



Marker compound contents and antioxidant capacities of the taproot and lateral root of Danshen (*Salvia miltiorrhiza* Radix)

Gi-Un Seong¹ · Shin-Kyo Chung¹

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Abstract In this study, the marker compound contents of both the taproot and lateral root of Danshen (*Salvia miltiorrhiza* Radix), which is cultivated in Korea, were investigated. The salvianolic acid B content was the highest in the taproot (5.17–6.75%) and lateral root (3.99–5.69%). The cryptotanshinone, tanshinone I, and tanshinone IIA contents were higher in the lateral root than in the taproot of Danshen ($p < 0.05$). Principal component analysis results revealed that the taproot was correlated to the salvianic acid A, rosmarinic acid, salvianolic acid B, and salvianolic acid A contents, whereas the lateral root was correlated to the cryptotanshinone, tanshinone I, and tanshinone IIA contents. The total phenolic content and total flavonoid content of the taproot were higher than those of the lateral root ($p < 0.05$); however, the antioxidant activities of the taproot and lateral root of Danshen were similar. The salvianolic acid B content was correlated to the TPC of the taproot ($r = 0.748$) and the 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging activity of the lateral root ($r = 0.847$). This study could provide useful information for the classification of Danshen as a herbal medicinal product.

Keywords Antioxidant · Danshen · Principal component analysis · Salvianolic acid B · Tanshinone IIA

Shin-Kyo Chung (✉)
E-mail: kchung@knu.ac.kr

¹School of Food Science and Biotechnology, Kyungpook National University, Daegu 41566, Republic of Korea

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Introduction

Danshen (*Salvia miltiorrhiza* Radix) is widely used in traditional medicine for cardiovascular diseases such as coronary heart disease, hyperlipidemia, and cerebrovascular disease [1]. It has been cultivated in East Asian countries including China and Korea [2]. Its bioactivities such as antioxidant, antibacterial, anti-inflammatory, antitumor, and whitening effects and use in health-promoting foods have been investigated [3–6]. Moreover, its stability has been investigated [7]. The marker compounds contributing to the physiological activities of Danshen include salvianolic acids such as salvianolic acid A and salvianolic acid B and tanshinones such as cryptotanshinone, tanshinone I, and tanshinone IIA [8–10]. The rapid and accurate analysis of the marker compounds of Danshen by high-performance liquid chromatography (HPLC) with mass spectrometry has been reported [11]. Furthermore, the marker compound contents of Danshen according to the cultivation regions have been determined by HPLC and ultra-performance liquid chromatography-mass spectrometry [12].

The contents of marker compounds in medicinal plant roots and their activities differ depending on the breed, growing season, harvesting time, cultivation area, and region [13]. In particular, the contents of the marker compounds and their activities in the leaves and roots of Danshen have been reported [14]. However, there are no studies on the marker compound contents of the thick reddish-brown taproot and thin, tan-colored lateral root according to their morphological characteristics. The purpose of this study was to improve the classification, and quality of the taproot and lateral root and to enhance herbal medicinal products. Moreover, the marker compound contents and antioxidant activities of Danshen were measured, and the characteristics were assessed by principal component analysis (PCA) and correlation analysis.

Materials and Methods

Materials and sample preparation

Danshen was collected from four regions (Bonghwa, Gochang, Jangheung, and Yeongyang) in Korea. The collected Danshen had a long cylindrical taproot, and the lateral root was bent in various angles similar to the shape of a mustache (Fig. 1). The roots were dried in the shade and separated into two portions (the taproot and lateral root) (Fig. 1). The taproot of Danshen was dark reddish-brown, had a rough surface with thick vertical wrinkles, and was around 10–20 cm long. On the other hand, the lateral root of Danshen was thin and showed a different morphology. The diameter was measured at the upper portion of the roots using a dial caliper (ALLTRADE, Long Beach, CA, USA). The diameters of the taproot and lateral root of Danshen are shown in Table 1. Regardless of the cultivation region of Danshen, the diameter of the taproot (6.77–10.02 mm) was thicker than that of the lateral root (1.34–2.31 mm), and the color of the surface was darker. This can be attributed to the increased suberization of the root surface depending on the growth period [13]. In addition, new lateral roots may grow in response to the external environment [15,16]. Samples for analysis were sonicated for 30.0 min with 50 mL of 75% ethanol and filtered through a 0.45 μ m PTFE syringe filter.

Chemicals and machines

The marker compounds of Danshen, such as salvianic acid A, rosmarinic acid, salvianolic acid B, salvianolic acid A, cryptotanshinone, tanshinone I, and tanshinone IIA, and other chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO,

Table 1 Diameters of the taproot and lateral root of Danshen

Sample		Diameter (mm)
Taproot	A ¹⁾	10.02±1.64 ^{2)A3)}
	B	6.77±0.66 ^D
	C	7.32±0.96 ^{CD}
	D	7.40±1.10 ^{BCD}
	E	8.16±0.97 ^B
	F	7.75±1.24 ^{BC}
Lateral root	A	1.34±0.46 ^F
	B	2.31±0.54 ^E
	C	1.73±0.21 ^{EF}
	D	1.78±0.20 ^{EF}
	E	2.30±0.55 ^E
	F	2.03±0.56 ^{EF}
Taproot (Mean±SD)		7.90±1.51 ^A
Lateral root (Mean±SD)		1.92±0.55 ^B

¹⁾A-B, Bonghwa; C, Gochang; D-E, Jangheung; F, Yeongyang

²⁾Data are shown as the mean ± SD, n = 10

³⁾A-F Means followed by the same letters within the column are not significantly different ($p < 0.05$)

USA). A UV-Vis spectrophotometer (Optizen POP; Meacasy, Daejeon, Korea) and a microplate reader (Multiskan GO; Thermo Scientific, MA, USA) were used to determine antioxidant contents and antioxidant activities. A Waters (Milford, MA, USA) HPLC system equipped with 2695 Separations module and a 2996 photodiode array detector was used for marker compound analysis.

HPLC analytical conditions

The marker compound contents were analyzed according to a previous method [12] using a HPLC instrument with a UV detector (280 nm) and column (ODS H80, 4.6×250 mm, i.d. 4 μ m; YMC Co., Kyoto, Japan). The mobile phases used were as follows: A-1.0% formic acid (v/v) in distilled water; B-1.0% formic acid (v/v) in acetonitrile; operating conditions-flow rate of 0.8 mL/min and injection volume of 10 μ L. The gradient elution for HPLC analysis over 40.0 min was as follows: 75.0% A/25.0% B at 0.0 min, 75.0% A/25.0% B at 10.0 min, 40.0% A/60.0% B at 20.0 min, 15.0% A/85.0% B at 25.0 min, and 15.0% A/85.0% B at 40.0 min. The marker compound contents were confirmed by comparing their individual retention times with those of standards, and the results are expressed as a percentage (%; dry weight basis).

Antioxidant activities of Danshen

The antioxidant activities of Danshen were determined by α,α -Diphenyl- β -picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity assays [17,18]. The values of DPPH assay are expressed

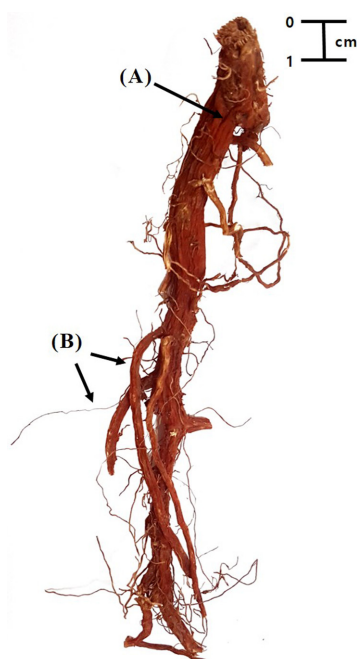


Fig. 1 Photo of the (A) taproot and (B) lateral root of Danshen

as ascorbic acid equivalent of the sample (mM AS), and those of FRAP and ABTS assays are expressed as Trolox equivalent of the sample (mM TE).

Total phenolic and flavonoid contents of Danshen

The total phenolic content (TPC) was determined by the Folin-Ciocalteu method [19] and expressed as milligrams of gallic acid equivalent per gram of the sample (mg GAE/g). The total flavonoid content (TFC) was determined by aluminum chloride colorimetric assay [20] and expressed as milligrams of quercetin equivalent per gram of the sample (mg QE/g).

PCA of marker compounds

The marker compound contents of Danshen were analyzed by PCA using the Statistical Analysis System (SAS, Version 9.4; SAS Institute Inc., Cary, NC, USA), which is a technique used to represent the pattern of similarity of the observations and the variables [21]. MS Excel software was used to visualize cluster formation for the taproot and lateral root of Danshen.

Statistical analysis

Values are expressed as the mean \pm standard deviation of three measurements. The data were analyzed by Duncan's multiple range test ($p < 0.05$) using SAS software. Correlation analysis between antioxidant capacities and marker compound contents of the taproot and lateral root of Danshen was performed by Pearson's correlation test ($p < 0.05$).

Results and Discussion

Marker compound contents and PCA of Danshen

The marker compound contents of Danshen are shown in Table 2. The salvianic acid A, rosmarinic acid, salvianolic acid B, salvianolic acid A, cryptotanshinone, tanshinone I, and tanshinone IIA contents were 0.00–0.02%, 0.22–0.30%, 3.99–6.75%, 0.04–0.07%, 0.09–0.17%, 0.01–0.09%, and 0.22–0.60%, respectively. Among the marker compounds, salvianolic acid B was the highest in the taproot (5.76%) and lateral root (4.97%), which is consistent with the findings of previous studies (5.53%) that have examined the marker compound contents of Danshen [12,22]. The salvianolic acid B content of the taproot was higher than that of the lateral root. In contrast, the cryptotanshinone, tanshinone I, and tanshinone IIA contents of the lateral root (0.13, 0.05, and 0.43%, respectively) were higher than those of the taproot (0.11, 0.02, and 0.28%, respectively).

PCA, a multivariate analysis method, was used to simplify the complexity in high-dimensional data. It achieves this by transforming the data into fewer dimensions, which act as summaries of features [23]. It is used to identify the relationship between groups, using correlation analysis to determine the relationship between two variables [24]. The marker compound contents of the taproot and lateral root were set as a measurement variable. The components in PCA may be used to explain the variance within the variables. The PCA scores revealed that PC1 (Component 1) was 45.33%, PC2 (Component 2) was 30.59%, and cumulative

Table 2 Marker compound contents of the taproot and lateral root of Danshen

Sample	Salvianic acid A	Rosmarinic acid	Salvianolic acid B	Salvianolic acid A	Cryptotanshinone	Tanshinone I	Tanshinone IIA
Taproot	A ¹⁾	n.d. ²⁾	0.22 \pm 0.04 ^{AB}	6.75 \pm 0.40 ^A	0.04 \pm 0.01 ^C	0.13 \pm 0.01 ^{BC}	0.04 \pm 0.01 ^{BC}
	B	n.d.	0.25 \pm 0.05 ^{AB}	5.72 \pm 0.08 ^{BC}	0.05 \pm 0.01 ^{ABC}	0.11 \pm 0.01 ^{CD}	0.02 \pm 0.02 ^{CD}
	C	0.02 \pm 0.03 ^{3)4)A5)}	0.29 \pm 0.13 ^A	5.30 \pm 0.24 ^{CDE}	0.06 \pm 0.02 ^{AB}	0.11 \pm 0.02 ^{CDE}	0.01 \pm 0.02 ^{CD}
	D	n.d.	0.25 \pm 0.02 ^{AB}	5.56 \pm 0.35 ^{BCDE}	0.04 \pm 0.01 ^{BC}	0.11 \pm 0.00 ^{DE}	0.01 \pm 0.00 ^D
	E	0.01 \pm 0.01 ^A	0.23 \pm 0.07 ^{AB}	5.17 \pm 0.22 ^{EF}	0.05 \pm 0.01 ^{ABC}	0.09 \pm 0.01 ^E	0.01 \pm 0.01 ^D
	F	n.d.	0.30 \pm 0.04 ^A	5.91 \pm 0.12 ^B	0.07 \pm 0.01 ^A	0.10 \pm 0.00 ^{DE}	0.02 \pm 0.00 ^{CD}
Lateral root	A	n.d.	0.25 \pm 0.04 ^{AB}	5.28 \pm 0.16 ^{DE}	0.05 \pm 0.01 ^{ABC}	0.16 \pm 0.01 ^A	0.11 \pm 0.02 ^A
	B	n.d.	0.24 \pm 0.06 ^{AB}	4.63 \pm 0.12 ^G	0.05 \pm 0.01 ^{ABC}	0.17 \pm 0.02 ^A	0.09 \pm 0.03 ^A
	C	n.d.	0.24 \pm 0.03 ^{AB}	4.84 \pm 0.18 ^{FG}	0.05 \pm 0.01 ^{ABC}	0.11 \pm 0.00 ^{CDE}	0.02 \pm 0.01 ^{CD}
	D	n.d.	0.26 \pm 0.04 ^A	5.41 \pm 0.25 ^{CDE}	0.04 \pm 0.01 ^{BC}	0.14 \pm 0.01 ^B	0.05 \pm 0.01 ^B
	E	0.00 \pm 0.01 ^A	0.14 \pm 0.07 ^B	3.99 \pm 0.21 ^H	0.04 \pm 0.01 ^C	0.11 \pm 0.01 ^{CDE}	0.02 \pm 0.03 ^{CD}
	F	n.d.	0.19 \pm 0.04 ^{AB}	5.69 \pm 0.11 ^{BCD}	0.05 \pm 0.01 ^{BC}	0.11 \pm 0.01 ^{CD}	0.04 \pm 0.02 ^{BC}
Taproot (Mean \pm SD)	0.00 \pm 0.01 ^A	0.26 \pm 0.06 ^A	5.76 \pm 0.67 ^A	0.05 \pm 0.02 ^A	0.11 \pm 0.01 ^B	0.02 \pm 0.02 ^B	0.28 \pm 0.08 ^B
Lateral root (Mean \pm SD)	0.00 \pm 0.00 ^A	0.22 \pm 0.06 ^A	4.97 \pm 0.60 ^B	0.05 \pm 0.01 ^A	0.13 \pm 0.03 ^A	0.05 \pm 0.04 ^A	0.43 \pm 0.13 ^A

¹⁾A-B, Bonghwa; C, Gochang; D-E, Jangheung; F, Yeongyang

²⁾not detected

³⁾Data are shown as the mean \pm SD, n=3

⁴⁾The values are expressed on a dry weight basis (%)

⁵⁾A-F Means followed by the same letters within the column are not significantly different ($p < 0.05$)

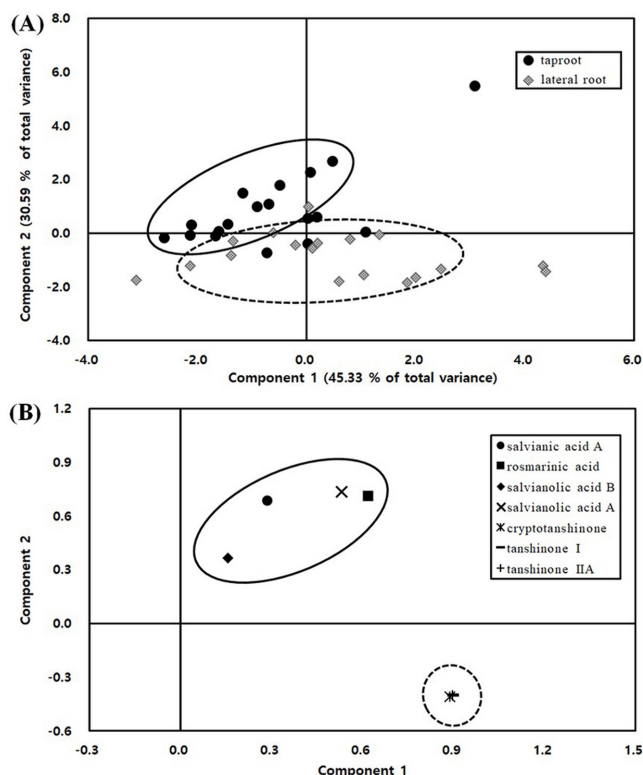


Fig. 2 Principal component analysis (PCA) of the marker compounds of the taproot and lateral root of Danshen. (A) score plots; (B) loading plots

proportion was 75.92%, which can explain the overall results [25]. Depending on the position on the graph, the taproot of Danshen was clustered at the top, whereas the lateral root of Danshen was

at the bottom; thus, Danshen can be distinguished by the root portion (taproot and lateral root) (Fig. 2A). Fig. 2B shows the correlation between the marker compounds of Danshen. At the top of the graph, phenolic acids including salvianic acid A, rosmarinic acid, salvianolic acid B, and salvianolic acid A were clustered. On the other hand, tanshinones including cryptotanshinone, tanshinone I, and tanshinone IIA were closely clustered at the bottom of the graph [23]. The results are similar to those of another study, which found that the contents of ginsenosides are different depending on the morphological characteristics of the taproot and lateral root of ginseng [26]. Therefore, the taproot of Danshen is closely correlated to the component of salvianolic acids, and the lateral root of Danshen is closely correlated to the component of tanshinones. These results revealed the different marker compound contents of the taproot and lateral root, which could be used for the classification of Danshen.

Antioxidant capacity and correlation analysis of Danshen

The DPPH radical scavenging activity, FRAP, and ABTS radical scavenging activity assays were performed, and the TPC and TFC were determined to evaluate the antioxidant capacities of the taproot and lateral root of Danshen. The results are shown in Table 3. FRAP activity was significantly higher in the taproot (7.73 mM TE) than in the lateral root (6.68 mM TE) ($p < 0.05$). However, DPPH and ABTS activities were similar between the taproot (4.21 mM AS and 1.42 mM TE, respectively) and lateral root (4.23 mM AS and 1.50 mM TE, respectively) ($p < 0.05$). The results demonstrated the higher TPC and TFC of the taproot (83.40 and 69.33 mg QE/g, respectively) compared with those of the lateral root (76.34 and 59.90 mg QE/g, respectively) ($p < 0.05$). The antioxidant capacities of water and ethanol extracts of Danshen showed similar results [27].

Table 3 Antioxidant activities and contents of the taproot and lateral root of Danshen

Sample		Antioxidant activity			Antioxidant content	
		DPPH (mM AS)	FRAP (mM TE)	ABTS (mM TE)	TPC (mg GAE/g)	TFC (mg QE/g)
Taproot	A ¹⁾	4.28±0.03 ^{2)CD3)}	7.97±0.04 ^A	1.53±0.03 ^C	98.54±3.31 ^A	88.46±5.64 ^A
	B	4.28±0.01 ^{CD}	7.37±0.05 ^C	1.34±0.06 ^{DE}	83.68±1.97 ^{BCD}	60.00±1.67 ^{DEF}
	C	4.35±0.03 ^{BC}	7.48±0.10 ^C	1.30±0.07 ^E	72.02±4.01 ^F	58.51±5.30 ^{EF}
	D	4.05±0.08 ^{EF}	7.96±0.12 ^A	1.66±0.04 ^A	81.18±2.05 ^{BCDE}	77.66±1.31 ^B
	E	4.18±0.06 ^{DE}	7.77±0.06 ^B	1.40±0.08 ^D	85.77±5.21 ^{BC}	69.04±1.73 ^C
	F	4.09±0.04 ^{EF}	7.85±0.07 ^{AB}	1.30±0.10 ^E	79.24±6.77 ^{CDEF}	62.31±1.80 ^{DE}
Lateral root	A	4.54±0.02 ^A	7.14±0.03 ^D	1.55±0.01 ^{BC}	88.26±4.42 ^B	74.57±2.06 ^B
	B	4.17±0.07 ^{DE}	6.85±0.06 ^F	1.51±0.02 ^C	77.57±8.60 ^{CDEF}	59.81±2.25 ^{DEF}
	C	4.48±0.03 ^{AB}	7.05±0.09 ^{DE}	1.52±0.04 ^C	60.91±3.78 ^G	52.39±3.37 ^G
	D	4.14±0.02 ^{DEF}	6.51±0.17 ^G	1.63±0.03 ^{AB}	82.71±1.50 ^{BCD}	65.38±2.18 ^{CD}
	E	4.05±0.24 ^{EF}	5.53±0.05 ^H	1.25±0.04 ^E	72.99±3.60 ^{EF}	52.76±1.09 ^G
	F	4.00±0.24 ^F	6.97±0.16 ^{EF}	1.53±0.02 ^C	75.63±3.15 ^{DEF}	54.49±3.98 ^G
Taproot (Mean±SD)		4.21±0.12 ^A	7.73±0.24 ^A	1.42±0.15 ^A	83.40±9.00 ^A	69.33±11.40 ^A
Lateral root (Mean±SD)		4.23±0.23 ^A	6.68±0.57 ^B	1.50±0.12 ^A	76.34±9.61 ^B	59.90±8.51 ^B

¹⁾ A-B, Bonghwa; C, Gochang; D-E, Jangheung; F, Yeongyang

²⁾ Data are shown as the mean±SD, n=3

³⁾ A-H Means followed by the same letters within the column are not significantly different ($p < 0.05$)

Table 4 Correlation coefficients between the antioxidant capacities and contents and the marker compound contents of the taproot and lateral root of Danshen

	Variable	DPPH	FRAP	ABTS	TPC	TFC
Taproot	Salvianic acid A	0.374	-0.156	-0.043	-0.156	-0.090
	Rosmarinic acid	0.136	0.035	0.036	-0.112	-0.137
	Salvianolic acid B	0.196	0.474*	0.335	0.748**	0.667**
	Salvianolic acid A	0.145	-0.096	-0.282	-0.274	-0.403
	Cryptotanshinone	0.544* ¹⁾	0.038	0.308	0.485*	0.455
	Tanshinone I	0.432	0.264	0.230	0.703**	0.543*
	Tanshinone IIA	0.662** ²⁾	0.013	0.292	0.640**	0.449
Lateral root	Salvianic acid A	0.246	-0.329	-0.297	-0.140	-0.214
	Rosmarinic acid	0.603**	0.588*	0.738**	0.406	0.561*
	Salvianolic acid B	0.114	0.703**	0.847**	0.407	0.437
	Salvianolic acid A	0.581*	0.649**	0.492*	0.333	0.467
	Cryptotanshinone	0.352	0.387	0.441	0.697**	0.726**
	Tanshinone I	0.386	0.449	0.450	0.807**	0.814**
	Tanshinone IIA	0.472*	0.544*	0.543*	0.764**	0.810**

¹⁾*Significant at $p < 0.05$ ²⁾**Significant at $p < 0.01$

The correlations between the antioxidant capacities and marker compound contents of the taproot and lateral root are shown in Table 4. The TPC of Danshen taproot had the highest positive correlation with the salvianolic acid B content ($r=0.748$), followed by the tanshinone I content ($r=0.703$) and tanshinone IIA content ($r=0.640$) ($p < 0.01$). The marker compounds (rosmarinic acid, salvianolic acid B, cryptotanshinone, tanshinone I, and tanshinone IIA) of the lateral root of Danshen showed a significant positive correlation with the TPC, TFC, FRAP activity, and ABTS activity. The salvianolic acid B content of Danshen lateral root had the highest positive correlation with ABTS activity ($r=0.847$), followed by FRAP activity ($r=0.703$) ($p < 0.01$). Specifically, the tanshinone I content of the lateral root showed a high positive correlation with the TFC ($r=0.814$) and TPC ($r=0.807$) ($p < 0.01$). Moreover, the tanshinone IIA content showed a significant positive correlation with the TFC ($r=0.810$) and TPC ($r=0.764$) ($p < 0.01$). Among the phenolic compounds, salvianolic acid B, which has been reported to show high antioxidant activity, could decrease the production of reactive oxygen species and NADPH oxidase activity [28,29]. Moreover, antioxidant capacity could be enhanced through the polymerization of flavone or salvianolic acid B due to an increase in hydroxyl groups [30]. Therefore, the antioxidant capacities of the taproot and lateral root are highly correlated to the contents of salvianolic acid B and tanshinone IIA.

These studies revealed the different marker compound contents and antioxidant capacities of the taproot and lateral root, and could provide useful information for the classification of Danshen as a herbal medicinal product.

Author's contributions GUS and SKC conceived and designed the experiments. GUS performed most of experiments and wrote the manuscript. SKC revised and edited the manuscript and supervised the work. All authors have read and approved the final manuscript.

Competing interests The authors declare that they have no competing interests.

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