Kim KS, Shim SH, Jang JM, Cheong JH, Kim BK. A study on hair-growth activity of *Artemisia apiacea* Hance. J Pharm Soc Korean 1999;43:798-801.
18. Kim KS, Lee S, Lee YS, Jung SH, Park Y, Shin KH, *et al.* Anti-oxidant activities of the extracts from the herbs of *Artemisia apiacea*. J Ethnopharmacol 2003;85:69-72.
19. Ryu JC, Park SM, Hwangbo M, Byun SH, Ku SK, Kim YW, *et al.* Methanol extract of *Artemisia apiacea* Hance attenuates the expression of inflammatory mediators via NF- ? B inactivation. Evid Based Complement Alternat Med 2013;2013:494681.
20. Tapiero H, Townsend DM, Tew KD. Phytosterols in the prevention of human pathologies. Biomed Pharmacother 2003;57:321-5.
21. Gabay O, Sanchez C, Salvat C, Chevy F, Breton M, Nourissat G, *et al*. Stigmasterol: A phytosterol with potential anti-osteoarthritic properties. Osteoarthritis Cartilage 2010;18:106-16.
22. Gomes A, Saha A, Chatterjee I, Chakravarty AK. Viper and cobra venom neutralization by beta-sitosterol and stigmasterol isolated from the root extract of *Pluchea indica* Less. (*Asteraceae*). Phytomedicine 2007;14:637-43.
23. Panda S, Jafri M, Kar A, Meheta BK. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. Fitoterapia 2009;80:123-6.
24. Park SJ, Kim DH, Jung JM, Kim JM, Cai M, Liu X, *et al.* The ameliorating effects of stigmasterol on scopolamine-induced memory impairments in mice. Eur J Pharmacol 2012;676:64-70.
25. Choi JM, Lee EO, Lee HJ, Kim KH, Ahn KS, Shim BS, *et al.* Identification of campesterol from *Chrysanthemum coronarium* L. and its antiangiogenic activities. Phytother Res 2007;21:954-9.
26. Lee JH, Lee JY, Park JH, Jung HS, Kim JS, Kang SS, *et al.* Immunoregulatory activity by daucosterol, a beta-sitosterol glycoside, induces protective Th1 immune response against disseminated Candidiasis in mice. Vaccine 2007;25:3834-40.
27. Jiang LH, Yang NY, Yuan XL, Zou YJ, Zhao FM, Chen JP, *et al.* Daucosterol promotes the proliferation of neural stem cells. J Steroid Biochem Mol Biol 2014;140:90-9.
28. Fabio F, Luigi G. Herbal medicine today: Clinical and research issues. Evid Based Complement Alternat Med 2007;4:37-40.
29. Lee B, Weon JB, Yun BR, Lee J, Eom MR, Ma CJ. Simultaneous determination of five major compounds in the traditional medicine Pyeongwee-San by high performance liquid chromatography-diode array detection and liquid chromatography-mass spectrometry/mass spectrometry. Pharmacogn Mag 2014;10:S22-9.
30. Ying L, Si-Wang W, Hong-Hai T, Wei C. Simultaneous quantification of six main active constituents in Chinese Angelica by high-performance liquid chromatography with photodiode array detector. Pharmacogn Mag 2013;9:114-9.

Cite this article as: Lee J, Weon JB, Yun BR, Eom MR, Ma CJ. Simultaneous determination three phytosterol compounds, campesterol, stigmasterol and daucosterol in *Artemisia apiacea* by high performance liquid chromatography-diode array ultraviolet/visible detector. Phcog Mag 2015;11:297-303.

Source of Support: Nil, **Conflict of Interest:** None declared.