

Original Research Article

Simultaneous Determination of Two Isomers of Asarone in *Piper sarmentosum* Roxburgh (Piperaceae) Extracts using Different Chromatographic Columns

Mohd Shahrul Ridzuan Hamil¹, Abdul Hakeem Memon¹, Amin Malik Shah Abdul Majid² and Zhari Ismail^{1*}

¹Department of Pharmaceutical Chemistry, ²Department of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden 11800, Pulau Pinang, Malaysia

*For correspondence: **Email:** ismailzhari@gmail.com; **Tel:** +604653888 ext 4222; **Fax:** +604-6570017

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Abstract

Purpose: To develop a rapid and reliable reverse phase high performance liquid chromatography (RP-HPLC) method to quantify the two isomers of asarones in *P. sarmentosum* extracts using two different columns under similar analytical conditions.

Methods: Two isomers, α - and β -asarone, were analyzed using two types of C-18 columns with 0.1 % orthophosphoric acid: acetonitrile: methanol (50: 40: 10) as mobile phase. The developed method was applied to determine the contents of α - and β -asarone in extracts of different parts of *P. sarmentosum*.

Results: Column A retention times for the elution of α - and β -asarone were 11.890 ± 0.008 and 10.80 ± 0.004 min, respectively, and were significantly shorter than those of column B (15.110 ± 0.024 and 13.290 ± 0.018 , respectively, $p < 0.001$). Column B showed better resolution (1.82 ± 0.025 of the isomers than column A (1.10 ± 0.01 , $p < 0.001$). Both columns showed comparable sensitivity, precision and selectivity of the compounds investigated. α -Asarone level was in the range 0.36 - 5.14 % in ethanol and 50 % ethanol extracts, but absent in all water extracts. β -Asarone occurred in the range of 0.01 - 0.15 % in ethanol and 50 % ethanol extracts but was absent in all water extracts of *P. sarmentosum*.

Conclusion: The results indicate that the developed method is a suitable quality assurance method for determining α - and β -asarone isomers in herbal extracts and food preparations.

Keywords: α - Asarone, Isomers, *Piper sarmentosum*, Herbal extracts, Retention time

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INTRODUCTION

Piper sarmentosum Roxburgh (family: Piperaceae) is a herbaceous shrub, and is most widely distributed in Southeast Asia including Malaysia, Cambodia, Laos, Philippines, Myanmar, Thailand, Vietnam and China [1]. The plant has been investigated for a number of pharmacological activities such as hypoglycemic [2], anti-amoebic [3], antibacterial [4],

neuromuscular blocking [5], anti-malarial [6], antioxidant [7], anti-tuberculosis [8], anticancer [9] and anti-angiogenic activity [10].

The chemical constituents identified from the plant include (2E, 4E)-N-isobutyldecadienamide, N-(2-phenylpropanoyl) pyrrole, sarmentine, sarmentosine, 1-(3,4-methylenedioxyphenyl)-(1E)-tetradecane, 1-allyl-2,6-demethoxy-3,4-methylenedioxybenzene, asaricin, α -asarone, γ -

asarone, hydrocinnamic, oxalic acids, sitosterol and naringenin [11,12]. Asarones are mostly found in isomers i.e. cis- and trans-forms. The trans-asarone or α -asarone chemically known as (E)-1,2,4-trimethoxy-5-(prop-1-en-1-yl) benzene while cis-asarone or β -asarone known as (Z)-1,2,4-trimethoxy-5-(prop-1-en-1-yl)benzene.

Apart from showing biological activities, i.e. anthelmintic, pesticidal and neuroprotective [13, 14], both α - and β -asarones are also known as carcinogenic and genotoxic compounds [15,16]. β -asarone possesses toxic and sterilizing effects [17] whereas α -asarone showed greater cytotoxic effect [18].

In food and herbal industries it is required that the quantity of asarones be limited to ascertain the products safety [19-21]. Therefore, analysis of these asarone isomers in *P. sarmentosum* is important for its quality control in herbal remedy preparation.

This study was conducted in order to develop and validate a simple and robust RP-HPLC method for the quantification of α - and β -asarones in different extracts of *P. sarmentosum* without using large quantity of organic solvents and special stationary phase or modifiers for the separation of asarone isomers.

EXPERIMENTAL

Preparation of raw material

P. sarmentosum plant was collected from Batu Gajah, Malaysia. The plant samples were identified by Dr. Rahmad Zakaria from School of Biological Sciences, Universiti Sains Malaysia and the specimen vouchers were deposited at School of Biological Sciences, Universiti Sains Malaysia with voucher number USM/Herbarium/11481. The plant samples which were divided into four different parts (leaf, stem, root and fruit) were ground into powder form using an electric grinder SM-100 (Retsch, Germany).

Chemicals and reagents

All solvents used were of HPLC grade and were acquired from Merck Sdn. Bhd. (Selangor, Malaysia). Authentic α - and β -asarones were obtained from Sigma-Aldrich (M) Sdn. Bhd (Kuala Lumpur, Malaysia). Two C-18 columns (250 x 4.6 mm, 5 μ m); (A) ZORBAX Eclipse Plus Phenyl-Hexyl and (B) ZORBAX Eclipse Plus were purchased from Agilent Technologies (USA).

Preparation of extracts

All extracts were prepared by maceration of powdered material with 99 % ethanol, 50 % ethanol and water solvents at 60 °C for 48 h. All extracts were filtered; the solvent was evaporated to dryness using rotary evaporator R-100 (Buchi, Switzerland) at 40 °C. Extracts were labeled as PSL-E, PSL-EW, PSL-W, PSS-E, PSS-EW, PSS-W, PSR-E, PSR-EW, PSR-W, PSF-E, PSF-EW and PSF-W, respectively, where PS = *P. sarmentosum*, L = leaves, S = Stem, R = roots, F = fruits, EW = ethanol: water (50: 50), W = water, E = ethanol.

Instrumentation and HPLC conditions

All experiments were performed using an Agilent Technologies 1260 infinity (USA) HPLC system with UV detector, quaternary pump, online degasser, and column incubator. Separation was done on two different C-18 columns (250 x 4.6 mm, 5 μ m) (A) ZORBAX Eclipse Plus Phenyl-Hexyl, C-18 column and (B) ZORBAX Eclipse Plus C-18 made by Agilent Technologies (USA), using an isocratic mobile phase consisting 0.1 % phosphoric acid : acetonitrile : methanol 50 : 40 : 10, the flow rate was set at 1 ml/min, column temperature was maintained at 30 °C, and detection was performed at 210 nm. Injection volume was 10 μ L and data acquisition was carried out by Agilent ChemStation software for LC systems.

Preparation of the standard mixture

A stock solution of a mixture of α - and β -asarone was prepared at 1 mg/mL in HPLC grade methanol, and serial dilutions were prepared in the range 0.44 – 250 μ g/mL. *P. sarmentosum* extracts were also prepared at 10 mg/mL in the same solvent and were further diluted to 1 mg/mL with methanol. Both, samples and standard stock solution were filtered through 0.45 μ m syringe filters.

Method validation

The proposed method was validated on both columns according to the ICH guidelines [22]. The following validation characteristics were evaluated: selectivity, linearity, precision, accuracy and the limits of detection and quantification (LOD and LOQ).

Linearity

Linearity was determined by injecting 10 μ L of the standard mixture in the concentration range

0.448 - 250 µg/mL. The calibration curves were obtained by plotting peak area versus concentration, and linearity (R²) was determined by regression analysis of the calibration graphs.

Selectivity

The selectivity of method was determined by comparing the retention time of target compounds obtained in the sample extracts with their reference counterparts, and by spiking the extracts with known concentration of the reference compounds.

Precision

Precision was determined as the coefficient of variation (% CV) of peak area and retention time. The standard mixture was analyzed at 5 concentrations in the range 7.81 - 125 µg/mL, and the intraday, interday and intermediate precisions were determined (n = 5).

Accuracy

Accuracy was determined as a percentage recovery of α- and β-asarone at 3.16, 6.25 and 12.5 µg/mL added to the ethanol extract at 1000 µg/mL. The peak area corresponding to the compounds in the ethanol extract (B), the individual reference compounds (C) and their combinations (A) were recorded. The percentage recovery was then calculated using the following formula: % Recovery = ((A - B) / C) × 100. The results are presented average ± SD (n = 3).

Limits of detection (LOD) and of quantification (LOQ)

The LOD and LOQ were calculated from the slope and standard deviation method as in Eqs 1 and 2.

$$\text{LOD} = (3.3 \times \delta) / S \dots\dots\dots (1)$$

$$\text{LOQ} = (10 \times \delta) / S \dots\dots\dots (2)$$

Where δ = is the standard deviation of the Y intercept of the linear regression equation. S = the slope of the linear regression equation [22].

Determination of α- and β-asarone concentration in *P. sarmentosum* extracts

Three different extracts from 4 parts of *P. sarmentosum* were analyzed for asarone contents (Figures 2 and 3). Concentrations of α- and β-asarones were calculated by applying the linear regression equations of the reference compounds. The identifications of asarones were

done by comparing the retention times to those of standards, as well as adding the individual standard to the samples. *P. sarmentosum* extracts (10 µL) were injected at 1000 µg/mL, and the peak areas corresponding to α- and β-asarone were recorded. The linear regression equations of the standard calibration curves were applied to calculate the concentrations of the marker compounds, and the results are presented as mean % wt/wt using the formula: % wt/wt = (the found concentration/1000 µg/mL) × 100

Statistical analysis

Statistical calculations were carried out using SPSS 20.0 software package. Independent sample t-test was applied and the differences were considered significant at *p* < 0.05.

RESULTS

It is reported that trans/α- isomers stays for a longer time in column as compared cis/β-isomer [23]. So in the present study, it was observed that with each column the cis/β-asarone had a lower retention time than the trans/α-asarone. In terms of column comparison, we found that column A has significantly shorter retention time for the elution of α- and β-asarone which were 11.89 ± 0.008 min and 10.80 ± 0.004 min compared to column B 15.11 ± 0.024 min and 13.29 ± 0.018 min, (Figure 1). The dimethylphenylhexylsilane stationary phase in column A was able to elute the target compounds faster than column B which contained dimethyl-n-octadecylsilane stationary phase. Column B showed better resolution (1.82 ± 0.025 min difference between) of the isomers as compared to column A (1.10 ± 0.01 min difference) which was statistically significant, *p* < 0.001. We also found that both columns showed comparable sensitivity, precision and selectivity of the compounds investigated. Column A had higher sensitivity with lower LOD and LOQ compared to column B.

Selectivity

The selectivity of the method was determined by comparing the retention times of α- and β-asarone obtained in the sample extracts with those of the reference compounds on both columns. The retention time of α- and β-asarone was 11.8 ± 0.002 and 10.79 ± 0.021 min on column A while it was 15.09 ± 0.01 and 13.26 ± 0.02 min for column B respectively.

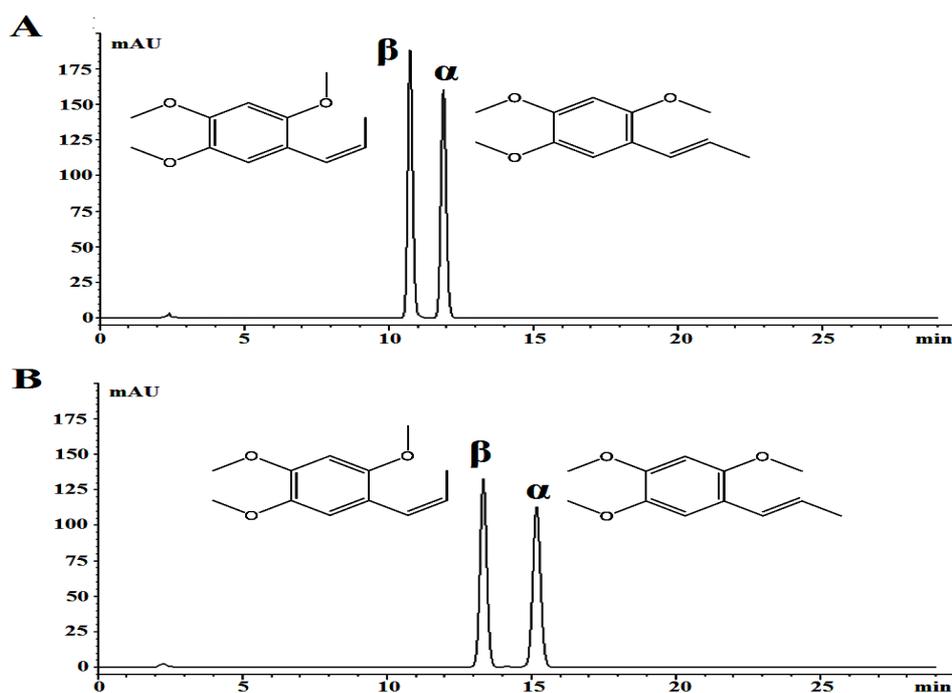


Figure 1: HPLC chromatograms of α - and β -asarone at 210 nm. A = ZORBAX Eclipse Plus Phenyl-Hexyl C-18 column, B = ZORBAX Eclipse Plus C-18 column

Their counterparts in *P. sarmentosum* extracts were eluted at 11.81 ± 0.002 and 10.8 ± 0.03 min on column A while they eluted 15.28 ± 0.67 and 13.33 ± 0.03 min, respectively, on column B. Spiking the extracts with α - and β -asarone increased the peak areas of the compounds without any shift in the retention times and appearance of extra peaks. This further confirmed the identity of the compounds and the method's selectivity on both columns.

Linearity

Linearity was presented in terms of the regression coefficient (R^2) of the regression equations of reference compounds (R^2) was 0.999 ± 0.001 for both α - and β -asarone which indicate good linearity of the proposed method using two different columns.

Precision

The precision was calculated in terms of the % CV of the retention time and peak area (Table 1 and 2). The α - and β -asarone were eluted at 11.800 ± 0.002 and 10.790 ± 0.021 min on column A with CV of < 0.05 %, and 15.09 ± 0.01 min and 13.26 ± 0.02 min with a % CV of < 0.42 % on column B, respectively. The % CV of the peak areas were calculated in the concentration range 7.81 - 125 $\mu\text{g/mL}$ with an average of < 0.05 and < 0.20 % on columns A and B

respectively. These results indicated good reproducibility of retention times and peak areas on both columns.

Accuracy and recovery

The results are presented as recovery of the reference compounds, viz, 31.25, 62.5, and 125 $\mu\text{g/mL}$ ranging from 97 ± 0.50 and 99 ± 0.5 % for columns A and B, respectively.

LOD and LOQ

The LOD and LOQ of α - and β -asarone with column A and B were calculated and presented in Table 2.

α - and β -asarone in extracts of *P. sarmentosum*

Asarone contents in the extracts of different parts of *P. sarmentosum* varied between the samples and are shown in Table 3. The α -asarone was found from 0.36 - 5.14 % in ethanol and 50 % ethanol extracts of *P. sarmentosum* parts and absent in all water extracts. β -asarone was found in lesser amounts in the ranges from 0.01 - 0.15 % in ethanol and 50 % ethanol extract and was absent in all water extracts of different parts of *P. sarmentosum*.

Table 1: Precision analysis of α - and β -asarone using both columns

Precision	Conc. ($\mu\text{g/mL}$)	Column A		α -asarone		Column B				
		PA (mAU*S) AV \pm SD	% CV	RT (min) AV \pm SD	% CV	PA (mAU*S) AV \pm SD	% CV	RT (min) AV \pm SD	% CV	
Intraday	125	8021.0 \pm 1.42	0.02	11.89 \pm 0.005	0.04	8005.16 \pm 3.10	0.04	15.08 \pm 0.01	0.08	
	62.5	4236.8 \pm 0.45	0.02	11.89 \pm 0.005	0.04	4263.42 \pm 2.31	0.05	15.09 \pm 0.00	0.03	
	31.25	2080.8 \pm 0.84	0.04	11.90 \pm 0.007	0.05	2160.56 \pm 1.89	0.09	15.15 \pm 0.01	0.04	
	15.625	1075.8 \pm 1.09	0.10	11.89 \pm 0.004	0.03	1097.52 \pm 2.78	0.25	15.14 \pm 0.01	0.04	
	7.8125	542.0 \pm 0.00	0.00	11.87 \pm 0.043	0.36	568.20 \pm 1.07	0.19	15.15 \pm 0.01	0.06	
Interday	125	8022.4 \pm 1.14	0.01	11.90 \pm 0.01	0.01	7967.56 \pm 1.67	0.02	15.09 \pm 0.00	0.02	
	62.5	4235.8 \pm 1.09	0.02	11.89 \pm 0.02	0.02	4263.87 \pm 1.99	0.05	15.09 \pm 0.01	0.04	
	31.25	2082 \pm 0.70	0.03	11.90 \pm 0.03	0.01	2138.23 \pm 2.49	0.12	15.09 \pm 0.01	0.07	
	15.625	1075.2 \pm 0.83	0.07	11.90 \pm 0.01	0.01	1097.75 \pm 1.97	0.18	15.09 \pm 0.01	0.06	
	7.8125	543.8 \pm 0.83	0.15	11.89 \pm 0.03	0.00	563.98 \pm 3.42	0.61	15.09 \pm 0.01	0.05	
Inter-mediate	125	8022.4 \pm 1.14	0.01	11.90 \pm 0.09	0.02	7986.36 \pm 1.00	0.01	15.09 \pm 0.01	0.04	
	62.5	8021.7 \pm 0.99	0.01	11.89 \pm 0.002	0.01	4263.65 \pm 0.22	0.01	15.09 \pm 0.00	0.01	
	31.25	4236.3 \pm 0.71	0.01	11.89 \pm 0.001	0.00	2149.39 \pm 0.42	0.02	15.12 \pm 0.00	0.02	
	15.625	2081.4 \pm 0.85	0.04	11.90 \pm 0	0.01	1097.64 \pm 0.58	0.05	15.11 \pm 0.00	0.02	
	7.8125	1075.5 \pm 0.42	0.03	11.89 \pm 0.001	0.11	566.09 \pm 1.66	0.29	15.12 \pm 0.00	0.00	
β-asarone										
Intraday	125	8389.6 \pm 1.1	0.01	10.79 \pm 0.005	0.05	7977.84 \pm 1.71	0.02	13.31 \pm 0.00	0.00	
	62.5	4485.8 \pm 1.7	0.03	10.79 \pm 0.005	0.05	4190.78 \pm 2.60	0.06	13.31 \pm 0.00	0.00	
	31.25	2243.6 \pm 1.1	0.05	10.79 \pm 0.008	0.08	2193.30 \pm 3.99	0.18	13.30 \pm 0.01	0.04	
	15.625	1237.8 \pm 1.4	0.13	10.79 \pm 0.005	0.05	1116.04 \pm 2.76	0.25	13.30 \pm 0.00	0.00	
	7.8125	646.2 \pm 0.83	0.12	10.80 \pm 0.004	0.04	7977.84 \pm 1.71	0.02	13.31 \pm 0.00	0.00	
Interday	125	8389.8 \pm 1.2	0.02	10.8 \pm 0.00	0.06	8018.86 \pm 3.25	0.04	13.27 \pm 0.02	0.18	
	62.5	4487.4 \pm 1.5	0.03	10.796 \pm 0.00	0.05	4198.37 \pm 3.82	0.09	13.27 \pm 0.02	0.18	
	31.25	2243.6 \pm 1.1	0.05	10.798 \pm 0.00	0.07	2196.84 \pm 3.31	0.15	13.27 \pm 0.02	0.18	
	15.625	1235.4 \pm 1.3	0.10	10.796 \pm 0.00	0.05	1115.91 \pm 2.46	0.22	13.26 \pm 0.02	0.18	
	7.8125	647 \pm 0.00	0.00	10.8025 \pm 0.00	0.04	581.46 \pm 2.36	0.41	13.26 \pm 0.02	0.18	
Inter-mediate	125	8389.7 \pm 0.14	0.00	10.797 \pm 0.00	0.03	7998.35 \pm 1.09	0.01	13.29 \pm 0.02	0.13	
	62.5	4486.6 \pm 1.13	0.02	10.796 \pm 0.00	0.00	4194.58 \pm 0.86	0.02	13.29 \pm 0.02	0.13	
	31.25	2243.6 \pm 0.00	0.00	10.797 \pm 0.00	0.01	2195.07 \pm 0.48	0.02	13.29 \pm 0.01	0.10	
	15.625	1236.6 \pm 1.69	0.13	10.795 \pm 0.00	0.01	1115.98 \pm 0.21	0.02	13.28 \pm 0.02	0.12	
	7.8125	646.6 \pm 0.56	0.08	10.8022 \pm 0.00	0.00	581.89 \pm 0.06	0.01	13.28 \pm 0.01	0.09	

Table 2: Summary of α - and β -asarone calibration data on column A and B. The regression equation is ($y = ax + b$), where (a) is the slope and (b) is the y intercept

Column A	α -Asarone				Column B			
	Precision	a (AV \pm SD)	b(AV \pm SD)	LOD (μ g/mL) (AV \pm SD)	LOQ (μ g/mL) (AV \pm SD)	a (AV \pm SD)	b (AV \pm SD)	LOD (μ g/mL) (AV \pm SD)
Intraday	63.928 \pm 0.01	94.712 \pm 0.63	0.032 \pm 0.00	0.099 \pm 0.00	63.558 \pm 0.13	142.06 \pm 4.25	0.22 \pm 0.000	0.67 \pm 0.001
Interday	63.928 \pm 0.01	95.170 \pm 0.66	0.034 \pm 0.00	0.10 \pm 0.00	62.926 \pm 0.87	143.54 \pm 0.00	0.30 \pm 0.001	0.90 \pm 0.010
β-asarone								
Intraday	66.058 \pm 0.01	200.64 \pm 0.54	0.027 \pm 0.00	0.081 \pm 0.00	62.950 \pm 0.03	162.60 \pm 2.86	0.09 \pm 0.001	0.27 \pm 0.004
Interday	66.066 \pm 0.01	200.24 \pm 0.68	0.034 \pm 0.01	0.103 \pm 0.00	63.252 \pm 0.12	155.64 \pm 0.01	0.19 \pm 0.001	0.59 \pm 0.001

Table 3: Summary of α - and β -asarone concentration in three extracts of different parts of *P. sarmentosum*

Samples	Column A				Column B			
	RT (min)	α -asarone (% \pm SD)	RT (min)	β -asarone (% \pm SD)	RT (min)	α -asarone (% \pm SD)	RT (min)	β -asarone (% \pm SD)
PSL-E	11.95	1.56 \pm 0.001	10.78	0.08 \pm 0.001	15.16	1.53 \pm 0.001	13.32	0.07 \pm 0.003
PSL-EW	11.95	0.85 \pm 0.001	10.78	0.09 \pm 0.003	15.15	0.83 \pm 0.001	13.32	0.09 \pm 0.002
PSL-W	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
PSS-E	11.92	0.42 \pm 0.00	N/D	N/D	15.20	0.40 \pm 0.002	N/D	N/D
PSS-EW	11.93	0.38 \pm 0.002	N/D	N/D	15.20	0.36 \pm 0.001	N/D	N/D
PSS-W	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
PSR-E	11.92	5.14 \pm 0.001	10.78	0.15 \pm 0.001	15.10	5.11 \pm 0.003	13.27	0.14 \pm 0.000
PSR-EW	11.96	1.14 \pm 0.002	10.8	0.01 \pm 0.001	15.12	1.13 \pm 0.001	13.28	0.01 \pm 0.001
PSR-W	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
PSF-E	11.96	3.84 \pm 0.001	10.8	0.11 \pm 0.001	15.13	3.83 \pm 0.01	13.36	0.12 \pm 0.000
PSF-EW	11.96	3.75 \pm 0.001	10.8	0.11 \pm 0.001	15.16	3.70 \pm 0.001	13.36	0.10 \pm 0.000
PSF-W	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D

N/D: Not detected

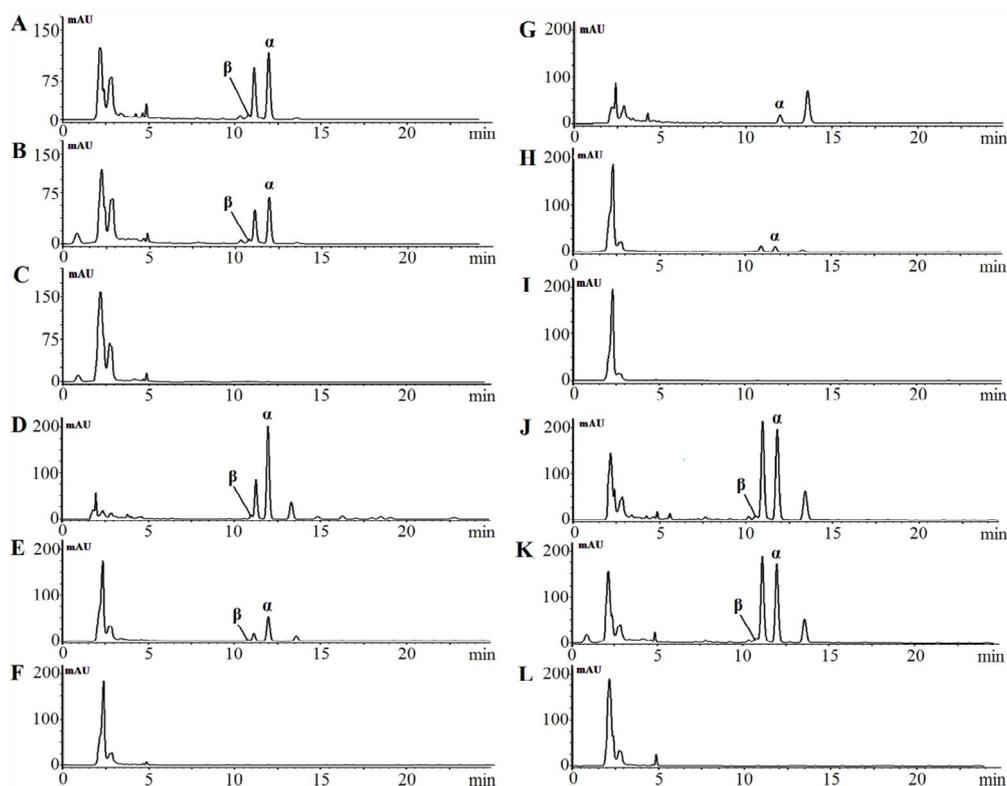


Figure 2: HPLC chromatograms of α - and β -asarone and *P. sarmentosum* extracts at 210 nm on ZORBAX Eclipse Plus Phenyl-Hexyl C-18 column. A = PSL-E, B = PSL-EW, C = PSL-W, D = PSR-E, E = PSR-EW, F = PSR-W, G = PSS-E, H = PSS-EW, I = PSS-W, J = PSF-E, K = PSF-EW and L = PSF-W

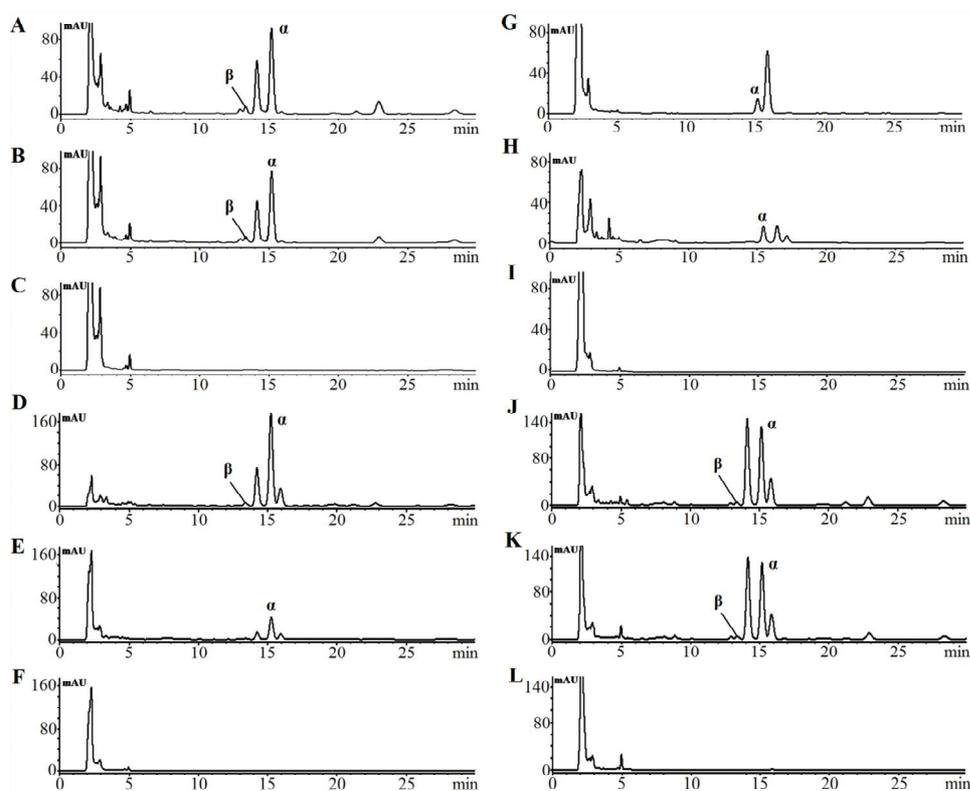


Figure 3: HPLC chromatograms of α - and β -asarone and *P. sarmentosum* extracts at 210 nm on ZORBAX Eclipse Plus C-18 column. A = PSL-E, B = PSL-EW, C = PSL-W, D = PSR-E, E = PSR-EW, F = PSR-W, G = PSS-E, H = PSS-EW, I = PSS-W, J = PSF-E, K = PSF-EW and L = PSF-W

DISCUSSION

For a long time, *P. sarmentosum* has been used as herbal remedy and food supplement, thus reliable procedures are needed for the quantitative analysis of its phytochemical constituents. In this study, *P. sarmentosum* extracts are analysed for their content of α - and β -asarone. Both α - and β -asarone are phenylpropanoids which have been reported to have cytotoxic, genotoxic, carcinogenic, psychoactive effects in both *in vitro* and *in vivo* models [18,21].

Based on the HPLC data, α -asarone is present as the major isomer in *P. sarmentosum* extracts compared to β -asarone. This study showed that in different parts of *P. sarmentosum* the concentration of α - and β -asarone were different and also varied between the extraction methods.

The importance of measuring asarone in *P. sarmentosum* is to determine the best extraction method in order to produce a safer extract which is free from asarone or produce extract with asarone concentration within acceptable limit set by the food regulation bodies, i.e., 115 $\mu\text{g/day}$ [20].

One case study on *Acorus calamus* herbal preparation which contained asarone has produced side effects such as tachycardia, dizziness, tremor, irregular breathing, pallor, anxiety, nausea and vomiting [24]. From this analysis, we found that water extracts of *P. sarmentosum* are free from asarones and practically safe for consumption.

CONCLUSION

The developed HPLC method is simple, rapid and compatible with two different C-18 columns for the quantification of α - and β -asarone in *P. sarmentosum* extracts.

The analysis of different extracts of various parts of the plant showed variations in asarone content. The method is promising for determining asarone isomers in medicinal herbs.

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REFERENCES

1. Wiert C. *Medicinal Plants of Asia and The Pacific*. Florida, USA: CRC Press; 2006; p 336.
2. Peungvicha P, Thirawarapan SS, Temsiririrkkul R, Watanabe H, Prasain JK, Kadota S. Hypoglycemic Effect of The Water Extract of *Piper sarmentosum* in rats. *J Ethnopharmacol* 1998; 60: 27-32.
3. Sawangjiaroen N, Sawangjiaroen K, Poonpanang P. Effects of *Piper longum* fruit, *Piper sarmentosum* root and *Quercus infectoria* nut gall on caecal amoebiasis in mice. *J Ethnopharmacol* 2004; 91: 357-360.
4. Masuda T, Inazumi A, Yamada Y, Padolina WG, Kikuzaki H, Nakatani N. Antimicrobial Phenylpropanoids from *Piper sarmentosum*. *Phytochemistry* 1991; 30: 3227-3228.
5. Ridditid W, Rattanaprom W, Thaina P, Chittrakarn S, Sunbhanich M. Neuromuscular blocking activity of methanolic extract of *Piper sarmentosum* leaves in the rat phrenic nerve-hemidiaphragm preparation. *J Ethnopharmacol* 1998; 61: 135-142.
6. Rahman NNNA, Furuta T, Kojima S, Takane K, Mohd MA. Antimalarial Activity of Extracts of Malaysian Medicinal Plants. *J Ethnopharmacol* 1999; 64: 249-254.
7. Hutadilok NT, Chaiyammutti P, Panthong K, Mahabusarakam W, Rukachaisirikul V. Antioxidative and Free Radical Scavenging Activities of Some Plants Used in Thai Folk Medicine. *Pharm Biol* 2006; 44: 221-228.
8. Rukachaisirikul T, Siriwatanakit P, Sukcharoenphol K, Wongvein C, Ruttanaweang P, Wongwattanavuch P, Suksamrarn A. Chemical constituents and bioactivity of *Piper sarmentosum*. *J Ethnopharmacol* 2004; 93: 173-176.
9. Ariffin SHZ, Wan Omar WHH, Ariffin ZZ, Safian MF, Senafi S, Megat Abdul Wahab R. Intrinsic anticarcinogenic effects of *Piper sarmentosum* ethanolic extract on a human hepatoma cell line. *Cancer Cell Int* 2009; 9: 1-9.
10. Hussain K, Ismail Z, Sadikun A, Ibrahim P, Malik A. *In vitro* Antiangiogenesis Activity of Standardized Extracts of *Piper sarmentosum* Roxb. *J Riset Kimia* 2009; 1: 146-150.
11. Parmar VS, Jain SC, Bisht KS, Jain R, Taneja P, Jha A, Tyagi OD, Prasad AK, Wengel J, Olsen CE, Boll, PM. *Phytochemistry of the genus Piper*. *Phytochem* 1997; 46: 597-673.
12. Subramaniam V, Adenan MI, Ahmad AR, Sahdan R. Natural Antioxidants: *Piper sarmentosum* (Kadok) and *Morinda elliptica* (Mengkudu). *Malays J Nutr* 2003; 9: 41-51.
13. Chan P, Soon-II K, Young-Joon A. Insecticidal activity of asarones identified in *Acorus gramineus* rhizome against three coleopteran stored-product insects. *J Stored Prod Res* 2003; 39: 333-342.
14. Cho J, Kim YH, Kong J-Y, Yang CH, Park CG. Protection of cultured rat cortical neurons from excitotoxicity by asarone, a major essential oil component in the
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- rhizomes of *Acorus gramineus*. *Life Sci* 2002; 71: 591-599.
15. Wiseman RW, Miller EC, Miller JA, Liem A. Structure-activity studies of the hepatocarcinogenicities of alkenylbenzene derivatives related to estragole and safrole on administration to preweanling male C57BL/6J x C3H/HeJ F1 mice. *Cancer Res* 1987; 47: 2275-2283.
 16. Hasheminejad G, Caldwell J. Genotoxicity of the alkenylbenzenes α - and β -asarone, myristicin and elemicin as determined by the UDS assay in cultured rat hepatocytes. *Food Chem Toxicol* 1994; 32: 223-231.
 17. Schmidt GH, Strelke M. Effect of *Acorus calamus* (L.) (Araceae) oil and its main compound β -asarone on *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). *J Stored Prod Res* 1994; 30: 227-235.
 18. Unger P, Melzig MF. Comparative Study of the Cytotoxicity and Genotoxicity of Alpha- and Beta-Asarone. *Sci Pharm* 2012; 80: 663-668.
 19. Commission E. Opinion of the Scientific Committee on Food on the presence of β -asarone in flavourings and other food ingredients with flavouring properties, 2002 [cited 18 June 2014]. Available from: http://europa.eu.int/comm/food/fs/sc/scf/index_en.html
 20. Agency EM. Public statement on the use of herbal medicinal products containing asarone. London; 2005.
 21. WHO. Toxicological Evaluation of Certain Food Additives - Beta-Asarone, 1981 [cited 19 June 2014]. Available from: www.inchem.org/documents/jecfa/jecmono/v16je04.htm
 22. ICH. Guidance for Industry, Q2B: Validation of Analytical Procedures: Methodology. USA: International Conference of Harmonisation; 1997.
 23. Baxter RM, Fan MC, Kandel SL. Cis-Trans Trans isomers of asarone, their liquid-gas chromatographic behavior and that of certain other propenylphenolethers. *Can J Chem* 1962; 40: 151-157.
 24. Zuba D, Byrska B. Alpha- and beta-asarone in herbal medicinal products. A case study. *Forensic Sci Int* 2012; 223: 5-9.