SUPPLEMENTARY MATERIAL

Chemical profiling of the tuber of *Stephania cambodica* Gagnep. (Menispermaceae) and analytical control by UHPLC-DAD

Chhavarath Dary[abc], Sok-Siya Bun[a*], Gaëtan Herbette[d], Fathi Mabrouki[a], Hot Bun[e], Sothea Kim[f], Florian Jabbour[c], Sovanmoly Hul[c], Béatrice Baghdikian[a], Evelyne Ollivier[a]

Affiliation

[a]*Aix Marseille Univ, UMR-MD3, Laboratory of Pharmacognosy and Ethnopharmacology, Faculty of Pharmacy, 27 Blvd Jean Moulin CS 30064, 13005 Marseille, France.*

[b]*University of Health Sciences, Research Unit, 73 Blvd Monivong, Phnom Penh, Cambodia.*

[c]*Muséum national d'Histoire naturelle, Institut de Systématique, Évolution, Biodiversité, UMR 7205 ISYEB MNHN/CNRS/UPMC/EPHE, Sorbonne Université, 57 rue Cuvier, CP 39, 75005 Paris, France.*

[d]*Aix Marseille Univ, Spectropole, FR1739, Service 511, 13397 Marseille, France.*

[e]*Aix Marseille Univ, Laboratory of Pharmacokinetics and Toxicokinetics, Faculty of Pharmacy, 27 Blvd Jean Moulin CS 30064, 13005 Marseille, France.*

[f]*University of Health Sciences, Laboratory of Phytochemistry, Faculty of Pharmacy, 73 Blvd Monivong, Phnom Penh, Cambodia.*

Correspondance

Dr. Sok-Siya Bun, Faculty of Pharmacy, Aix Marseille University, Marseille cedex 5, France.

E-mail: sok-siya.bun@univ-amu.fr.

Acknowledgment

The research was supported by French government scholarships (bourse du gouvernement français).

ABSTRACT

A new aporphine glycoside (**1**), named "angkorwatine", and eight known alkaloids: oblongine (**2**), stepharine (**3**), asimilobine-β-D-glucopyranoside (**4**), isocorydine (**5**), tetrahydropalmatine (THP) (**6**), jatrorrhizine (**7**), palmatine (PAL) (**8**) and roemerine (ROE) (**9**) were simultaneously isolated from the tuber of *Stephania cambodica*. The development and validation of UHPLC-DAD method was carried out for the quantification of marker compounds (PAL, ROE, THP) of *S.*

cambodica. In addition to good selectivity and linearity (r^2>0.997), trueness, precision, and accuracy of the method did not exceed the acceptance limit of ±10% for ROE, THP and ±20% for PAL. Consequently, this method is able to provide accurate results between 1.39−4.18 µg/mL, 2.01−30.72 µg/mL and 4.29−64.42 µg/mL for PAL, ROE, and THP, respectively. This study shows that the validated UHPLC method is a rapid, innovative and effective analytical approach to control quality of tubers of *S. cambodica* and to regulate the usage of this plant in traditional medicine.

Keywords: Accuracy profile; alkaloid; method validation; palmatine; quantification; roemerine; tetrahydropalmatine.

Supplementary file (Figure and Table) Legend

Figure S1. Habit of *Stephania cambodica*. Sampled parts of the plant (tubers and stem) are numbered and the year of collection given in parentheses.

Figure S2. UHPLC chromatogram of hydroethanolic tuber extract of *Stephania cambodica.* **1**= Angkorwatine, **2**= Oblongine, **3**= Isocorydine, **4**= Asimilobine-β-D-glucopyranoside, **5**= Stepharine, **6**= Tetrahydropalmatine, **7**= Jatrorrhizine, **8**= Palmatine, **9**= Roemerine.

Figure S3 HR-ESI-MS of compound **(1**).

Figure S4 1H NMR of compound (**1**).

Figure S5 COSY of compound (**1**).

Figure S6 HSQC of compound (**1**).

Figure S7 HMBC of compound (**1**).

Figure S8 NOESY of compound (**1**).

Figure S9 Key correlations of NOESY and HMBC in compound (**1**).

Figure S10. Accuracy profile for quantitative methods validation of THP (A), PAL (B), and ROE (C) in tuber of *S. cambodica.* (—) = bias (%), (----) = acceptance limit (±10%) for THP and ROE, (±20%) for PAL, (·····) = 95% β-expectation tolerance interval.

Table S1. ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (150 MHz, CD_3OD) chemical shifts of compound (**1)** (angkorwatine)

Table S2. Injection repeatability and column performance parameters in system suitability test.

Table S3. Results of validation of UHPLC-DAD method dedicated to the quantification of THP, PAL, ROE.

Table S4. Content of palmatine (PAL), roemerine (ROE) and tetrahydropalmatine (THP) in vegetal material of *S. cambodica.*

Figure S1 Habit of *Stephania cambodica.* Sampled parts of the plants (tubers and stem) are numbered and the year of collection given in parentheses.

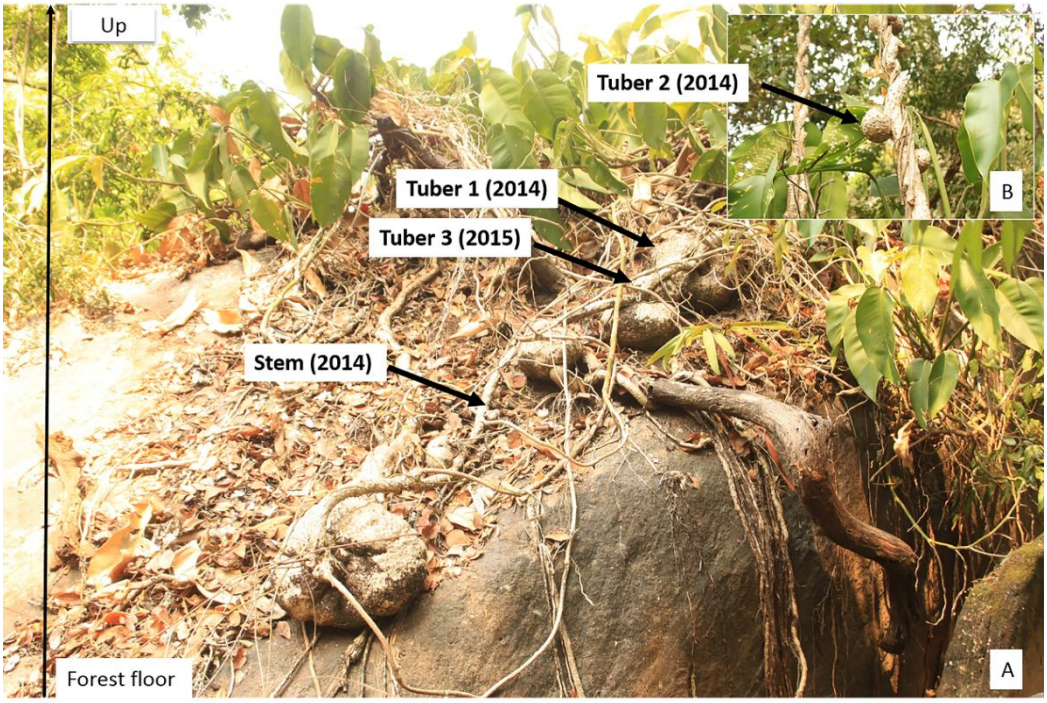

Figure S2. UHPLC chromatogram of hydroethanolic tuber extract of *Stephania cambodica.* **1**= Angkorwatine, **2**= Oblongine, **3**= Isocorydine, **4**= Asimilobine-β-D-glucopyranoside, **5**= Stepharine, **6**= Tetrahydropalmatine, **7**= Jatrorrhizine, **8**= Palmatine, **9**= Roemerine.

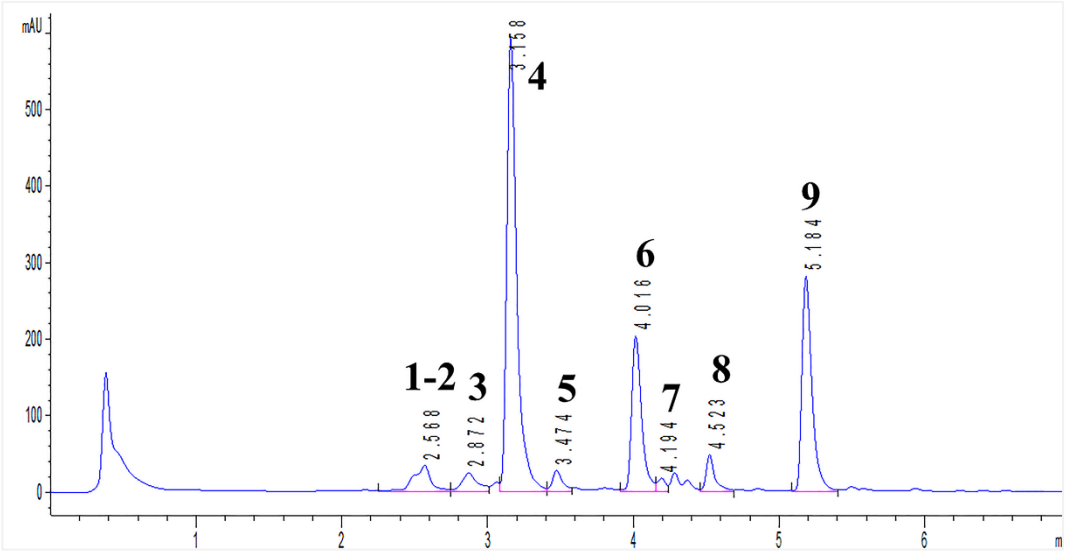

Figure S3 HR-ESI-MS of compound (**1)**.

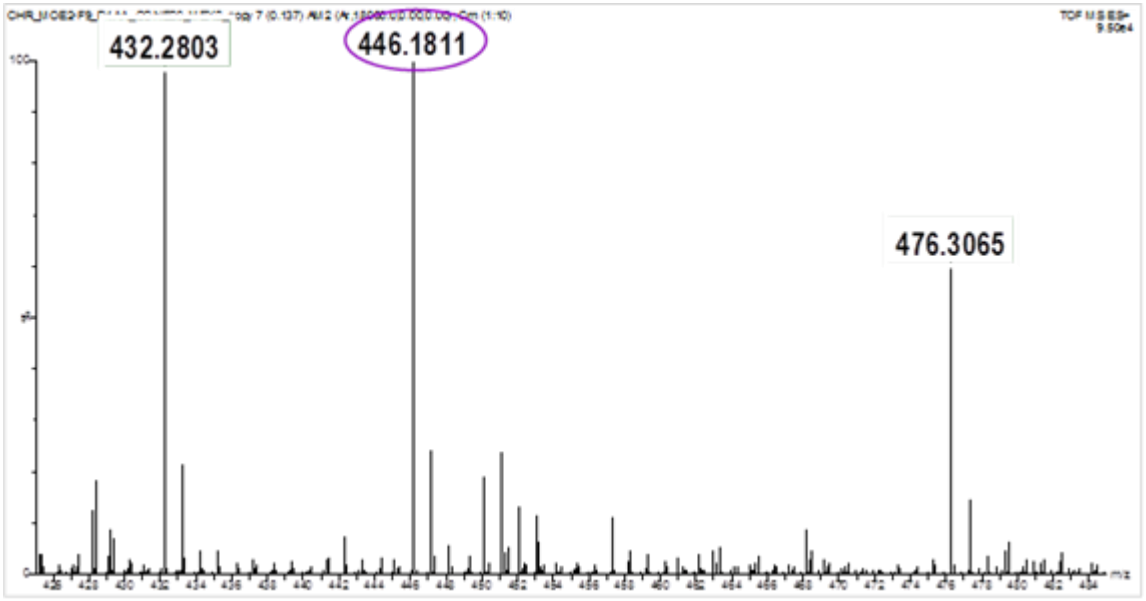

Figure S4 1H NMR of compound (**1**).

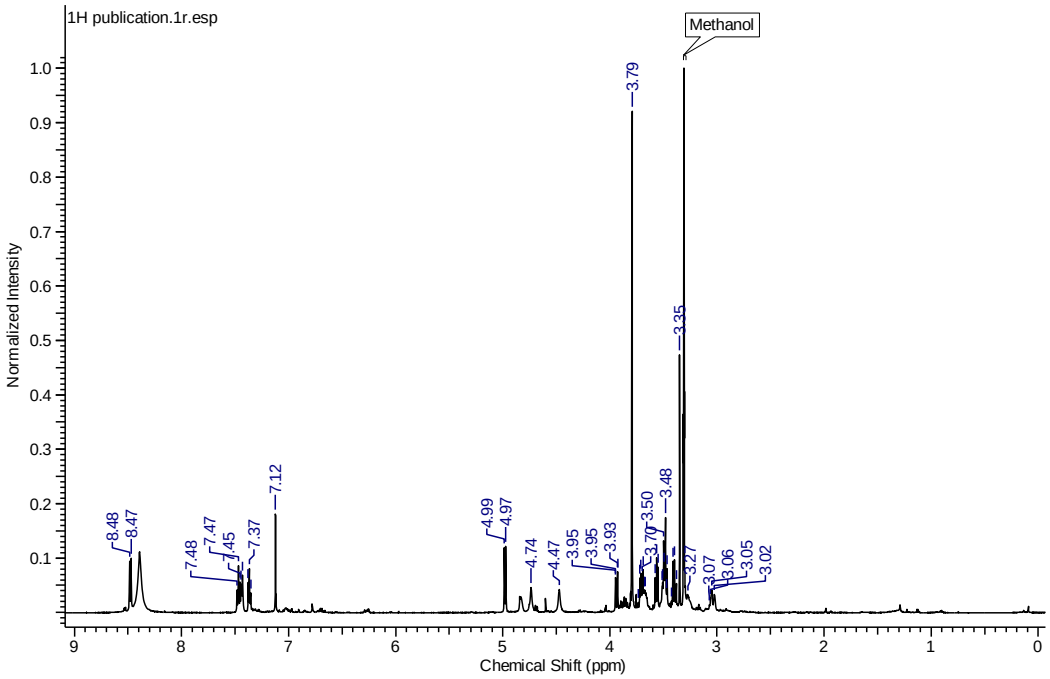

Figure S5 COSY of compound (**1**).

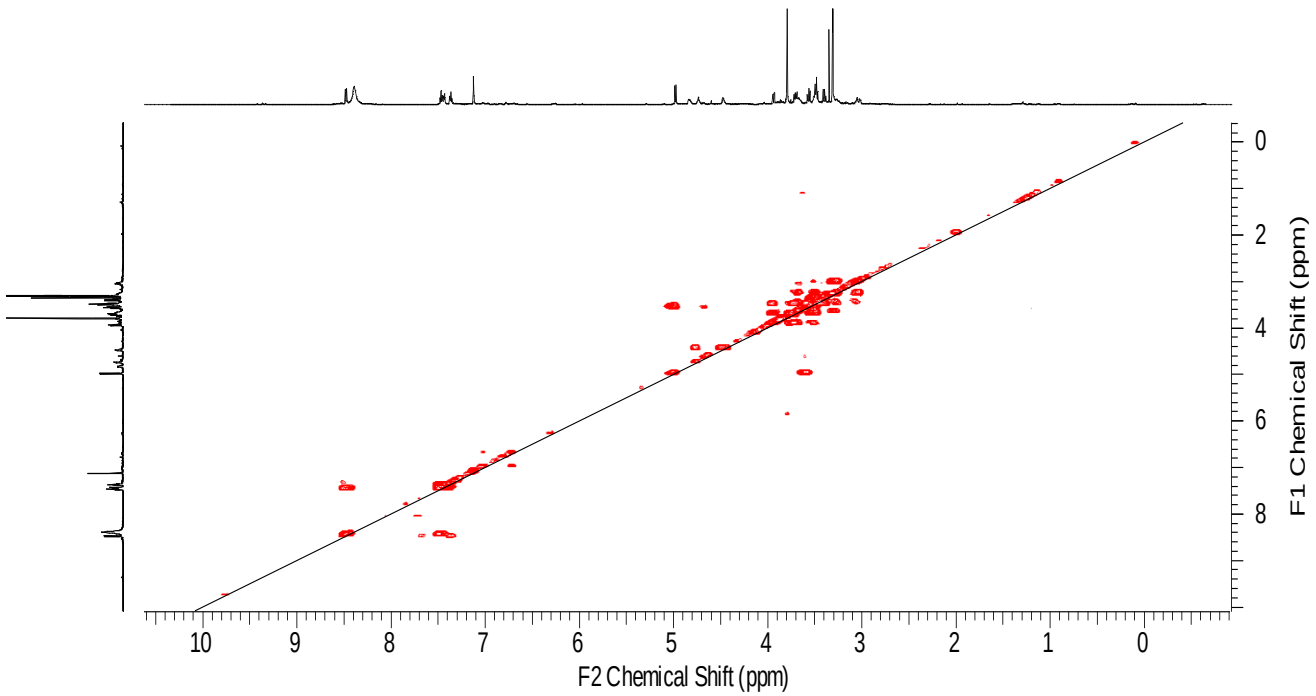

Figure S6 HSQC of compound (**1**).

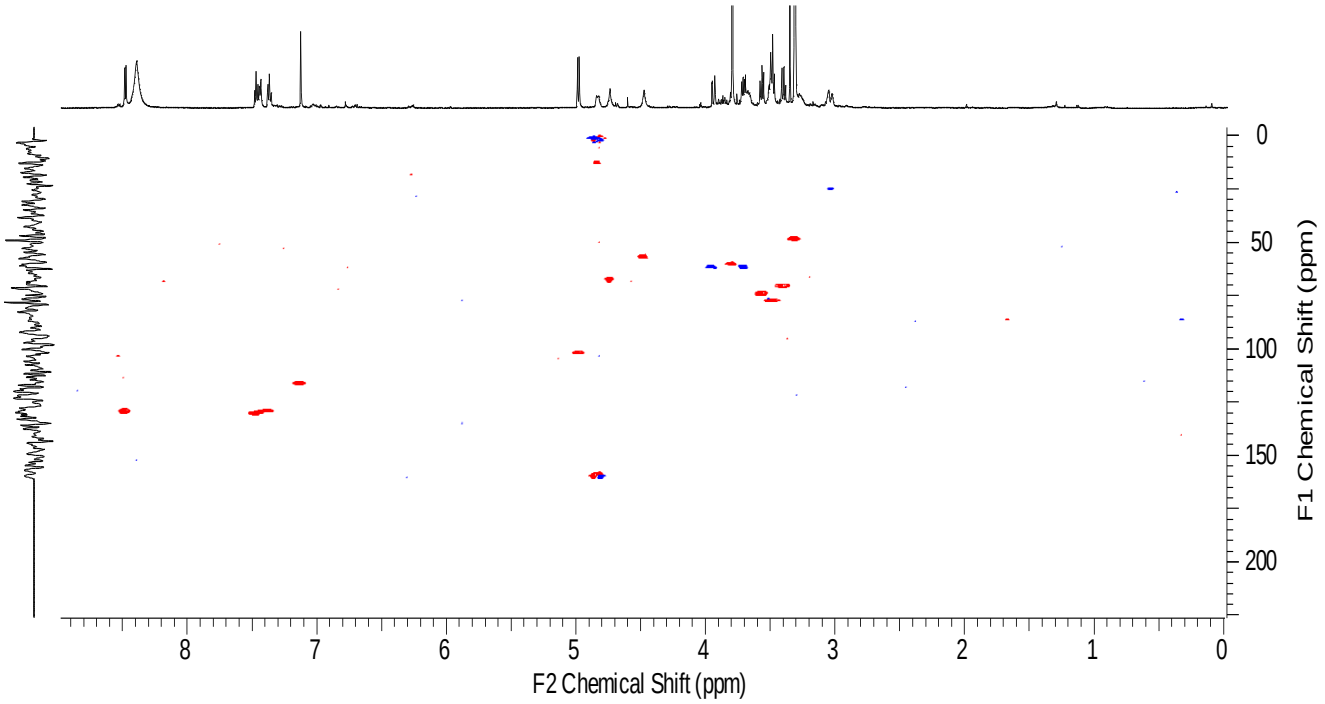

Figure S7 HMBC of compound (**1**).

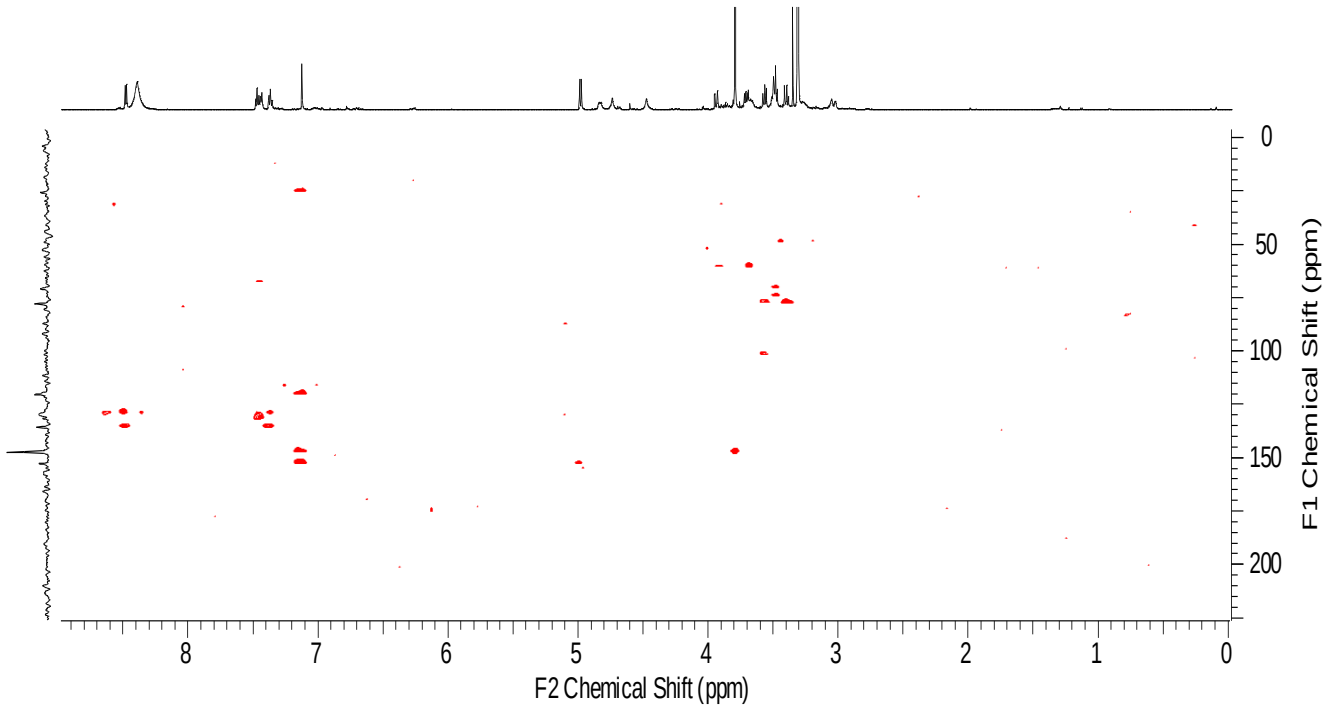

Figure S8 NOESY of compound (**1**).

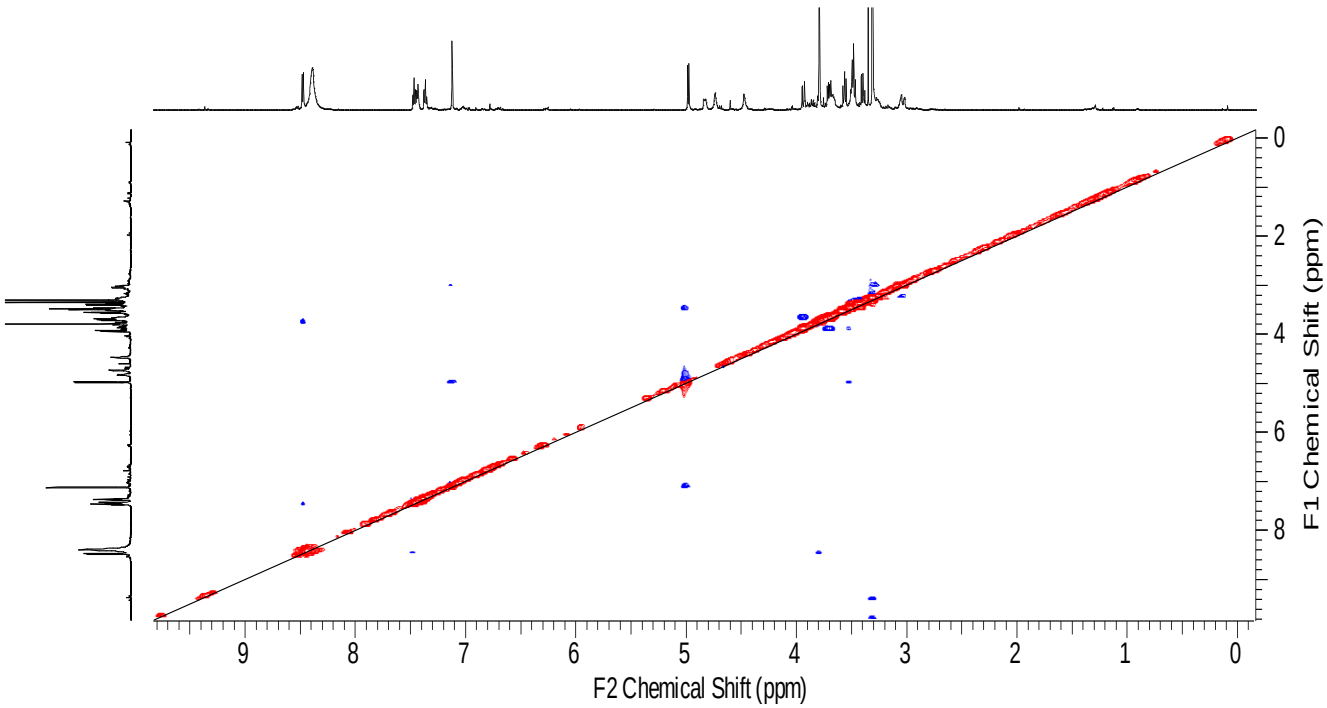

Figure S9 Key correlations of NOESY and HMBC in compound (**1**).

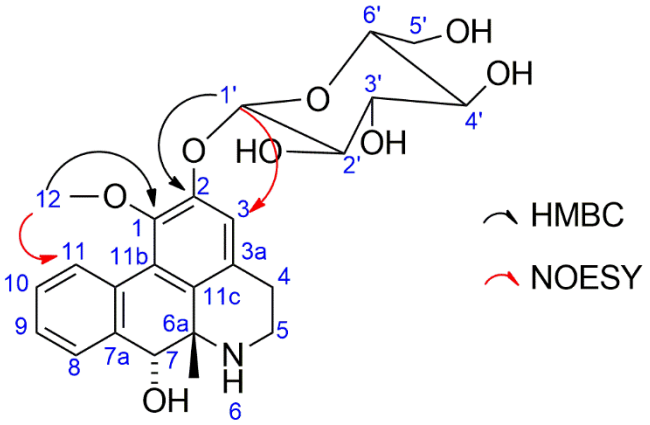

Figure S10. Accuracy profile for quantitative methods validation of THP (A), PAL (B), and ROE (C) in tuber of *S. cambodica*. (—) = bias (%), (----) = acceptance limit (±10%) for THP and ROE, (±20%) for PAL, (·····) = 95% β-expectation tolerance interval.

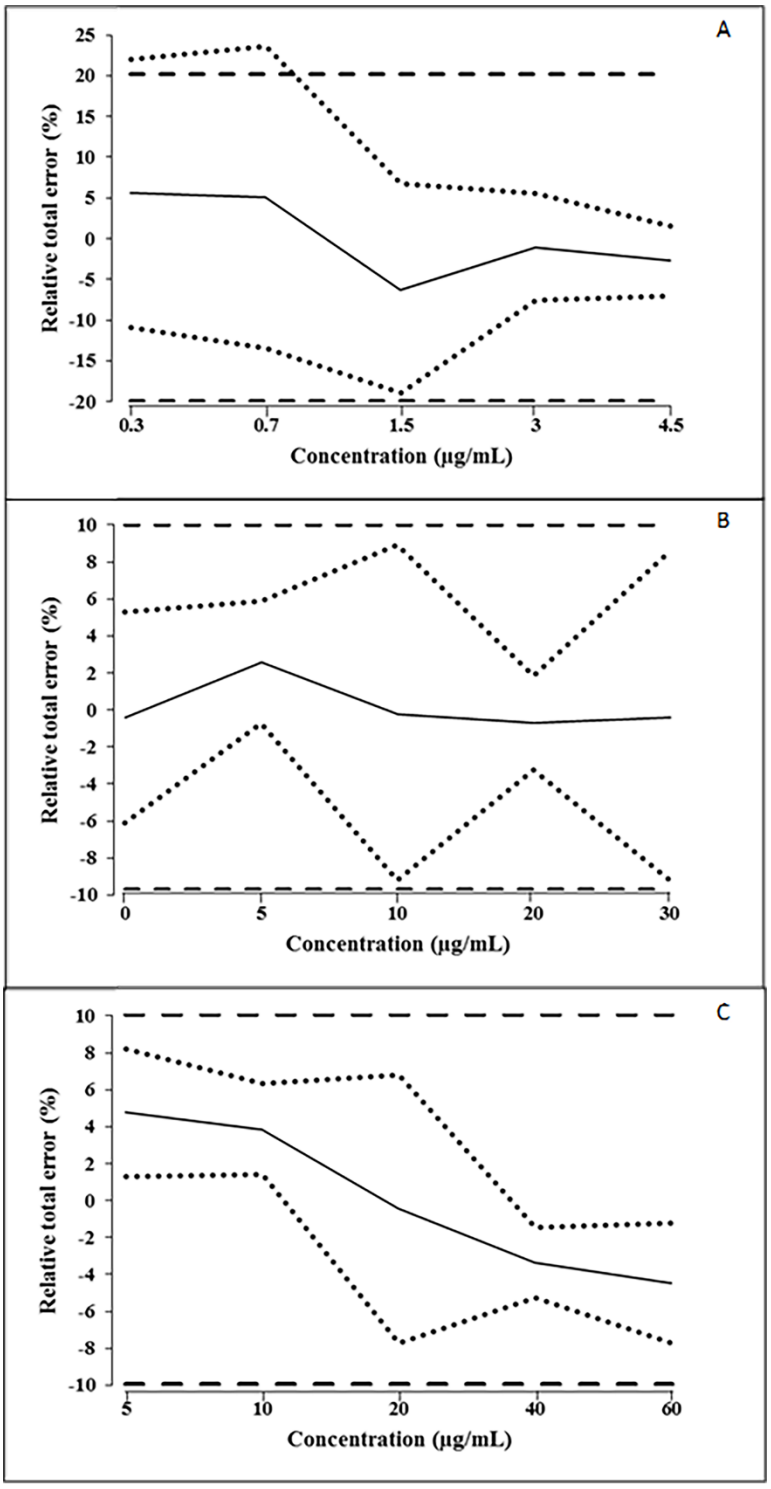

Table S1. ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (150 MHz, CD_3OD) chemical shifts of angkorwatine (**1**).

Position	^{1}H-NMR	^{13}C-NMR
1	—	147.5
2	—	153.3
3	7.21 ; s	117.3
3a	—	127.1
4	3.27 ; m	26.2
	3.03; brdd (17.0; 3.5)	
5	3.69 ; m	42.6
	3.49 ; m	
6a	4.47; brs	57.9
7	4.74 ; brs	68.7
7a	—	135.9
8	7.43 ; dd (7.4; 1.0)	130.3
9	7.36 ; td (7.4; 0.9)	129.5
10	7.47 ; td (7.5; 1.7)	130.9
11	8.48 ; dd (8.0; 0.7)	130.0
11a	—	131.9
11b	—	126.9
11c	—	120.6
12	3.79; s	61.1
1'	4.98; d (7.8)	102.5
2'	3.56; dd (9.1; 7.8)	74.9
3'	3.48; dd (9.0)	78.4
4'	3.40; dd (9.4; 9.0)	71.5
5'	3.50; ddd (9.4; 5.9; 2.4)	78.4
6'	3.94 ; dd (12.1; 2.4)	62.3
	3.71 ; dd (12.1; 5.9)	

Table S2. Injection repeatability and column performance parameters in system suitability test.

Test parameters	THP	PAL	ROE	FDA specifications
Resolution	18	5	4	≥ 2
Theoretical plates	23320	35801	39302	> 2000
K’	6	7	8	> 2
Symmetry factors	0.6	0.6	0.6	≤ 2

Table S3. Results of validation of UHPLC-DAD method dedicated to the quantification of THP, PAL, ROE.

Validation criterion	**PAL**		**ROE**		**THP**	
Response function (m=3; n=2)						
Y=ax						
Day 1	R^2=0.9992		R^2=0.9997		R^2=0.9996	
Day 2	R^2=0.9992		R^2=0.9993		R^2=0.9996	
Day 3	R^2=0.9996		R^2=0.9997		R^2=0.9995	
Linear Range (µg/ml)	1.67−33.20		1.54−30.80		4.24−84.70	
Trueness (k=3; p=3; n=3)	Concentration (µg/ml)	Relative biais (%)	Concentration (µg/ml)	Relative biais (%)	Concentration (µg/ml)	Relative biais (%)
Level 1	0.31	5.42	2.01	-0.57	4.29	4.68
Level 2	0.70	4.95	5.12	2.46	10.74	3.80
Level 3	1.39	-2.39	10.24	-0.42	21.47	-0.51
Level 4	2.79	2.63	20.48	-0.87	42.94	-3.44
Level 5	4.18	0.83	30.72	-0.59	64.42	-4.53
Precision (p=3; n=3)	Repeatability RSD (%)	Intermediate Precision RSD (%)	Repeatability RSD (%)	Intermediate Precision RSD (%)	Repeatability RSD (%)	Intermediate Precision RSD (%)
Level 1	6.59	6.59	1.79	2.08	1.38	1.38
Level 2	7.42	7.42	1.34	1.34	0.87	0.94
Level 3	5.03	5.20	3.64	3.71	2.91	2.91
Level 4	2.64	2.64	0.88	0.98	0.76	0.76
Level 5	1.72	1.72	2.05	2.97	1.29	1.29
Accuracy (p=3; n=3)	β-expectation lower and upper tolerance limits of the relative errors (%)		β-expectation lower and upper tolerance limits of the relative errors (%)		β-expectation lower and upper tolerance limits of the relative errors (%)	
Level 1	-11.00; 21.84		-6.34; 5.19		1.22; 8.12	
Level 2	-13.56; 23.45		-8.79; 5.79		1.35; 6.25	
Level 3	-19.48; 6.60		-9.69; 8.86		-7,77; 6.74	
Level 4	-7.71; 5.43		-3.44; 1.69		-5.34; -1.53	
Level 5	-7.13; 1.44		-9.71; 8.53		-7.74; -1.31	
Linearity						
Slope	0.9721		0.9939		0.9623	
r^2	0.9975		0.9993		0.9996	
Lower LOQ	1.39 µg/mL		2.01 µg/mL		4.29 µg/mL	
Upper LOQ	4.18 µg/mL		30.72 µg/mL		64.42 µg/mL	
LOD	0.19 µg/mL		0.38 µg/mL		2.36 µg/mL	

m: number of calibration points; p: days; n: replicates. PAL=palmatine, ROE=roemerine,

THP: tetrahydropalmatine. LOD= limits of detection, LOQ= limit of quantification.

Table S4. Content of palmatine (PAL), roemerine (ROE) and tetrahydropalmatine (THP) in vegetal material of *S. cambodica.*

Samples	Collection date	PAL (%)	ROE (%)	THP (%)
Stem (Dary18)	March 2014	0.20	0.74	2.89
Tuber 1 (Dary18)	March 2014	0.16	1.04	2.24
Tuber 2 (Dary18)	March 2014	0.23	1.23	3.48
Tuber 3 (Hul et al.5020)	July 2015	0.35	1.14	2.30