line, *International Journal* of *Pharmacy* and *Pharmaceutical Sciences*, 6(8) 263-268. **6**. Sagadevan P., Suresh S. N., Rathishkumar S., Gayathri S., Eswari D. V. (2013), Anticancer activity of methanolic leaf extract of *Andrographis paniculata* (Nees) and *Cardiospermum halicacabum* (Linn.) against human breast cell cancer cell line (MCF-7), *International Journal of Pharmaceutical and Life Sciences*, 4(9), 2983-2986. **7**. Jacob B., Natarajan S., Pattiyappan S., Sivaraman R., Suresh M. S. (2015), Study on anti-carcinogenic and apoptotic properties of methanolic stem extract of *Cardiospermum halicacabumin* MCF7 cell line, *Journal of Scientific and Innovative Research*, 4(1), 43-48. **8**. Ba Thi Cham, Nguyen Thi Thuy Linh, Nguyen Thi Hoang Anh, Nguyen Phi Hung, Do Thi Thao, Nguyen Quyet Tien, Nguyen Thanh Tam, Tran Duc Dai, Dang Ngoc Quang, Nguyen Thi Thu Hoa, Tran Khac Vu, Le Thi Hong Nhung, Domenico Delfino, Trinh Thi Thuy (2021), Cytotoxic and immunosuppressive activity of methyl caffeate isolated from *Balanophora laxiflora, Journal of Science and Technology,* 57(6), 114-118. **9**. Vu Kim Thu, Le Thi Kieu Anh, Dang Ngoc Quang, Nguyen Van Thang, Hoang Le Tuan Anh, Nguyen Xuan Nhiem, Dan Thi ThuyHang, Chau Van Minh, Phan Van Kiem (2015), Triterpenes from the leaves of *Glochidiono bliquum*, *Vietnam Journal of Science, Technology,* 53(2e), 103-106. **10.** Yu L., Tang X., Chen L., Wang M., Jian J., Cao S., Qiu F. (2012), Oleanane-type triterpenoid saponins from *Xanthoceras sorbifolia* Bunge, *Fitoterapia*, 83(8), 1636-1642. **11**. Dai J. R., Cardellina J. H., Mahon J. B., Boyd M. R. (1997), Zerumbone, an HIV-Inhibitory and cytotoxic sesquiterpene of *Zingiber aromaticum* and *Z. zerumbet*, *Natural Product Letters*, 10(2), 115-118. **12**. Sadhu S. K., Khatun A., Ohtsuki T., Ishibashi M. (2007), First isolation of sesquiterpenes and flavonoids from *Zingiber spectabile* and identification of zerumbone as the major cell growth inhibitory component, *Natural Product Research,* 21(14), 1242-1247. **13**. Somchit M. N., Mak J. H., Bustamam A. A., Zuraini A., Arifah A. K., Adam Y., Zakaria Z. A. (2012), Zerumbone isolated from *Zingiber zerumbet* inhibits inflammation and pain in rats, *Journal* of *Medicinal Plants Research*, 6(2) 177-180. **14.** Yalcin F. N., Ersoz T., Akbay P., Calis I., (2003), Phenolic, megastigmane, nucleotide, acetophenon and monoterpene glycosides from *Phlomis samia* and *P. carica, Turkish Journal of Chemistry,* 27(2003), 703-711. **15.** Voutquenne L., Lavaud C., Massiot G., Sevenet T., Hadi H. A. (1999), Cytotoxic polyisoprenes and glycosides of long-chain fatty alcohols from *Dimocarpus fumatus*, *Phytochemistry,* 50(1), 63-69. **16.** Ferrar L., Schettino O., Montesano D., (1996). Triterpenoids from *Cardiospermum halicacabum*, *Phytotherapy* Research, 10 (1), 192-194. **17**. Nguyen Thi Thuy Linh, Ba Thi Cham, Nguyen Thi Hoang Anh, Nguyen Thanh Tam, Dao Duc Thien, Tran Duc Quan, Tran Van Sung, Sabrina Adorisio, Domenico Delfino, Trinh Thi Thuy (2019), Ecdysteroids from *Vitex leptobotrys* inhibit proliferation of acute myeloid leukemia cells, *Vietnam Journal of Science and Technology*, 13-16. **18**. Trinh Thi Thuy, Ba Thi Cham, Nguyen Thi Thuy Linh, Phan Thi Ngoc Bich, Sabrina Adorisio, Domenico Delfino (2022), Phenolic acid analogues from *Balanophora laxiflora* inhibit proliferation of *in vitro* acute myeloid leukemia cells, *Records of Natural Products*, 16(3), 253-258. **19**. Zingue S., Gbaweng Y. A. J., Michel T., Ndinteh D. T., Rutz J., Auberon F., Blaheta R. A. (2019), Bioguided identification of daucosterol, a compound that contributes to the cytotoxicity effects of *Crateva adansonii* DC. (Capparaceae) to prostate cancer cells, *Journal of Ethnopharmacology,* 112251. doi:10.1016/j.jep.2019.112251. **20**. Conta A. C., Stelzner D. J. (2007), Immunomodulatory effect of the purine nucleoside inosine following spinal cord contusion injury in rat, *Spinal Cord*, 46(1), 39-44. **21.** Zimmermann-Klemd A. M., Reinhardt J. K., Morath A., Schamel W. W., Steinberger P., Leitner J., Gründemann C. (2020), Immunosuppressive activity of *Artemisia argyi* extract and isolated compounds, *Frontiers in Pharmacology,*11, 402. doi:10.3389/fphar.2020.00402.

Journal of Medicinal Materials, 2022, Vol. 27, No. 4 (pp. 218 - 222)

CALCIUM OXALATE CRYSTALLIZING INHIBITION OF EXTRACTS FROM SEVERAL *PANDANUS* SPECIES IN VIETNAM

Do Hoang Giang[1], Truong Thi Lien[1], Dang Viet Hau[1], Vu Duc Nam[1], Nguyen Thi Thu Thuy[2], Nguyen Tien Dat[1,*]

[1]Center for Research and Technology Transfer, Vietnam Academy of Science and Technology;
[2]Institute of Tropical Medicine, Vietnam - Russia Tropical Center, Vietnam
*Corresponding author: ntdat@ctctt.vast.vn
(Received June 20th, 2022)

Summary

Calcium Oxalate Crystallizing Inhibition of Extracts from Several *Pandanus* Species in Vietnam

Plants from the genus *Pandanus* were used as traditional medicines for urolithiasis treatment. Evaluation of calcium oxalate crystallizing inhibition of 14 extracts from 4 *Pandanus* species revealed that the extracts from *P. nanofrutex* and *P. amaryllifolius* exhibited stronger effects than those of *P. odoratissimus* and *P. tectorius*. The chemical quantitation and correlation analysis results indicated that phenolics and flavonoids significantly affect the calcium oxalate crystallizing inhibitory effect. These showed promising results for further investigation of antiurolithiatic compounds from *Pandanus* species.

Keywords: *Pandanus, Phenolic, Flavonoid, Alkaloid, Calcium oxalate.*

1. Introduction

Pandanus is one of the most common genera in the family of Pandanaceae which includes about 600 species. Among them, 17 species have been found in Vietnam, such as *Pandanus tectorius* Parkinson ex Du Roi, *Pandanus amaryllifolius* Roxb., *Pandanus odoratissimus* Linn., *Pandanus tonkinensis* B. Stone, *Pandanus nanofrutex* B. Stone, *Pandanus humillis* Lour… [1],[2]. Plants of the genus may vary in size with

heavy rough fruits, wide-branching trunks, and strap-shaped leaves [2],[3]. In Vietnamese traditional medicine, *P. tectorius* was used for the treatment of urolithiasis [1],[3], while parts of *P. odoratissimus* were used to treat fever or syphilis [4], whereas, leaves of *P. amaryllifolius* were commonly used as a food additive. Previous investigations reported lignans [5],[6], flavonoids [7], and alkaloids [8],[9],[10] from species of the *Pandanus* genus with anti-cancer, antioxidant, or anti-inflammatory activities However, the antiurolithiatic effect of natural products from *Pandanus* species has not been reported. Calcium oxalate was one of the most common chemicals in kidney stones. The growth of its crystals may seriously damage the kidney. Calcium oxalate crystallizing inhibitors can reduce the size of the crystals or prevent the reaction between calcium and oxalate ions. Previous studies reported the considerable inhibition of some plant extracts on the crystalization of calcium oxalate [11],[12],[13]. In this study, we evaluated the inhibition of calcium oxalate crystalization (nucleation) of extracts from 4 species of the *Pandanus* genus, which are *P. tectorius, P. amaryllifolius, P. odoratissimus, P. nanofrutex* and quantified the total contents of phenolics, flavonoids, and alkaloids in these extracts to predict the correlation between chemical compositions and the bioactivity.

2. Materials and methods

2.1. Materials

The aerial parts of *Pandanus tectorius* (5 samples, P.tec 1 - P.tec 5) were collected in Hanoi in February 2020 and March 2021. The aerial parts of *P. odoratissimus* (3 samples, P.od 1 - P.od 3) were collected in Nha Trang and Cam Ranh Bay, Khanh Hoa province in May 2021. The aerial parts of *P. amaryllifolius* (3 samples, P.ama 1 - P.ama 3) were collected in Ha Noi and Vinh Phuc in April 2021. The aerial parts of *P. nanofrutex* (3 samples, P.nano 1 - P.nano 3) were collected in Quang Nam province in March 2020. All of the samples were identified by Dr Bui Van Thanh, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. The samples were cleaned with water, dried at 60°C, and powdered.

2.2. Instruments and chemicals

UV-Vis absorbance was measured on a BioTek Synergy HTX multimode reader, Agilent, US. The incubator and the sonication bath were supplied by Daihan Scientific, Korea. Micropipette and pipette tips were supplied by Isolab, Germany.

The solvents methanol (MeOH), dichloromethane (DCM), ethyl acetate (EA), DMSO, water, together chemicals, such as NaOH, $NaNO_2$, $AlCl_3$, Na_2CO_3, $CaCl_2$, $Na_2C_2O_4$, NaCl (over 98% of purity) were purchased from Daihan Scientific, Korea. Tris-HCl was purchased from BioBasic, Canada. Folin-Ciocalteu reagent, gallic acid, and quercetin were supplied by Merck, Germany.

2.3. Extraction of the samples

100 g of each sample was extracted in triplicate with 1 L of ethanol 50% in a sonication bath at 50°C. The solution was filtered and evaporated to yield the extract.

2.4. Calcium oxalate nucleation assay

The effects of *Pandanus* extracts on calcium oxalate crystalization were evaluated using the nucleation assay [12]. $CaCl_2$ 5 mM and $Na_2C_2O_4$ 7.5 mM were prepared in the buffer mixture of Tris-HCl 0.05 M and NaCl 0.15 M (pH 6.5). Analytes were prepared at several concentrations in DMSO. Mixed 1 mL of the sample with 3 mL of $CaCl_2$ 5 mM, then added 3 mL of $Na_2C_2O_4$ 7.5 mM and incubate the mixture for 30 min at 37°C. The optical density of the mixtures was then measured at 620 nm wavelength. The inhibition percentage of nucleation by *Pandanus* extracts was calculated using the below formula and compared to that of positive control Cystone, a common polyherbal drug for antiurolithiasis in India [11],[12],[13],[14].

$\% I_i = [1 - (A_i/A_O)] \times 100\%$

$\% I_i$: inhibition percentage of sample i; A_i and A_O were the optical densities of sample i and blank. The IC_{50} value of each extract was measured based on experiments in several concentrations. The statistically significant difference in IC_{50} values was evaluated by one-way ANOVA and Tukey's HSD posthoc analysis.

2.5. Total phenolic assay

The external calibration was done with different concentrations of gallic acid (10-400 µg/mL). The analytes were prepared at 1 mg/mL or diluted if necessary. Each 100 µL of the sample or standard solution was mixed with 400 µL of Folin - Ciocalteu 10% and 500 µL of Na_2CO_3 6% and incubated for 15 min at 40°C. Its absorbance was measured with a UV-Vis. spectrophotometer at 750 nm. The total phenolic content was calculated as mg gallic acid equivalent (mg GAE/g) by using gallic acid calibration curve.

2.6. Total flavonoid assay

The external calibration was done with different concentrations of quercetin (10-200 µg/mL). The analytes were prepared at 1 mg/mL or diluted if necessary. Add 100 µL of $NaNO_2$ 5% to 500 µL sample or standard solution mix at

room temperature for 5 min, then add 100 µL of $AlCl_3$ 10% and 500 µL NaOH 1M to the mixture. Keep the mixture at room temperature for 15 min. Its absorbance was measured with a UV-Vis. spectrophotometer at 510 nm. The total flavonoid content was calculated as mg quercetin equivalent (mg QE/g) by using quercetin calibration curve.

2.7. Total alkaloid assay

5 grams of each extract was suspended in 50 mL of water and acidified to pH 2 by HCl 1N and partitioned with EA (75 mL × 3 times). The organic phase was removed and the water phase was basified by 1N NaOH to pH 11 then extracted with CH_2Cl_2 (4 times × 200 mL). The combined CH_2Cl_2 extracts were evaporated under reduced pressure to give total alkaloid mixtures. Total alkaloid content was measured by the weight of the alkaloid mixture in a gram of the total extract (mg/g).

2.8. Statistics

One-way ANOVA, Tukey's HSD posthoc analysis, and correlation analysis were performed in R Package. Values of $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Calcium oxalate crystallizing inhibition of Pandanus *extracts*

Calcium oxalate crystallizes due to the reaction between Ca^{2+} and $C_2O_4^{2-}$ at pH ≥ 7. Calcium oxalate crystalization inhibitors may have competing reactions to one of those ions then reduce the formation of calcium oxalate crystals that decrease the optical density of the suspension. The inhibition of *Pandanus* extracts on the crystalization of calcium oxalate was performed in Table 1. As shown, the difference among the inhibitory effects of the *Pandanus* extracts was statistically significant ($p < 0,05$) that could be observed via their IC_{50} values. In detail, IC_{50} values of *P. nanofrutex* and *P. amaryllifolius* extracts ranged respectively from 4.06 to 5.28 µg/mL, and from 3.28 to 19.55 µg/mL, which were dramatically lower than this of Cystone (IC_{50} at 57.25 µg/mL). Extracts of *P. nanofrutex* and *P. amaryllifolius* showed equal effects on calcium oxalate crystalizing inhibition ($p > 0.05$) and were statistically higher than those of the other two species. IC_{50} values of extracts from *P. odoratissimus* samples varied between 36.61 and 89.02 µg/mL, while those of *P. tectorius* extracts were much lower than the positive control, at 65.05-90.07 µg/mL.

Table 1. IC_{50} values for inhibition of *Pandanus* extracts on the crystalization of calcium oxalate (CaOx IC_{50}), together with total phenolic (TPC), flavonoid (TFC), and alkaloid (TAC) contents of those. Cystone was used as the positive control

Sample	CaOx IC_{50} (µg/mL)	TPC (mg GAE/g)	TFC (mg QE/g)	TAC (mg/g)
P.tec 1	70.11 ± 0.31	15.09 ± 1.82	10.29 ± 0.83	< 0.01
P.tec 2	65.05 ± 0.50	18.74 ± 1.71	7.24 ± 0.68	< 0.01
P.tec 3	70.54 ± 0.14	19.03 ± 2.21	16.86 ± 1.97	0.02 ± 0.01
P.tec 4	85.23 ± 0.21	20.85 ± 3.04	2.76 ± 0.18	0.05 ± 0.01
P.tec 5	90.07 ± 0.18	17.56 ± 0.39	3.14 ± 0.21	< 0.01
P.od 1	56.63 ± 0.32	27.67 ± 0.18	12.73 ± 0.56	< 0.01
P.od 2	89.02 ± 0.40	28.73 ± 3.24	11.71 ± 1.37	< 0.01
P.od 3	36.61 ± 0.45	42.15 ± 2.37	19.47 ± 0.21	< 0.01
P.ama 1	3.28 ± 0.31	58.01 ± 0.05	26.76 ± 1.01	9.10 ± 0.57
P.ama 2	19.55± 0.63	60.71 ± 1.3	32.66 ± 3.56	8.62 ± 0.27
P.ama 3	16.50 ± 0.13	58.72 ± 0.35	32.05 ± 1.74	9.05 ± 0.14
P.nano 1	4.06 ± 0.25	64.36 ± 5.42	38.56 ± 0.86	< 0.01
P.nano 2	5.28 ± 0.41	64.59 ± 3.35	36.52 ± 2.86	< 0.01
P.nano 3	4.17 ± 0.61	59.19 ± 4.89	39.58 ± 0.57	< 0.01
Cystone	57.25 ± 0.19			

3.2. Total phenolic, flavonoid and alkaloid contents of the Pandanus extracts

Previous studies indicated that phenolics, flavonoids, and alkaloids were the common secondary metabolites in *Pandanus* species [5],[6],[7],[8],[9],[10]. In this study, the contents of these compositions in *Pandanus* extracts were evaluated and compared using ANOVA and Tukey's HSD posthoc analysis. As shown in Table 1, extracts from *P. nanofrutex* and *P. amaryllifolius* had similar total phenolic contents ($p > 0.05$), which ranged from 58,01 - 64,59 mg GAE/g. Whereas, the total phenolic contents of *P. odoratissimus* and *P. tectorius* extracts varied between 15.09 - 42.15 mgGAE/g, which were significantly lower ($p < 0.05$) than those of *P. nanofrutex* and *P. amaryllifolius*. Similarly, the total flavonoid contents of extracts from *P.*

nanofrutex and *P. amaryllifolius* ranged from 26.76 - 39.58 mg QE/g, which were significantly higher than those of *P. odoratissimus* and *P. tectorius* extracts.

On the other hand, alkaloids were mostly detected in the extracts of *P. amaryllifolius* at 8.62 - 9.10 mg/g. Alkaloids were also found in two *P. tectorius* samples but at low concentrations. Previous studies indicated that several alkaloids were isolated from *P. amaryllifolius* [[8],[9],[10],[15],[16], however, this class of compound has never been detected in the rest three species.

3.3. Correlation between calcium oxalate crystalizing inhibition and chemical compositions of Pandanus extracts

The correlation between calcium oxalate crystalizing inhibition and chemical compositions of *Pandanus* extracts could be evaluated by calculating Pearson's correlation coefficient among inversed IC_{50}, TPC, TFC, and TAC values. Inversed IC_{50} values could be explained as the inhibition efficiency of the extracts. These were used due to a similar trend to the other three values, which were more convenient for the calculation of the correlation coefficient than using IC_{50} values. All of the quantitative data were standardized before calculation to comparably scale all of the factors.

As can be seen in Fig. 1, Inversed IC_{50} values deeply correlated to TPC and TFC values, that the correlation coefficients were 0.78 and 0.76, respectively. Meanwhile, inversed IC_{50} values showed a weak correlation to TAC values ($R^2 = 0.27$). Since the inhibition of *Pandanus* extracts on calcium oxalate crystalization may be considerably affected by phenolic and flavonoid contents, the alkaloids have a weak effect on the activity. Phenolics and flavonoids contain several phenol hydroxyl groups with acid properties that may have competing reactions to Ca^{2+} or $C_2O_4^{2-}$ in a solution or reverse the formation of calcium oxalate, then reduce the concentration of this salt in the solution. Meanwhile, alkaloids, which are commonly base, may not affect sharply the crystalization of calcium oxalate.

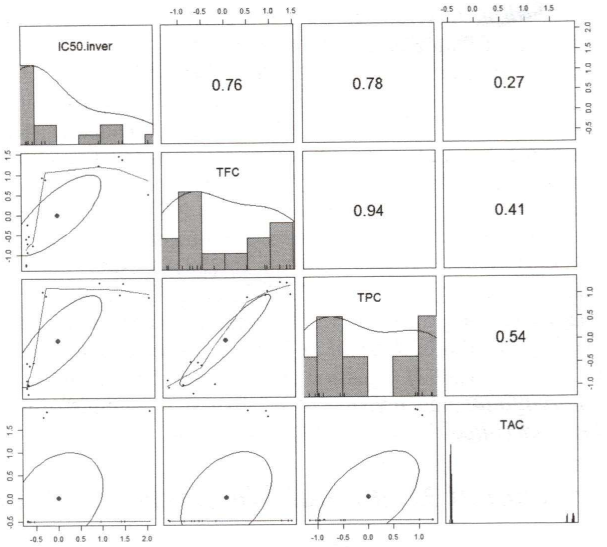

Fig. 1. Correlation matrix among inversed IC_{50} (IC50.inver), TFC, TPC, TAC. The numbers in the box were the Pearson's correlation coefficient between two factors in the corresponding row and column.

These results also explain that the higher calcium oxalate crystalizing inhibition of *P. nanofrutex* and *P. amaryllifolius* extracts may be due to their higher phenolic and flavonoid contents, rather than the alkaloids contents, than those of *P. odoratissimus* and *P. tectorius*.

4. Conclusion

Results of the study indicated that *P. nanofrutex* and *P. amaryllifolius* extracts exhibited stronger inhibition than those of the positive control (Cystone), *P. odoratissimus* and *P. tectorius* extracts in the formation of calcium oxalate crystals. Correlation analysis between the activity and chemical compositions revealed that phenolics and flavonoids were important inhibitors of calcium oxalate crystalization. These showed the promising direction for the development of antiurolithiatic products from *Pandanus* plants that further *in vivo* and clinical explorations should be conducted to confirm their efficiency.

Acknowledgement: *This study was funding by the Vietnam Academy of Science and Technology with the code QTBG01.03/20-21.*

References

1. Vo Van Chi, 1999, Dictionary of Vietnam Herbal Medicines, *Medical Publishing House*. **2.** Pham Hoang Ho, 2003, Vietnam Plants, *Tre Publishing House*. **3.** Do Tat Loi, 2004, Medicinal plants and remedies in Vietnam, *Medical Publishing House*. **4.** Vo Van Chi, Tran Hop, 1999, Valuable plants in Vietnam, *Vietnam Education Publishing House*. **5.** Ting J. T., Whang C. S. (1998), Antioxidative activities of constituents isolated from *Pandanus odoratissimus*, *Phytochemistry*, 49(7), 2145-2148. **6.** Cuong N., Son N., Van D., Tram N., Thao D., Su P., Thuan N. (2015), Isolation of natural products from fruits of *Pandanus odoratissimus* L., *Vietnam Journal of Chemistry*, 53(4), 432-435. **7.** Zhang X., Guo P., Sun G., Chen S., Yang M., Fu N., Wu H., Xu X. (2012), Phenolic compounds and flavonoids from the fruits of *Pandanus tectorius* Soland, *Journal of Medicinal Plants Research*, 6(13), 2622-2626. **8.** Takayama H., Ichikawa T., Kitajima M., Aimi N., Lopez D., Nonato M. G. (2001), A new alkaloid, pandanamine; finding of an anticipated biogenetic intermediate in *Pandanus amaryllifolius* Roxb,

Tetrahedron Letters, 42(16), 2995-2996. **9.** Tan M. A., Kitajima M., Kogure N., Nonato M. G., Takayama H. (2010), Isolation of Pandamarilactonine-H from the Roots of *Pandanus amaryllifolius* and Synthesis of epi-Pandamarilactonine-H, *Journal of Natural Products*, 73(8), 1453-1455. **10.** Tan M. A., Kitajima M., Kogure N., Nonato M. G., Takayama H. (2010), Isolation and total syntheses of two new alkaloids, dubiusamines-A, and -B, from *Pandanus dubius*, *Tetrahedron*, 66(18), 3353-3359. **11.** Kasote D., Jagtap S., Thapa D., Khyade M., Russell W. (2017), Herbal remedies for urinary stones used in India and China: A review, *Journal of Ethnopharmacology*, 203, 55-68. **12.** Mosquera D., Ortega Y., Quero P., Martínez R., Pieters L. (2020), Antiurolithiatic activity of *Boldoa purpurascens* aqueous extract: An *in vitro* and *in vivo* study, *Journal of Ethnopharmacology*, 253, art.ID 112691. **13.** Suman S., Suresh K. (2020), Antiurolithiatic activity of ethanolic extract of *Piper cubeba* dried fruits: An *in-vitro* and *in-vivo* study, *Pharmacognosy Journal*, 12(6), 1289-1296. **14.** Sweta B., Archana N. S., Devesh T. (2018), Antiurolithiatic activity of *Daucus carota*: An *in vitro* Study, *Pharmacognosy Journal*, 10(5), 880-884. **15.** Takayama H., Ichikawa T., Kitajima M., Nonato M. G., Aimi N. (2001), Isolation and characterization of two new alkaloids, norpandamarilactonine-A and -B, from *Pandanus amaryllifolius* by spectroscopic and synthetic methods, *Journal of Natural Products*, 64(9), 1224-1225. **16.** Takayama H., Ichikawa T., Kitajima M., Nonato M. G., Aimi N. (2002), Isolation and structure elucidation of two new alkaloids, pandamarilactonine-C and -D, from *Pandanus amaryllifolius* and revision of relative stereochemistry of pandamarilactonine-A and -B by total synthesis, *Chemical and Pharmaceutical Bulletin*, 50(9), 1303-1304.

Journal of Medicinal Materials, 2022, Vol. 27, No. 4 (pp. 222 - 227)

DEVELOPMENT OF STACHYOSE QUANTITATIVE DETERMINATION IN *STACHYS AFFINIS* BUNGE BY LC - MS AND ITS APPLICATION FOR ESTABLISHMENT OF STACHYOSE REFERENCE STANDARD

Nguyen Thi Bich Ngoc[1,2], Dang Thi Xuan Quyen[3], Nguyen Thi Vy Phuong[3], Vo Thi Bach Hue[4], Phan Nguyen Truong Thang[5], Phan Van Ho Nam[1,*]

[1]*Department of Analytical Chemistry and Quality Control of Drug, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam;*

[2]*NTT High Technology Institute - Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam;*

[3]*Tipharco Pharmaceutical Joint Stock Company;*

[4]*Department of Analytical Chemistry and Quality Control of Drug, Lac Hong University, Dong Nai Province, Vietnam;*

[5]*Institute of Drug Quality Control Ho Chi Minh City, Vietnam.*

*Corresponding author: phanvanhonam@ump.edu.vn

(Received March 27th, 2022)

Summary

Development of Stachyose Quantitative Determination in *Stachys affinis* Bunge by LC - MS and its Application for Establishment of Stachyose Reference Standard

Stachyose is the main ingredient in *Stachys affinis* rhizomes and has many pharmacological effects such as lowering blood sugar, strengthening the immune system, and relieving constipation, etc. To monitor the quality of the new material and its products, the quantitative determination of stachyose in *Stachys affinis* Bunge rhizomes was developed according to the guidelines of ICH (2005) and AOAC (2012). Besides, the established procedure was applied to determine the purity of stachyose as following requirements of a reference standard.

The procedure was UPLC-MS system (ACQUITY QDa Waters); Phenomenex Gemini NX-C_{18} (4.6 × 150 mm; 5 µm) column; acid formic 0.05% and MeOH as mobile phase in isocratic mode; flow rate of 0.5 mL/min; detector MS/ESI- (665 *m/z*); cone voltage 40 V; capillary voltage 0.8 kV. Standards and test samples are all diluted in water before analysis.

Keywords: *Stachys affinis* Bunge, *Stachyose, Reference standard, LC-MS.*

1. Introduction

Vietnam is considered as a country with great potential for medicinal herbs in Southeast Asia and in the world. According to the Agency of Traditional Medicine Administration, out of a total of nearly 5,000 known medicinal plant and mushroom species, there are many species that have the potential to be exploited to create medicinal materials to serve market demand [1]. However, according to the report of the Agency of Traditional Medicine Administration, Vietnam can only supply 25% of the domestic demand for medicinal herbs, and most of the 75% depends on imports from mainly pharmaceuticals China. Therefore, the quality control of imported medicinal herbs is of great concern at present.

Stachyose ($C_{24}H_{42}O_{21}$) is a tetrasaccharide containing two α-D-galactose units, one α-D-glucose unit and one β-D-fructose unit linked as follows: *O*-α-D-galactose-(1→6)-*O*-α-galactose-