**16.** Ma C. T., Eom T., Cho E., Wu B., Kim T. R., Oh K. B., Han S. B., Kwon S. W., Park J. H (2017), Aquilanols A and B, macrocyclic humulene-type sesquiterpenoids from the agarwood of *Aquilaria malaccensis*. *Journal of Natural Products*, 80, 3043-3048. **17.** Dagne E., Asmellash S., Abate D. (1994), An antifungal polyacetylenic compound from the culture filtrate of the fungus *Trametes pubescens*. *Journal of Natural Products*, 57(3), 390-392. **18.** Zhou X. J. (2014), Structural characterisation and antioxidant activity evaluation of phenolic compounds from cold-pressed *Perilla frutescens* var. arguta seed flour. *Food Chemistry*, 164(1), 150-157. **19.** Cuenca M. D. R., Catalan C. A. N. (1991), Monoterpenes and lignans from *Mikania saltensis*. *Journal of Natural Products*, 54(4), 1162-1164. **20.** Pham H. Y., Dan T. T. H., Nguyen H. H., Ngo A. B., Duong T. H. Y., Nguyen A. H., Nguyen T. V. T., Bui H. T., Nguyen X. N., Phan V. K. (2022), Lignans from *Gymnema sylvestre*. *VNU Journal of Science: Natural Sciences and Technology*, 38(4), 18-24. **21.** Wang L., Yang Y., Yang H., Qiu R., Huang S. (2017), Direct analysis of succinic acid fermentation broth by $^{1}$H diffusion-ordered NMR spectroscopy and quantitative $^{1}$H NMR technique, *ACS Sustainable Chemistry & Engineering*, 5(4), 2824-2828.

***Journal of Medicinal Materials, 2023, Vol. 28, No. 3 (pp. 155 - 160)***

# DISCRIMINATION OF SPECIALTY TEA VARIETIES COLLECTED IN NORTHERN VIETNAM BASED ON FREE AMINO ACIDS PROFILE

***Nguyen Thi Kieu Oanh *, Le Hoang Linh, Nguyen Van Tat Thanh, Dao Yen Vy, Nguyen Duc Luong***

*Department of Life Sciences, University of Science and Technology of Hanoi,*
*Vietnam Academy of Science and Technology, Hanoi, Vietnam*

*Corresponding author: nguyen-thi-kieu.oanh@usth.edu.vn

(Received March 14th, 2023)

**Summary**

**Discrimination of Specialty Tea varieties Collected in Northern Vietnam Based on Free Amino Acids Profile**

Green tea (*Camellia sinensis* L.) is one of the most popular drinks in Vietnam and has become an agricultural product with high export value. There are a lot of specialty tea varieties with distinguished flavor and taste; however, little information was found in the literature about the chemical profiles of these varieties. This study focused on the analysis of free amino acid content of *C. sinensis* L. varieties collected in Northern mountainous Agriculture and Forestry Science Institute (NOMAFSI) by using an Amino acid analyzer system and the discrimination of these samples by PCA statistical analysis. Our data contributed to explain the distinction in quality among unprocessed and processed tea leaves from different *C. sinensis* varieties. The results of this study provide a simple tool for further research on the quality control and traceability of tea products.

**Keywords:** *Camellia sinensis, PCA, Free amino acid, NOMAFSI, Discrimination, Green tea variety.*

## 1. Introduction

Tea is one of the most popular drinks in Vietnam and has become an agricultural product with high export value. Different tea products are prepared from the young leaves of *Camellia sinensis* L, normally classified as green tea (unfermented tea), Olong tea (semi-fermented tea), and black tea (fully fermented tea). Wide varieties of this species have been breeding, providing abundant resources for producing and manufacturing tea.

In Vietnam, the public institution taking charge of research and development on cultivation techniques, growing and processing green tea is the Northern mountainous Agriculture and Forestry Science Institute (NOMAFSI). This center was thriving on researching the tea propagation by the line selection method, thus generating many precious tea varieties such as VN15, PH11, PH12, Kim Tuyen…The Institute has 250 ha of land for scientific research and has kept a genetic collection with 170 tea varieties [1]. However, research on the chemical composition of these varieties and markers for tea quality is still limited in this country. To date, there is almost no information of the metabolite diversity of *C. sinensis* speciality teas of Vietnam, both raw materials and products.

Free amino acids are crucial in the chemical composition of tea as they are precursors for bioactive compounds and essential proteins [2],[3],[4]. The correlation between amino acids content and the sensory characteristics of tea was revealed by Horie et al. [5]. Amino acids individually contributed to the taste and flavor of tea leaves and also play a role in forming the aroma during tea processing [6],[7]. The amount of each amino acid and the combination of the complete profile directly affect the taste of tea extract and thus, the quality of tea. These indicators and other components like metals were used as input data for the successful geographical classification of tea and other products by chemometric approaches. In these investigations, the Principal component analysis (PCA) technique, one of the most normally used technique to determine how one sample was differentiated from another, were applied for the purpose of classification [2]. As a result, measuring the level of free amino acids in tea samples is considered a critical aspect of quality control. The amino acids content of tea raw materials varied according to genetic resources (varieties or cultivars) and environmental factors such as: geographic region, shade, fertilizer, and climate condition etc. [8],[9]. On the other hand, the amino acids profile of tea products depend on

the manufacture process [10],[11]. In this study, free amino acid profiles of unprocessed and processed leaves of speciality *C. sinensis* varieties of Northern Vietnam were investigated using an automated amino acid analyzer Biochrom30+ system based on a UV-VIS spectroscopy detector. The peak areas of 23 amino acids in these samples were input data for the PCA to reveal the differences among categories and identify biomarkers for tea quality.

## 2. Materials and methods

### *2.1. Materials*

*Green tea samples*

The green tea samples including both raw materials and tea products were collected at NOMAFSI in Phu Tho, Vietnam. For unprocessed samples, fresh leaf buds of six specialty tea varieties, including Kim Tuyen (KT), Huong Bac Son (HBS), PH11, PH12, VN15, and LDP2, were harvested. With Kim Tuyen, the bud (NKT) and the leaves under the bud (GKT) were collected separately. These samples were dried in the oven at 45°C until unchanged weight. For processed ones, we selected six tea products of NOMAFSI, including Dong Phuong My Nhan (DPMN), Olong Kim Tuyen (KTO), Phuc Van Tien (PVT), Xanh Thom (XT), Mao Tiem (MT), and Bich Loa Xuan (BLX). The dried tea leaves might now be stored and used as samples. The samples were then pulverized in a blender to obtain the homogenous powder (pass through 52 mesh sieve). The information on each piece is described in Table 1.

**Table 1.** Sampling information of tea samples.

| No | Sample | Sample type | Raw material | Number of samples | Code name |
|---|---|---|---|---|---|
| 1 | Dong Phuong My Nhan | Processed | Kim Tuyen | 5 | DPMN |
| 2 | Olong Kim Tuyen | Processed | Kim Tuyen | 3 | KTO |
| 3 | Phuc Van Tien | Processed | Phuc Van Tien | 3 | PVT |
| 4 | Xanh Thom | Processed | Huong Bac Son | 3 | XT |
| 5 | Mao Tiem | Processed | VN15 | 3 | MT |
| 6 | Bich Loa Xuan | Processed | VN15 | 3 | BLX |
| 7 | Kim Tuyen | Unprocessed | | 8 | NKT, GKT |
| 8 | Huong Bac Son | Unprocessed | | 4 | HBS |
| 9 | PH11 | Unprocessed | | 9 | PH11 |
| 10 | PH12 | Unprocessed | | 5 | PH12 |
| 11 | VN15 | Unprocessed | | 5 | VN15 |
| 12 | LDP2 | Unprocessed | | 8 | LDP2 |

*Chemicals*

For the free amino acids analysis, oxidised hydrolysate standards including 23 amino acids (L-cysteic acid, taurine, D,L-methinonine sulfoxide, L-methionine sufone, L-aspartic acid, L-threonine, L-serine, L-glutamic acid, L-proline, Glycine, L-alanine, L-cystine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-ornithine, L-lysine, Ammonia, L-arginine) with the concentration of 2.5 mM was used as standard substances (analytical grade, Biochrom, United Kingdom). Ultra ninhydrin reagent kit and 10 buffers solutions (sodium oxidized buffer 1 with pH 3.35; sodium hydro/oxide buffer 2 with pH 4.25; sodium oxidized buffer 3 with pH 2.65; sodium oxidized buffer 4 with pH 8.60; sodium hydrolysate buffer 5 with pH 6.45; sodium regeneration buffer 6; sodium accelerated buffer 1; sodium accelerated buffer 2; sodium accelerated buffer 3; sodium accelerated buffer 4) were provided by Biochrom, United Kingdom with analytical grade. The coil flush solution was made by adding the MilliQ water and isopropanol 50% with the ratio 1:1. The reagent ninhydrin buffer was prepared by mixing the ultrasolve solution 1750 ml (Biochrom, United Kingdom) with ultra ninhydrin solution 250 ml (Biochrom, United Kingdom) under nitrogen gas.

### *2.2. Methods*

*Sample preparation:* The free amino acids were extracted by using the protocol of Jia et al. with slight modification [12] About 100 mg of the powder of each species was added with 1.5 ml of boiling water (about 100$^{o}$C)into the 2 ml-tube. The tubes were then put under ultrasonic at 80°C for 15 minutes and incubated in a Thermomixer at 90°C for 45 minutes. Subsequently, the samples were centrifuged at 14,000 rpm for 5 minutes. The supernatant was filtered through a 0.22 µm membrane into a 2 ml vial which was covered by a screw top-vial cap.

*Free amino acid analysis*: The procedure was performed using Biochrom 30+ Physiological Accelerated LC System machine, which was equipped with BioSys Control Software, Biochrom Alias Manager autosampler control software, and OpenLAB CDS EZChrom Edition

(Biochrom, UK). The Biochrom 30+ system was set up as follows: the Pressure Bar was heated at 50°C; the optimum temperature of the Reaction Coil was 135°C. The Photometer wavelength detection functioned at both 440 and 570 nm. All samples, including the standard supplied solution and fifty-nine extracts, were injected into the system with the volume of 10 µl. The obtained signal of each amino acid was represented by the area value and equivalent standard concentration (ESTD) which means the concentration was extrapolated by one-point external calibration.

*Data analysis*: The Principal Component Analysis (PCA) was performed by using R version 4.2.1 [13] and different packages: FactoMineR and factoextra [14] to discriminate the free amino acid profiles of the tea raw material and products.

**3. Results**

This study revealed a characterization of the amino acid composition in aqueous extracts derived from six unprocessed sample types including HBS, KT, LDP2, PH11, PH12, VN15 and six processed ones including MT, DPMN, XT, PVT, KTO, BLX. After removing the outliers, the data were obtained from a total of 59 samples. The limit of detection was 9-15 pmoles for each amino acid. The reproducibility of the peak area was better than 1.5% RSD at 10 nanomoles and that of the retention time was better than 0.5% RSD. The area for amino acids that were not detected by the method was set as ½ of the detection limit concentration. All data were normalized by log-transformation and used for further statistical analysis.

*3.1. Free amino acid profile of unprocessed tea leaves*

For unprocessed samples, the analysis revealed significant variations in the content of 23 amino acids. Specifically, the presence of D,L-methionine sulfoxide was limited to only LDP2 and KT, while free Asp was exclusively found in the HBS variety. Furthermore, Leu was quantified in only the VN15 and LDP2 varieties, while Arg was not detected in either of these two (Fig. 1). Additionally, Lys was detected in the VN15, LDP2, and HBS samples, but was not found in PH11, PH12, and KT.

The discrimination based on free amino acid profiles among tea samples was analyzed using principal component analysis (PCA). This technique is commonly used to distinguish samples. The PCA loading scatter plot visually displayed the separation between the clusters, showing the level of discrimination among six varieties (Fig. 2(b)). The two principal components accounted for 65.6% of the total variance, effectively illustrating the data. As shown in Fig. 1, the free amino acid profiles separated into two central regions, with VN15 and LDP2 close to each other but distinct, and a second cluster containing PH11, PH12, KT, and HBS. Interestingly, PH11 and PH12, which were both derived from the same Shan Tuyet variety, had significantly different free amino acid profiles.

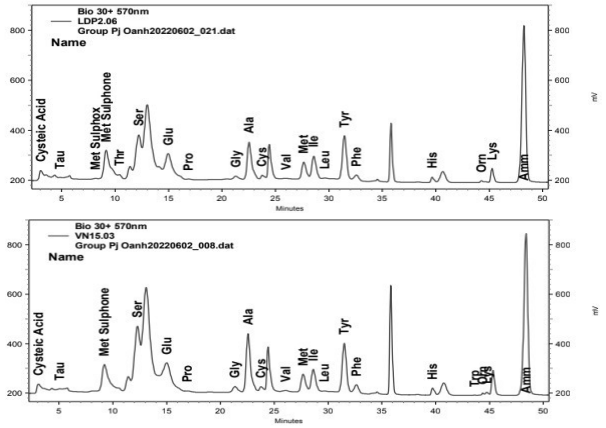


**Fig. 1.** The chromatograms of a LDP2 sample and a VN15 sample

We performed the PCA analysis within four species of the second cluster to see these samples' distribution more clearly on the PC1 and PC2 (Fig. 2(a)). Indeed, HBS and PH11 were close in the plot, suggesting that the amino acid profiles of the two species were quite similar. KT varied greatly among samples. The NKT and GKT samples were separated into two clusters, which can be explained by the fact that the leaves of NKT (the bud of KT) were younger than those of GKT (the first two leaves of KT).

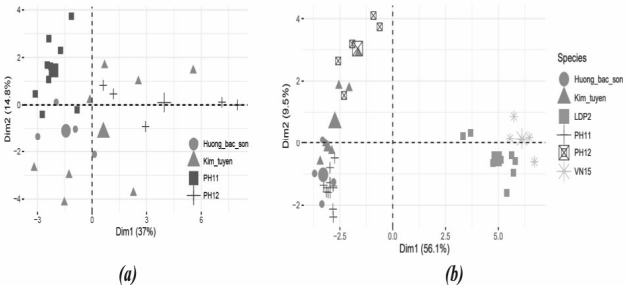


**Fig. 2.** PCA scatter plots of the free amino acids profile of a) four tea varieties: Huong Bac Son, Kim Tuyen, PH11, and PH12; and b) six tea varieties: Huong Bac Son, Kim Tuyen, LDP2, PH11, PH12, and VN15.

The function of *fviz_contrib* of *FactomineR* package allowed identifying the contribution percentage of each amino acid on the discriminative performance. Fig. 3 shows the bar chart of the contribution of variables to the first two components in the PCA analysis. It can be seen that Met, Tyr, Ile, Glu, Ser, and Phe contributed the most to the separation of these samples on the PCA scatter plot. These amino acids were considered markers for six tea varieties. Quantification of these chemicals can provide information for the

identification of the tea accession, then for the quality control or traceability.

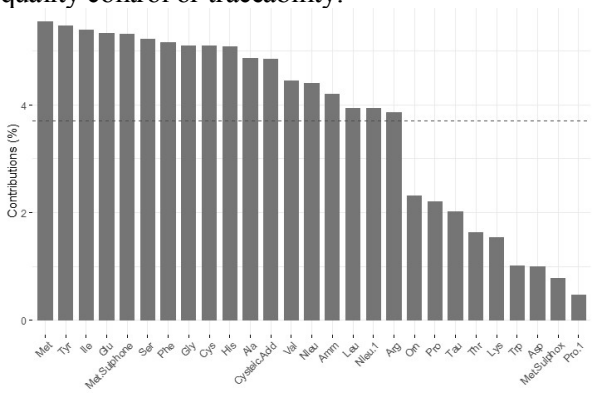


**Fig. 3.** The contribution of single amino acids on the discrimination of six unprocessed tea varieties on the first two components of PCA scatter plot.

### *3.2. Free amino acid profile of processed tea*

We also applied the same protocol for the analysis of the amino acid profile of specialty tea products of NOMAFSI, including BLX, DPMN, MT, KTO, PVT, and XT. In the processed tea product, D,L-Met sulfoxide, Asp, Leu, and Lys were not detected in all samples. Except Thr was not found in MT and BLX, other products contain remaining free amino acids. In the PCA plot of six products in Fig. 4(b), BLX, PVT, and MT separated well and made long distances with DPMN, KTO, and XT, suggesting that these three former products represented a big difference in free amino acid profiles among them and between them and the three latter samples. The first two dimensions explained 51.7% and 15.2% total variance that represented the reliability of discrimination analysis. Otherwise, when zooming in the distribution of DPMN, KTO and XT in Fig. 4(a), these samples are distinguished well, demonstrating that there are still slight differences in the free amino acid content. In this analysis, the first two components explained 61.7% variance. DPMN and KTO were produced from the same raw material KT, suggesting that the fermentation process affects the amino acid profiles of tea products.

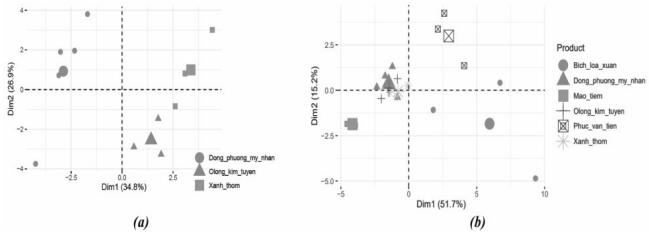


**Fig. 4.** PCA scatter plots of the free amino acids profile of a) three products: Dong Phuong My Nhan, Xanh Thom and Olong Kim Tuyen; and b) six products: Bich Loa Xuan, Dong Phuong My Nhan, Mao Tiem, Olong Kim Tuyen, Phuc Van Tien, Xanh Thom.

Fig. 5 represents the contribution percentage of variables to the first two components of PCA analysis, illustrating that Phe, Met, Orn, Cys, Met, and Gly contributed the most to the discrimination of these products. Therefore, these amino acids could be markers for quality control of these tea products.

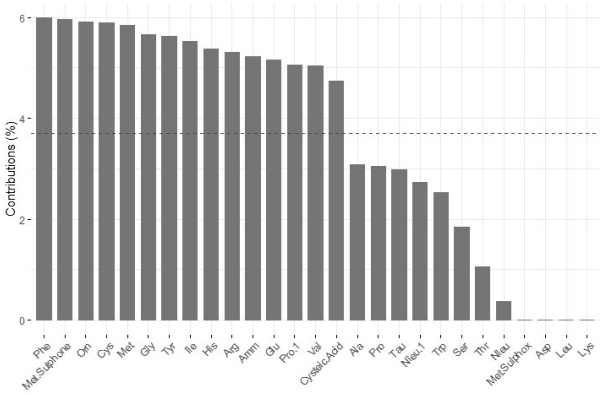


**Fig. 5.** The contribution of single amino acids to the discrimination of six processed tea products on the component 1 and 2 of the PCA plot

## 4. Discussion

The differentiation in the profiles of free amino acids among the tea products was a result of various oxidation processing protocols. The oxidation process, in which broken tea cells interact with enzymes and oxygen, influences the aroma and distinctive taste of various tea varieties. The PCA analysis of the amino acid profiles of eight tea materials and related products was performed to examine the impact of the oxidation process on the metabolic pathways of amino acids (Fig. 6(b)). Even though XT is a product from HBS fresh leaves, XT and HBS were far in the scatter plot suggesting a significant dissimilarity in the amino acid profiles. The distinction in the processing comes from: XT was processed by partial fermentation to retain the flavor and partly remove the bitter and acrid taste of HBS, which is processed only by drying in the oven at 45$^{o}$C. The activity of enzymes inside tea leaves metabolized amino acids to secondary compounds, thus changing the whole profile of XT compared to HBS. In contrast, DPMN, KTO, and KT were found to be close to each other, indicating that the fermentation process does not affect much the metabolism of amino acids in these samples. DPMN and KTO originated from the same material, KT. However, the precursor of DPMN comes from KT leaves which were exposed to tea jassid, an insect that absorbs water from the plant tissues. This biotic stress produced a slight

difference in free amino acid profiles among these samples (Fig. 6*(a)*). In terms of process, DPMN was made by complete fermentation, while KTO was made by semi-fermentation. The main difference between the two methods comes from the activity of enzymes in oxidation reactions. DPMN samples were found to be closer to GKT than NKT, indicating that DPMN contains a similar free amino acid profile to older leaves than younger ones.

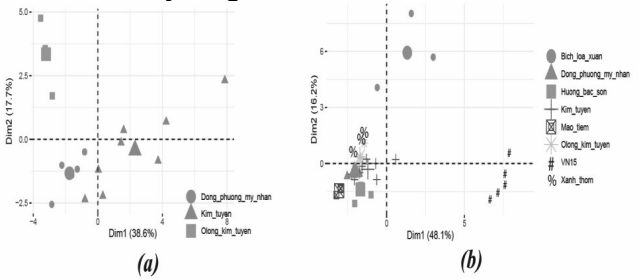


**Fig. 6.** The PCA scatter plot of a) three sample categories: Kim Tuyen, Olong Kim Tuyen and Dong Phuong My Nhan; and b) eight sample categories: Bich Loa Xuan, Dong Phuong My Nhan, Huong Bac Son, Kim Tuyen, Mao Tiem, Olong Kim Tuyen, VN15 and Xanh Thom.

It is widely acknowledged that free amino acids play a significant role in the aroma and flavor of specialty teas [15],[16],[17],[18],[19],[20]. Specifically, the presence of Glutamic acid imparts a sweetness and umami taste, while Tryptophan reduces bitterness [16]. Threonine, one of the nine essential amino acids required for proper human physiological functioning, has been found to contribute to the aroma profile, along with Alanine [17],[18],[19]. Serine and Tyrosine, on the other hand, impart wine-like aroma characteristics [20]. Thus, this discrimination analysis can provide insight into the variance in taste and flavor among tea materials and products. Moreover, the free amino acid profile was one of the key determining variables of tea quality due to each amino acid's contribution to nutrition and healthcare. Some amino acids, such as glutamine and theanine, were well known as benefit mental health. Therefore, identifying and quantifying amino acids is very important in quality control and proofing tea's pharmacological effect.

To date, numerous investigations have been performed to assess tea quality through chemical analysis. However, a majority of these studies have evaluated the impact of the withering, rolling, and fermentation stages on the overall amino acid content, rather than individual amino acids [10],[11]. This is a novel occasion where characterization has been performed on the entire amino acid profile and utilizing a significant sample size. This information can be utilized to explain the heterogeneity in the amino acid profiles among tea varieties and during the production process.

## 5. Conclusion

In this study, 23 amino acids were characterized in fifty-nine tea samples, including unprocessed and processed samples. The PCA scatter plot demonstrates the discrimination between fresh leaves and tea products. The diversity in the free amino acids profiles of *C. sinensis* varieties and tea products explained these leaves' different flavors and tastes. Interestingly, we indicated the metabolic change of amino acids from fresh material to tea products by comparing the profiles of unprocessed leaves and their different fermented products. This analysis tool also showed the contribution of single amino acids to the discrimination, suggesting that these amino acids could be markers for tea traceability. These results provide valuable insight into the amino acid composition of these plant varieties and products and highlight the need for further studies on the functional implications of these differences.

**Acknowledgments:** *This work was supported by Vietnam Academy of Science and Technology (grant number THTEXS.05/21-24). The authors would like to acknowledge Dr. Luu Ngoc Quyen and MSc. Phung Le Quyen (NOMAFSI) to support in sample collection.*

**References**

**1**. http://www.nomafsi.com.vn/en/main.html. **2**. Azevedo M. S., Seraglio S. K. T., Rocha G., Balderas C. B.,, Piovezan M., Gonzaga L. V., Falkenberg D. B, Fett R., Oliveira M. A. L., Costa A. C. O. (2017), Free amino acid determination by GC-MS combined with a chemometric approach for geographical classification of bracatinga honeydew honey (*Mimosa scabrella* Bentham), *Food Control*, 78, 383-392. **3**. Duan J. Y., Li Z. Y., Li J., Song H. B., Fu J. S., & Xie B. G (2020), Comparison of nutritional and flavor characteristics between four edible fungi and four fruits and vegetables based on components and characteristics of free amino acids, *Mycosystema*, 39(6), 1077-1089. **4**. Liang Y., Zhang L., Gao F., Zhang X., Li X., Zhang H., Yang S., Xiong J. (2019), Changes in NaCl, reducing sugar and amino acids and formation of tastes in Chongqing Shuidouchi during fermentation, *Food Fermentation Industries*, 45, 27-34. **5**. Horanni R. and Engelhardt U. H. (2013), Determination of amino acids in white, green, black, oolong, pu-erh teas and tea products, *Journal of Food Composition and Analysis*, 31, 94-100. **6**. Ni H., Jiang Q. X., Zhang T., Huang G. L., Li L. H., Chen F. (2020), Characterization of the aroma of an instant white tea dried by freeze drying, *Molecules*, 25, 3628. **7**. Feng Z. H., Li Y. F., Li M., Wang Y. J., Zhang L., Wan X. C., Yang X. (2019), Tea aroma formation from six model manufacturing processes, *Food Chemistry*, 285, 347-354. **8**. Lin Y. S., Tsai Y. J., Tsay J. S., Lin J. K. Factors affecting the levels of tea polyphenols and caffeine in tea leaves, *Journal of Agricultural and Food Chemistry*, 51, 1864-1873. **9**. Le Gall G., Colquhoun I. J., Defernez M. (2004), Metabolite profiling using 1H-NMR spectroscopy for quality assessment of green tea, *Camellia sinensis* (L.), *Journal of Agricultural and Food Chemistry*, 52, 692-700. **10**. Jabeen S., Alam S., Saleem M., Ahmad W., Bibi R., Hamid F. S., Shah H. U. (2019), Withering timings affect the total free amino acids and mineral contents of tea leaves during black tea manufacturing, *Arabian Journal of Chemistry*, 12(8), 2411-2417. **11**. Yu P., Huang H., Zhao X., Zhong N., & Zheng H. (2022), Dynamic variation of amino acid content during black tea processing: A review,

*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.*