Food Reviews International, 1-14. **12**. Jia X., Ye J., Wang H., Li L., Wang F., Zhang Q., Chen J., Zheng X., He H. (2018), Characteristic amino acids in tea leaves as quality indicator for evaluation of Wuyi Rock Tea in different cultured regions, *Journal of Applied Botany and Food Quality*, 91, 187-93. **13**. R Team, R. D. C. T. R: A language and environment for statistical computing. Vienna, Austria: R *Foundation for Statistical Computing*, 2014. URL https://www.R-project.org/. **14**. Kassambara A. (2018), Machine learning essentials: Practical guide in R, *Sthda*, 2018. **15**. Feng Z. H., Li Y. F., Li M., Wang Y.J., Zhang L., Wan X. C., Yang X. G. (2019), Tea aroma formation from six model manufacturing processes, *Food Chemistry*, 285, 347-354. **16**. Lee L. S., Choi J. H., Son N., Kim S. H., Park J. D., Jang D. J., Jeong Y., Kim H. J. (2013), Metabolomic analysis of the effect of shade treatment on the nutritional and sensory qualities of green tea, *Journal of Agricultural and Food chemistry*, 61(2), 332-338. **17**. Tang Q., Tan P., Ma N., Ma X. (2021), Physiological Functions of threonine in animals: beyond nutrition metabolism, *Nutrients*, 13(8), 2592. **18**. Hernández-Orte P., Cacho J. F., Ferreira V. (2002), Relationship between varietal amino acid profile of grapes and wine aromatic composition. Experiments with model solutions and chemometric study, *Journal of Agricultural and Food chemistry*, 50(10), 2891-2899. **19**. Wong K. H., Abdul Aziz S., & Mohamed S. (2008), Sensory aroma from Maillard reaction of individual and combinations of amino acids with glucose in acidic conditions, *International journal of food science & technology*, 43(9), 1512-1519. **20**. Yılmaz C., Özdemir F., Gökmen V. (2020), Investigation of free amino acids, bioactive and neuroactive compounds in different types of tea and effect of black tea processing, *LWT*, 117, 108655.

Journal of Medicinal Materials, 2023, Vol. 28, No. 3 (pp. 160 - 165)

EXTRACTION, ISOLATION, AND QUANTITATIVE ANALYSIS OF KADSURIN IN *KADSURA COCCINEA* FRUITS BY HPLC-DAD METHOD

Le Thi Loan[1], Vu Thi Diep[1], Nguyen Thi Thu[1], Nguyen Duc Thang[2], Pham Anh Tam[2], Nguyen Mau Toan[2], Nguyen Thi Mai[3], Do Thi Ha[1,*]

[1]National Institute of Medicinal Materials, Hanoi, Vietnam; [2]Management Board of Xuan Lien Nature Reserve, Thanh Hoa, Vietnam; [3]Hong Duc University, Thanh Hoa, Vietnam

*Corresponding author: hado.nimms@gmail.com

(Received June 06th, 2023)

Summary

Extraction, Isolation, and Quantitative Analysis of Kadsurin in *Kadsura coccinea* Fruits by HPLC-DAD Method

From fruits of *Kadsura coccinea*, kadsurin, a lignan was isolated and characterized. Kadsurin content in *K. coccinea* samples was quantitatively determined using an HPLC-DAD method, an Agilent XDB C_{18} reversed-phase column, a gradient condition of acetonitrile (A) and 0,1% phosphoric acid in water (B). The flow rate was 1.0 mL/min, the column temperature was set at 40ºC; the injection volume was 10 µL and the detection wavelengths were set at 217 nm. The method was validated for the specificity (RSD of peak area = 1.32%; RSD of tR = 0.04%); linearity ranging from 2.04 to 65.28 µg/mL (r = 0.9999), precision (RSD < 2.0%) and high accuracy (recovery: 98.84% - 99.00%). The limits of detection (LOD) and quantification (LOQ) were 0.026 µg/mL and 0.084 µg/mL, respectively. The method was then applied to determine the quantity of kadsurin in *K. coccinea* fruits collected in Xuan Lien Nature Reserve. The results revealed that the content of kadsurin varied from 0.12% to 0.47%.

Keywords: *Kadsura coccinea*, *Lignan, Kadsurin.*

1. Introduction

Kadsura coccinea (Lem) A. C. Smith is an evergreen climbing shrub of Schisandraceae, has bear fruits that are similar to sugar apples (*Annona squamosa*). In Vietnam, *Kadsura coccinea* is known as "Nắm cơm", "Na leo", "Na rừng". *Kadsura coccinea* contains compounds belonging to the group of lignans, triterpenoids, phenolics, flavonoids, and anthocyanins [1],[2],[3]. Lignans and its derivatives are the major components of the species *K. coccinea*. Up to now, there have been about 91 lignan compounds isolated from *K. coccinea* and were classified into 4 groups according to different frameworks, including dibenzo cyclooctadiene, spiro benzo furanoid, dibenzo cyclooctadiene, diaryl butane and aryl tetralin lignan [4]. In addition to lignans, triterpenoids are also major constituents of *K. coccinea* with about 111 compounds reported [4]. As a folk medicine, the roots and stems are often employed for reducing inflammation and relieving pain, such as gastroenteritis, rheumatoid arthritis, bruises, and dysmenorrhea. *K. coccinea* fruits cure kidney pain, backache, cough, bronchitis, and neurasthenia [5]. The results of research on biological effects show that *K. coccinea* has anti-cancer [6],[7], anti-oxidant [3], acetylcholinesterase enzyme inhibitory [8],[9], and anti-HIV effects [10].

Although *K. coccinea* is a widely used as a medicinal herb, however until now, the study on quality assessment and standard development for this medicinal herb is still very limited. This medicinal herb hasn't been mentioned in the Vietnamese pharmacopoeia V. Some studies evaluated the total phenolic content, and the anthocyanin content in *K. coccinea* fruits [1],[2],[11]. However, the results of analysis by UPLC-QTOF/MS showed that lignan compounds were the main compounds and had relatively high concentrations in stems of *K. coccinea* [12]. Therefore, in this publication, we extracted and isolated a major lignan compound from *K.*

coccinea fruits and developed a method to quantify this compound in *K. coccinea* fruits by the HPLC-DAD method.

2. Materials and methods

2.1. Plant material

The whole fruits of *K. coccinea* were collected at Xuan Lien Nature Reserve in July 2020. Fresh samples were washed, chopped, and dried at 50ºC, stored in sealed plastic bags before extraction.

2.2. General experimental procedures

The NMR spectra were obtained on a Bruker AM500 FT-NMR spectrometer (Karlsruhe, Germany) using TMS as an internal standard. The electrospray ionization mass spectroscopy (ESI-MS) was obtained on an LC-MS/MS (Shimadzu, Japan). *Silica gel* (Merck, 40 - 63 µm particle size) was used for column chromatography (CC). Thin-layer chromatography (TLC) was carried out with *silica gel* $60F_{254}$ and RP-$18F_{254}$ plates. Spots were detected under 254 and 366 nm UV light or by spraying with 10% sulfuric acid in 96% ethanol (EtOH) and heating. HPLC analysis was performed on a Shimadzu system, consisting of a binary pump LC-20AD, a SIL-20A autosampler, a CTO-20A column oven, and a diode-array detector SPD-M20A.

2.3. Extraction and isolation

The fruits *K. coccinea* were hot-extracted with 80% ethanol to obtain crude extract. The crude extract was then further isolated by normal-phase *silica gel* and reversed-phase *silica gel* column chromatography, monitored eluate by TLC, and detected under 254 and 366 nm UV light or by spraying with 10% sulfuric acid in 96% ethanol (EtOH) and heating. Purify by repeated termination in methanol. The chemical structures of the compounds were determined by NMR data (^{1}H- and ^{13}C-NMR, DEPT, HSQC, HMBC, COSY...), and combined with the characterizations. physicochemical (sensory, melting point) and comparison with published data.

2.4. HPLC analysis of kadsurin in K. coccinea fruits

Sample preparation: Standard stock solution of kadsurin was prepared by dissolving it in methanol, then diluted to establish calibration curves in ranges of 2 - 64 µg/mL. The stock and working solutions were stored at 4°C.

Sample extraction: sample preparation factors were investigated including extraction solvents (ethanol, methanol, ethanol 70%, methanol 70%, ethanol 50%, methanol 50%), extraction method (ultrasonic and heat reflux methods), extraction time (30 - 120 min), the ratio of the mass of the sample to the volume of the solvent (g/mL) (1/50, 1/125, 1/250). Select the test sample preparation method to get the highest content of kadsurin.

HPLC condition: The separation of the compound was performed on an Agilent C_{18} column (250 x 4.6 mm, 5 µm). The column temperature was maintained at 40°C. The mobile phase was a mixture of acetonitrile and 0.1% phosphoric acid in water (55 - 45, v/v). The flow rate was kept at 1.0 mL/min. The injection volume was 10 µL. Detection was set at a wavelength of 217 nm.

Method validation: the method was validated according to ICH guidelines [13].

The kadsurin content is calculated as follows:

$$X\ (\%) = \frac{C \times V \times 100 \times P \times 100}{M \times 1000 \times (100 - a) \times 100} = \frac{C \times V \times P}{M \times 10 \times (100 - a)}$$

C: the concentration of the analyzed compound in the sample solution from the calibration curve equation (µg/mL); V: volume of the sample solution (mL); m: weight of tested sample taken to prepare the sample solution (mg); a: the moisture of powder (%); P: purity of standard (%).

3. Results and Discussion

3.1. Extraction and isolation

The fruits of *K. coccinea* (2.4 kg) were powdered and hot-extracted with 80% EtOH (3 times, 3 hours each time, ratio of solvent to sample 8:1, L/kg). The combined extracts were filtered and evaporated under reduced pressure to yield a brown residue (500.0 g). The residue (450 g) was separated using *silica gel* CC and eluted with a gradient of *n*-hexane - acetone (100% *n*-hexane, 30:1 - 10:1, v/v) and dichloromethane - methanol (100% dichloromethane, 10:1 - 1:1, v/v) to obtain 12 fractions (Q1A- Q1M). Fraction Q1H (30,0 g) was fractionated by *silica gel* CC, using *n*-hexane - acetone (8:2 - 7:3, v/v) as mobile phase to yield 10 fractions (Q2A-Q2K). Fraction Q2D-F (5.0 g) was separated by RP-C_{18} gel CC employing methanol - water (7:3, v/v) to obtain 5 fractions (Q3A-Q3E). Fraction Q3B (900 mg) was crystallized in methanol to give compound **KCQ-1** (300.0 mg).

Compound **KCQ-1**: white powder, the purity 95.02% calculated by HPLC peak area, ^{1}H-NMR (500 MHz, CD_3OD): 5.30 (1H, br d, J = 3.0 Hz, H-25), 5.09 (1H, t, J = 7.0 Hz, H-42), 4.84 (1H, br s, H-46), 4.78 (1H, br s, H-46), 1.77 (3H, s, H-47), 1.66 (3H, s, H-44), 1.57 (3H, s, H-45), 1.01 (3H, s, H-48), 0.87 (3H, d, J = 6.5, H-39), 0.80 (3H, s, H-37), 0.73 (3H, s, H-36). ^{13}C-NMR (125 MHz, CD_3OD): 174.8 (C-3), 149.3 (C-4), 146.2 (C-8), 129.9 (C-25), 124.7 (C-24), 117.1 (C-7), 111.3 (C-28), 52.2 (C-17), 50.9 (C-14), 44.7 (C-

15), 43.1 (C-13), 38.4 (C-9), 35.7 (C-10), 35.5 (C-22), 35.1 (C-20), 33.5 (C-15), 33.3 (C-12), 28.8 (C-6), 28.6 (C-1), 28.3 (C-2), 27.5 (C-16), 27 (C-30), 25.1 (C-29), 24.8 (C-26), 24.2 (C-23), 23.4 (C-19), 21.1 (C-18), 17.9 (C-21), 17.9 (C-11), 17.1 (C-27).

The ^{1}H-NMR spectrum of **KCQ-1** displayed signals of two aromatic protons at δ_H 6.56 (1H, s, H-4) and 6.46 (1H, s, H-11), an OCH_2O group at δ_H 5.97 (1H, d, J = 1.0 Hz) and 5.95 (1H, d, J = 1.5 Hz), a hydroxymethine group at δ_H 5.64 (1H, s, H-9), a methylene group at δ_H 2.64 (2H, d, J = 5.0 Hz, H-6), two methine groups at δ_H 2.06 (1H, m, H-7) and 2.00 (1H, J = 1.0; 8.0 Hz, H-8), four methoxy groups at δ_H 3.80 (3H, s, 1-OCH_3), 3.87 (3H, s, 2-OCH_3), 3.89 (3H, s, 3-OCH_3), and 3.62 (3H, s, 14-OCH_3), and an acetyl group at δ_H 1.37 (3H, s). The ^{13}C-NMR and DEPT spectra of **KCQ-1** exhibited signals for 25 carbons, including an acetyl group (δ_C 170.1 and 20.7), four methoxy groups (δ_C 60.7, 60.3, 59.7, and 56.0), a methylene group (δ_C 38.8), an OCH2O group (δ_C 101.2), a hydroxymethine group (δ_C 82.3), two methine groups (δ_C 34.9 and 41.9), two methyl groups (δ_C 14.8 and 19.5), and ten carbons not bonded to hydrogen (δ_C 151.6, 150.9, 148.6, 141.4, 139.6, 136.0, 134.9, 133.3, 123.3, and 120.1). The 1D-NMR spectral data suggested that **KCQ-1** has a dibenzocyclooctadiene lignan structure. The position of the acetyl group was determined by the interaction between the proton H-9 (δ_H 5.64) and the carbonyl carbon (δ_C 170.1), as well as with carbon C-7 (δ_C 34.9), C-8 (δ_C 41.9), C-10 (δ_C 134.9), C-11 (δ_C 102.4), C-15 (δ_C 120.1), and C-17 (δ_C 19.5). The positions of the two methoxy groups were assigned to C-2 and C-3 based on the interaction between the proton of the methoxy group and the corresponding carbons (δ_H 3.87 → δ_C 139.6 and δ_H 3.89 → δ_C 151.6), as well as the interaction between H-4 (δ_H 6.56) and C-2 and C-3. The remaining two methoxy groups at C-1 and C-14 were also determined through the interaction between the proton of the methoxy group and the corresponding carbons (δ_H 3.62 → δ_C 150.9 and δ_H 3.80 → δ_C 141.4), combined with reference comparison [14]. The methyl group at position C-8 was identified through the interaction between H-17 (δ_H 1.06) and C-7, C-8, and C-9 (δ_C 82.3). The other methyl group at C-7 was also determined through the interactions observed in the HMBC spectrum between H-18 (δ_H 0.91) and C-6 (δ_C 38.8), C-7, and C-8. Furthermore, the HMBC spectrum indicated interactions between the protons of the OCH_2O group (δ_H 5.97 and 5.95) with the carbons C-12 (δ_C 148.6) and C-13 (δ_C 136.0), and between H-11 (δ_H 6.46) and the carbons C-9, C-10, C-12, C-13, and C-15 (δ_C 120.1), as well as interactions between H-4 and C-2, C-3, C-5, C-6, and C-16 (δ_C 123.3). Based on the aforementioned analyses and in combination with references [14],[15] **KCQ-1** can be identified as kadsurin (Fig. 1).

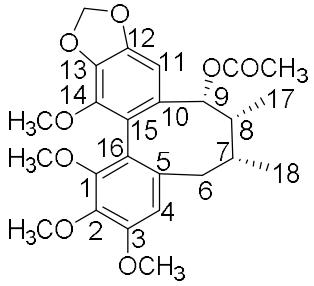

Fig. 1. Chemical structure of kadsurin

3.2. HPLC analysis of kadsurin in K. coccinea fruits

3.2.1. Optimization of chromatographic condition:

Based on the maximum absorption of kadsurin in the UV-Vis spectrum, the detection was set at 217 nm.

Other HPLC parameters, including mobile phase, flow rate, elution program, column oven temperature, sample injection volume were examined to choose the best separation conditions. The selected chromatographic conditions are as followed: Agilent C_{18} column (250 x 4.6 mm, 5 µm). The column temperature was maintained at 40°C. The mobile phase was a mixture of acetonitrile and 0.1% phosphoric acid in water (55-45, v/v). The flow rate was kept at 1.0 mL/min. The injection volume was 10 µL.

3.2.2. Optimization of extraction conditions:

The results are presented in Table 1. From the investigation results, the sample preparation procedure was selected as follows: 200,0 mg of fruit powder was refluxed with 25 mL methanol for 2 hours. The solution was filtered through a 0.45 µm membrane filter before HPLC analysis.

Table 1. The results of extraction conditions optimization

Extraction solvents (m= 200 mg, solvent volume 25 mL, heat reflux in 60 min)		**Extraction methods and extraction time** (m= 200mg, solvent volume 25 mL of methanol)	
Solvent	**Kadsurin (%)**	**Conditions**	**Kadsurin (%)**
Methanol	**0.288**	Sonication 30 min	0.235
Methanol 70%	0.278	Sonication 60 min	0.252
Methanol 50%	0.272	Sonication 90 min	0.275

Extraction solvents (m= 200 mg, solvent volume 25 mL, heat reflux in 60 min)		Extraction methods and extraction time (m= 200mg, solvent volume 25 mL of methanol)	
Ethanol 95%	0.282	Reflux 60 min	0.288
Ethanol 70%	0.276	Reflux 90 min	0.293
Ethanol 50%	0.270	**Reflux 120 min**	**0.299**
		Reflux 150 min	0.298
Ratio of the mass of the sample to the volume of the solvent (g/mL) (m= 200 mg, solvent methanol, reflux in 120 min)			
Conditions	**Kadsurin (%)**	**Conditions**	**Kadsurin (%)**
200 mg/ 10 mL	0.270	200 mg/50 mL	0.300
200 mg/ 25 mL	**0.299**	200 mg/ 25 mL, 2 times	0.300

3.2.3. Validation of developed HPLC method:

Specification

The experiment was performed with the blank, standard, and sample solutions according to the analytical procedure. The obtained chromatograms showed in Fig.s 2 and 3.

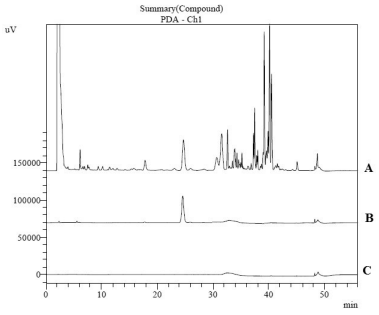

Fig. 2. Chromatograms for specificity study (A: sample solution; B: standard; C: blank)

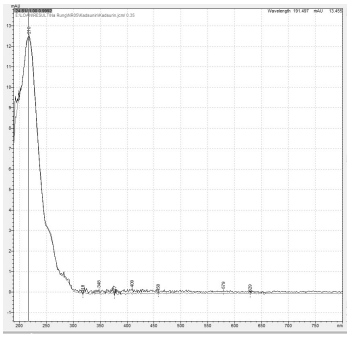

Fig. 3. Comparing the UV spectrums of kadsurin in standard and sample chromatograms

The results showed that there was no peak in the blank sample at the retention times of the targeted peaks in the standard solution. The obtained chromatograms showed clear separation peaks and low background noise. Additionally, when comparing the UV spectrum of standard solutions (match ratio) was 0.9992. Therefore the analytical method was specific for the determination of the targeted marker.

System suitability

Table 2 showed that the RSD % of retention time and peak area were all under 2%. These indicated that the developed method conforms to system suitability criteria.

Table 2. System suitability testing

No	Retention time (min)	Peak area (mAu.s)
1	24.558	233121
2	24.579	230625
3	24.581	226051
4	24.588	234578
5	24.580	230572
6	24.573	233395
Mean	24.577	231390.3
SD	0.010	3064.0
RSD (%)	0.04	1.32

Linearity

Different concentrations of kadsurin were prepared and injected into the HPLC system. The calibration curve was constructed by plotting the peak areas versus the concentrations of the analyte. The result showed satisfactory linearity in the concentration ranges of 2.04 - 65.28 µg/mL for kadsurin. The typical calibration curve equation of kadsurin was y = 49922x - 17364. Linear correlation value $r^2 = 0.9999$. Therefore, it can be concluded that this developed method conforms the linearity.

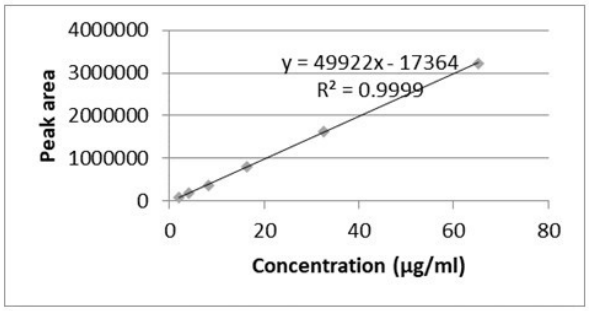

Fig. 4. Illustrating the linearity between kadsurin concentration and its corresponding peak area

Precision

The results of repeatability and intermediate precision study werer presented in Table 3. The average content of kadsurin is 0.297% and the RSD all under 2.0%. The result was in good

agreement with acceptable values for validation of analytical procedure.

Table 3. The precision results

No.	Kadsurin content (%)		
	Day 1	Day 2	
1	0.298	0.296	**Average**: 0.297 ± 0.002% **RSD**: 0.58%
2	0.301	0.299	
3	0.297	0.297	
4	0.296	0.298	
5	0.296	0.295	
6	0.299	0.296	
Average (%)	0.298	0.297	
SD (%)	0.002	0.001	
RSD (%)	0.66	0.50	

3.2.5. Accuracy

5.02 mg of kadsurin was weighed and dissolved in 10 mL of methanol. Dilute this solution with methanol to obtain solutions with a corresponding concentration of 5.02; 10.04 and 20.08 µg/mL. These solutions were added to the known samples, then extraction and analysis were done. The results in Table 4 showed that recovery rates of kadsurin were in the range of 95-105%. The recovery values were 98.01 - 99.80% indicating that the accuracy of the method was acceptable.

LOD and LOQ

The limits of detection (LOD) and the limits of quantification (LOQ) were calculated by diluting standard solutions until signal-to-noise ratios of 3 and 10, respectively. LOD and LOQ were estimated to be 0.026 µg/mL and 0.084 µg/mL for kadsurin, corresponding 3.61 µg/g and 11.67 µg/g in samples.

Table 4. The accuracy testing results

Amount added (µg/mL)	Amount found (µg/mL)	Recovery (%)	M ± SD (%)	RSD (%)
5.02	4.92	98.01	99.00 ± 0.91	0.92
5.02	4.98	99.20		
5.02	5.01	99.80		
10.04	9.89	98.51	98.84 ± 0.35	0.35
10.04	9.92	98.80		
10.04	9.96	99.20		
20.08	19.82	98.71	98.92 ± 0.20	0.20
20.08	19.90	99.10		
20.08	19.87	98.95		

The analytical method for the determination of kadsurin in *K. coccinea* fruits met the requirements of systematic suitability, selectivity, linearity, precision, and accuracy according to ICH criteria. Therefore, this method could be used to analyze kadsurin in *K. coccinea* fruits.

3.2.4. Sample analysis

The proposed method was applied for the determination of kadsurin in 6 samples *K. coccinea* collected in Xuan Lien Nature Reserve. Each sample was analyzed in triplicate to determine the meant content (%). The results were summarized in Table 5.

Table 5. Kadsurin content in the fruits of *K. coccinea*

No.	Sample name	Moisture (%)	Weight (mg)	Peak area (mAu.s)	Concentration (µg/mL)	Content (%)	M ± SD (%)
1	Q1	9.55	199.66	1323190	25.51	0.353	0.346 ± 0.006
			207.01	1326724	25.58	0.342	
			205.12	1325075	25.55	0.344	
2	Q2	10.75	208.79	1841145	35.37	0.474	0.470 ± 0.004
			197.42	1719012	33.04	0.469	
			204.45	1772562	34.06	0.467	
3	Q3	9.85	205.25	1141836	22.06	0.298	0.299 ± 0.002
			203.12	1140671	22.04	0.301	
			203.31	1127722	21.79	0.297	
4	Q4	21.6	207.33	383112	7.62	0.117	0.119 ± 0.002
			186.83	346046	6.92	0.118	
			186.83	353249	7.05	0.120	
5	Q5	8.90	208.19	683112	13.33	0.176	0.174 ± 0.002
			202.18	646046	12.62	0.171	
			201.85	653249	12.76	0.173	
6	Q6	9.35	202.18	703362	13.72	0.187	0.191 ± 0.004
			196.32	706612	13.78	0.194	
			199.05	717357	13.98	0.194	

Table 5 showed that the content of kadsurin in the fruits of *K. coccinea* collected in Xuan Lien Nature Reserve varied from 0.174 ± 0.002 to 0.470 ± 0.004%.

3.2.8. Discussion

According to an overview report on *K. coccinea* species by Yang et al. (2020), the main components of this species are lignan and

triterpenoid compounds [4]. In addition, some studies also show the presence of phenolics, flavonoids, and anthocyanins groups [1],[2],[3]. The results of our previous study showed that fruits contain tannins, reducing sugars, sterols, carotenes, saponins, amino acids, organic acids, flavonoids, fats, and polysaccharides. In this study, kadsurin was isolated from the fruits of *K. coccinea*. Kadsurin is a lignan belonging to the dibenzo cyclooctadiene structure, this is one of the four main groups of lignans isolated from *K. coccinea* with about 70 published compounds [4]. In addition, studies on the activity of this compound have also been published as an anti-tumor [16] and inhibiting lipid peroxidation [17]. Therefore, kadsurin was selected as a marker for the quality assessment of *K. coccinea* fruits.

In this paper, a method to quantify kadsurin in *K. coccinea* fruits by HPLC has been developed and validated. The validation results showed that the developed method is selective, specific, accurate, and suitable for the quantification of kadsurin *K. coccinea* fruits. Quantitative results showed that the studied samples have kadsurin marker content ranging from 0.119 ± 0.002% to 0.470 ± 0.004% by weight of dried fruits.

The standard used in this study, kadsurin, was isolated from the medicinal plant with purity calculated as HPLC peak area ratio. This may lead to inaccurate determination of kadsurin purity and its content in *K. coccinea* fruits. This is the limitation of this research and should be improved in further studies.

Generally, the above results are important suggestions in selecting a marker for quality assurance of *K. coccinea* fruits.

4. Conclusion

In this study, the main lignan compound kadsurin (**KCQ-1**) from *K. coccinea* fruits was extracted, isolated, and selected as a reference for quality control of this medicinal herb. The method for quantifying kadsurin in *K. coccinea* fruits by HPLC has been developed and validated, showing that the method is selective, specific, accurate, and suitable for the quantification of kadsurin in *K. coccinea* fruits. In addition, the quantitative results showed that the *K. coccinea* fruit samples collected at Xuan Lien Nature Reserve had kadsurin content ranging from 0.119 ± 0.002% to 0.470 ± 0.002% by dried weight of dried fruits.

Acknowledgment: *This work was supported by the project: "Research, exploitation, and development of medicinal genetic resources of Na rừng (Kadsura coccinea (Lem.) A. C. Smith) in the North Central provinces", code No. NVQG-2019/DT.16.*

References

1. Hao J., Zhu H., Liu S., Li H. (2014), Characterization of anthocyanins in fruit of *Kadsura coccinea* (Lem.) AC Smith by UPLC/Q-TOF-MS analysis and evaluation of stability of the major anthocyanins, *Food Analytical Methods,* 7(6), 1312-1322. **2**. Sritalahareuthai V., Temviriyanukul P., On-Nom N., Charoenkiatkul S., Suttisansanee U. (2020), Phenolic profiles, antioxidant, and inhibitory activities of *Kadsura heteroclita* (Roxb.) Craib and *Kadsura coccinea* (Lem.) AC Sm, *Foods,* 9(9), 1222. **3**. Sun J., Yao J., Huang S., Long X., Wang J., García-García E. (2009), Antioxidant activity of polyphenol and anthocyanin extracts from fruits of *Kadsura coccinea* (Lem.) AC Smith, *Food Chemistry,* 117(2), 276-281. **4**. Yang Y. P., Hussain N., Zhang L., Jia Y. Z., Jian Y. Q., Li B., Choudhary M. I., Rahman A. U., Wang W. (2020), *Kadsura coccinea*: A rich source of structurally diverse and biologically important compounds, *Chinese Herbal Medicines,* 12, 214-223. **5**. Do Huy Bich, Dang Quang Trung, Bui Xuan Chuong, Nguyen Thuong Dong, Do Trung Dam, Pham Van Hieu, Vu Ngoc Lo, Pham Duy Mai, Pham Kim Man, Doan Thi Nhu, Nguyen Tap, Tran Toan (2002), *Medicinal Plants and Animal in Vietnam* (in Vietnamese), Science and Technology Publisher. **6**. Gao X. M., Pu J. X., Xiao W. L., Huang S. X., Lou L. G., Sun H. D. (2008), Kadcoccilactones K-R, triterpenoids from *Kadsura coccinea*, *Tetrahedron,* 64(51), 11673-11679. **7**. Trần Mạnh Hùng, Nguyễn Hải Đăng, Trần Phương Thảo (2018), Bước đầu nghiên cứu hoạt tính gây độc tế bào ung thư tụy của lanostan triterpenoid từ lá cây Na rừng, *Tạp chí Dược liệu,* 23(4), 210-216. **8**. Huang S. Z., Duan L. P., Wang H., Mei W. L., Dai H. F. (2019), Two new AChE inhibitors isolated from Li Folk Herb Heilaohu "*Kadsura coccinea"* Stems, *Molecules,* 24(19), 3628. **9**. Woo M. H., Nguyen D. H., Choi J. S., Park S. E., Thuong P. T., Min B. S., Le D. D. (2020), Chemical constituents from the roots of *Kadsura coccinea* with their protein tyrosine phosphatase 1B and acetylcholinesterase inhibitory activities, *Archives of Pharmacal Research,* 43(2), 204-213. **10**. Nguyen H. L., Tran T. H., Phuong T. T., Phan T. N. (2018), *In vitro* inhibitory effect of lanostane triterpenoids of *Kadsura coccinea* on the human immunodeficiency virus type-1 protease, *Indian Journal of Pharmaceutical Sciences,* 80 (4), 755-761. **11**. Sun J., Jiang Y., Amin I., Li Z., Prasad K. N., Duan X., Yang B., Xu L. (2011), An exotic fruit with high nutritional value: *Kadsura coccinea* fruit, *International Food Research Journal,* 18(2). **12**. Liu J., Wei X., Zhang X., Qi Y., Zhang B., Liu H., Xiao P. (2019), A comprehensive comparative study for the authentication of the *Kadsura* crude drug, *Frontiers in Pharmacology,* 9, 1576. **13**. ICH Harmonised Tripartite Guideline (2005), Validation of analytical procedures: text and methodology, *Q2 (R1),* 1(20), 05. **14**. Ookawa N., Ikeya Y., Taguchi H., Yosioka I. (1981), The constituents of *Kadsura japonica* Dunal. I. The structures of three new lignans, acetyl-, angeloyl-and caproyl-binankadsurin A, *Chemical and Pharmaceutical Bulletin,* 29(1), 123-127. **15**. Chen Y. P., Liu R., Hsu H. Y., Yamamura S., Shizuri Y., Hirata Y. (1977), The new schizandrin-type lignans, kadsurin and kadsurarin, *Bulletin of the Chemical Society of Japan,* 50(7), 1824-1826. **16**. Chen D. F., Zhang S. X., Kozuka M., Sun Q. Z., Feng J., Wang Q., Mukainaka T., Nobukuni Y., Tokuda H., Nishino H., Wang H. K. (2002), Interiotherins C and D, two new lignans from *Kadsura interior* and antitumor-promoting effects of related neolignans on epstein - Barr Virus activation, *Journal of Natural Products,* 65(9), 1242-1245. **17.** Yang X. W., Hattori M., Namba T., Chen D. F., Xu G. J. (1992), Anti-lipid peroxidative effect of an extract of the stems of *Kadsura heteroclita* and its major constituent, kadsurin, in mice, *Chemical and Pharmaceutical Bulletin,* 40(2), 406-409.