positive control. IC_{50} of the Lam Binh samples ranged from 36.48 to 49.79 µg/mL while the data for marketed samples varied between 41.21 and 54.07 µg/mL. No significant difference between the NO inhibitions of the two sampling groups could be observed ($p > 0.05$). The MTT assay indicated that all of the extracts had no cytotoxicity to the cells (data not shown).

4. Conclusion

Results of chemicals quantification and bioassays indicated that *A. sylvestris* leaves had a number of secondary metabolites with considerable free radical-scavenging and anti-inflammatory effects. The *A. sylvestris* samples from the Lam Binh have the phenolics, and flavonoids contents at 12.61 - 18.96 mg GAE/g and 7.85 - 13.27 mg QE/g, respectively, which were significantly higher than those of current commercial samples in the market. Meanwhile, tannins and alkaloid contents of the Lam Binh *A. sylvestris* samples leaves were at 2.54% - 3.94% and 1.39 - 3.94 mg/g, respectively, which were similar to the marketed samples. The DPPH radical-scavenging activity of *A. sylvestris* leaves extracts was deeply affected by phenolics and flavonoids contents while the anti-inflammatory composition of the plant should be further investigated. These could be promising results for the further development of *A. sylvestris* cultivation in the region.

Acknowledgement: *This study was funded by the Department of Science and Technology, Tuyen Quang Provincial People's Committee in the project with the approval decision code 1953/QĐ-UBND dated December 23, 2020.*

References

1. Chi V. V. (2012), *Dictionary of Vietnamese Medicinal Plants*, Medicine Publishing House. **2.** Ho P. H. (2003), *Plants of Vietnam*, Tre Publishing House. **3.** Anh N. H., Ripperger H., Schmidt J., Porzel A., Sung T. V., Adam G. (1996), Resorcinol derivatives from two *Ardisia* species, *Planta Medica*, 62(05), 479-480. **4.** Anh P. T. V., Dien V. V., Huong N. T. G., Phuong N. T. (2000), Primary investigation of phytochemicals and bioactivities of *Ardisia sylvestris* Pitard, *Journal of Medical and Pharmaceutical Information*, 6, 25-28. **5.** Biet H. V., Hong P. V. C., Toan T. Q., Nga N. T. T., Phuong N. T. N. (2020), Phytochemical, antioxidant, anti-bacteria, anti-inflammatory effects of the extract from *Ardisia silvestris* leaves, *Journal of Agriculture and Development*, 4(19), 100-105. **6.** Fattahi S., Zabihi E., Abedian Z., Pourbagher R., Motevalizadeh A. A., Mostafazadeh A., Akhavan-Niaki H. (2014), Total phenolic and flavonoid contents of aqueous extract of stinging nettle and *in vitro* antiproliferative effect on Hela and BT-474 cell lines, *International Journal of Molecular and Cellular Medicine*, 3(2), 102-107. **7.** Ministry of Health of Vietnam (2017), *Vietnamese Pharmacopoeia V*, Medical Publishing House. **8.** Brand-Williams W., Cuvelier M. E., Berset C. (1995), Use of a free radical method to evaluate antioxidant activity, *LWT - Food Science and Technology*, 28(1), 25-30. **9.** Vien L. T., Hanh T. T. H., Huong P. T. T., Dang N. H., Thanh N. V., Lyakhova E., Cuong N. X., Nam N. H., Kiem P. V., Kicha A., Minh C. V. (2016), Pyrrole oligoglycosides from the starfish *Acanthaster planci* suppress lipopolysaccharide-induced nitric oxide production in RAW264.7 macrophages, *Chemical and Pharmaceutical Bulletin*, 64(11), 1654-1657. **10.** Mosmann T. (1983), Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays, *Journal of Immunological Methods*, 65(1), 55-63. **11.** Ahmad S., Israf D. A., Lajis N. H., Shaari K., Mohamed H., Wahab A. A., Ariffin K. T., Hoo W. Y., Aziz N. A., Kadir A. A., Sulaiman M. R., Somchit M. N. (2006), Cardamonin, inhibits pro-inflammatory mediators in activated RAW 264.7 cells and whole blood, *European Journal of Pharmacology*, 538(1), 188-194. **12.** Arulselvan P., Fard M. T., Tan W. S., Gothai S., Fakurazi S., Norhaizan M. E., Kumar S. S. (2016), Role of antioxidants and natural products in inflammation, *Oxidative Medicine Celullar Longevity*, art.ID. 5276130. **13.** Le A. S., Do T. H., Vu T. D.., Dau B. T, Nguyen T. T. (2017), Chemical constituents, antioxidant, and cytotoxicity of *Ardisia gigantifolia* leaves extract, *Journal of Medicinal Materials*, 22(6), 346-351.

Journal of Medicinal Materials, 2023, Vol. 28, No. 2 (pp. 103 -109)

QUANTITATIVE DETERMINATION OF MIMOSINE IN THE MEDICINAL HERB *MIMOSA PUDICA* MIMOSACEAE BY HPLC-PDA

Nguyen Thi Thanh Van[1], Vo Quoc Ngu[2], Huynh Lam Diem My[2], Duong Hong To Quyen[3], Phan Van Ho Nam[1], Hua Hoang Oanh[1],*

[1]University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam;

[2]University of Natural Sciences, Vietnam National University, Ho Chi Minh City, Vietnam;

[3]Hospital of Traditional Medicine, Ho Chi Minh City, Vietnam

*Corresponding author: hhoanh@ump.edu.vn

(Received February 04th, 2023)

Summary

Quantitative Determination of Mimosine in the Medicinal Herb *Mimosa pudica* Mimosaceae by HPLC-PDA

An HPLC method was proposed for quantification of mimosine in the aerial parts of *Mimosa pudica*. The chromatography was established as: using the C_{18} (250 mm × 4.6 mm, 5 µm) column; the PDA detector was set at 275 nm; the mobile phase consisted of methanol and pH 2.0 buffer with ion-pairing agent sodium hexane-1-sulfonate, gradient

elution; flow rate was 1 mL/min; the column temperature was set at 35°C; injection volume was 20 µL. The method was validated for the system suitability (RSD of peak area = 1.18%; RSD of t_R = 0.03%); specificity; linearity ranging from 0.5 - 20.0 µg/mL (R^2 = 1), precision (RSD < 3.0%) and accuracy (recovery: 92.43% - 98.11%). As for practical application, the tested samples showed the content of mimosine ranging from 64.5 µg/g to 363.1 µg/g, on dried basis.

Keywords: *Mimosine, Mimosa pudica, Specification, HPLC-PDA.*

1. Introduction

Sensitive plant (*Mimosa pudica* L., Mimosaceae) has long been used in traditional medicine for sedative, hypnotic, anxiolytic and treatment of bronchitis, acute conjunctivitis, hepatitis, small bowel inflammation, urinary stones, rheumatism, paralysis, high blood pressure [1]. Currently, there are several medicinal products in the market that contain it. However, the latest edition (5^{th}) of the Vietnamese Pharmacopoeia does not include the monograph *M. pudica* [2]. The presence of mimosine, a non-protein amino acid, is exclusive to *M. pudica* and some species of *Leucaena* [3]. There are some reports of mimosine quantitation in *M. pudica* by RP-TLC, LC-MS but very few works using HPLC-PDA [4],[5],[6]. In order to control and improve the quality of the herb in the market, we develop a quantitative method for the determination of mimosine in this plant.

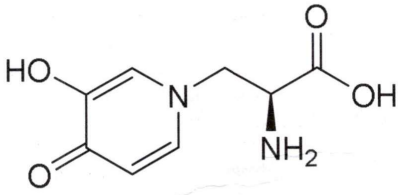


Fig. 1. Chemical structure of *L*-mimosine

2. Materials and methods

2.1. Materials

The aerial parts of Mimosa (*Mimosa pudica* L.) were provided by the Hospital of Traditional Medicine in Ho Chi Minh City and was identified by DNA barcoding at the University of Natural Sciences, Vietnam National University, Ho Chi Minh City. Homogenize the sample by grinding it into a powder, passing it through a 355 sieve.

Four sample batch numbers were coded to analysis: VTNC_ XH 0108022022, VTNC_XH 020802022, VTNC_XH 0109022022, VTNC_XH 0209022022.

2.2. Chemicals, apparatus

Standards: *L*-mimosine (98.0%) from Aladdin (Shanghai, China; CAS: 500-44-7, Lot. D2102244).

Reagents, solvents: methanol, ethanol, acetonitrile, sodium hexane-1-sulfonate, hydrochloric acid fuming 37%, ortho-phosphoric acid 85% (Merck, Germany), distilled water.

Apparatus: an HPLC system (Shimadzu, Japan, model: LC-2030C 3D plus) was equipped with high-pressure pumps, an autosampler, a PDA detector, a column Shim-pack GIST C_{18} (250 × 4.6 mm; 5 µm), and a precolumn C_{18} (10 × 4.6 mm; 5 µm) (Shimadzu, Japan); pH meter Lab 865 (SI analytics, Germany); analytical balance XP26, 0.001 mg readability and MS204, 0.1 mg readability (Mettler Toledo, Switzerland), ultrasonic bath (Elma, Germany), drying oven UN55 (Memmert, Germany), water bath WNB 14 (Memmert, Germany) and other analytical grade apparatus.

2.3. Methods

Sample preparation: Select the sample preparation method so that the content of mimosine is the highest (greatest extraction efficiency). Sample preparation factors were investigated including: Extraction solvents (water, 0.2 N hydrochloric acid, ethanol 50%), apparatus (sonication at room temperature and 50 °C, on water bath at 50 °C), extraction duration (10, 20 and 30 minutes), number of extraction times (one, two times), ratio of the *mass* of the *sample* to the *volume* of the *solvent* (1:15; 1:20 and 1:25) g/ml.

Chromatographic condition: The chromatographic condition was investigated to separate the mimosine peak from other peaks on the chromatogram and achieve chromatographic purity.

Validation: The procedure is validated according to ICH guidelines: system suitability, specificity, linearity range, accuracy, precision, limit of detection, and limit of quantitation [7].

Application on real samples: Use the validated procedure to quantify mimosine content in sensitive plants (*M. pudica*) from different sources.

3. Results

3.1. Sample preparation

The results are presented in Table 1. From the investigation results, the sample preparation procedure was selected as follows:

Weigh 400 mg of medicinal herbs (355), wet the sample with a minimum amount (about 1.0 ml) of ethanol 50% (due to the light density, difficult permeability nature), add 7 ml of 0.2 N

HCl solution, sonicate for 20 mins at room temperature. Transfer the extract to a 20 ml volumetric flask, continue to extract the medicinal herbs with 8 ml of 0.2 N HCl solution. Combine the extracts, rinse, and make up to the mark with 0.2 N HCl solution.

Table 1. Sample preparation method investigation results

Extraction solvents	**Water**	**HCl 0.2 N**	**Ethanol 50%**
Mimosine content (µg/g)	102.9	118.1	12.2
Apparatus	Sonication	Sonication at 50 °C	Waterbath at 50 °C
Mimosine content (µg/g)	118.1	112.3	97.5
Sample:solvent ratio	1:15	1:20	1:25
Mimosine content (µg/g)	93.1	118.1	103.6
Duration	10 min	20 min	30 min
Mimosine content (µg/g)	110.6	118.1	99.6
Number of extractions	One time	Two times	
Mimosine content (µg/g)	118.1	125.6	

3.2. Optimization of mobile phase composition

The mobile phase condition optimization and chromatogram are presented in Table 2 and Fig. 2, respectively.

Table 2. Investigated mobile phase conditions

Condition	**Elution program**
I	Methanol - pH 2.0 buffer (10:90)
II	Acetonitrile - pH 2.0 buffer (10:90)
III	As below in Procedure (selected condition)

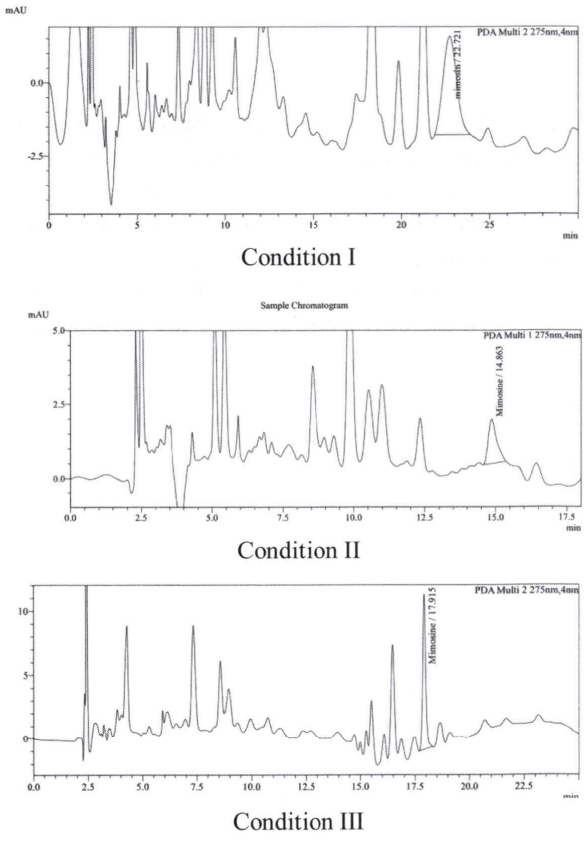


Fig. 2. Investigated chromatogram of sample solution

The results showed that condition III has the greatest separation efficiency within 25 minutes. In condition II, the mimosine peak eluted early but did not have the necessary separation performance. In condition I, the mimosine peak was broadened and there may be many compounds existing in matrix did not elute within the analysis time. Therefore, it was selected as the mobile phase in our procedure.

3.3. Procedure

Reference solution: dissolve 2.5 mg of mimosine standard in 0.2 N hydrochloric acid and dilute to 20 ml with the same solvent. Dilute 2 ml of the above solution to 100 ml with 0.2 N hydrochloric acid, filter through a 0.22 µm filter. The resulting solution has a concentration of 2.5 µg/ml mimosine.

Test solution: weigh 400 mg of herbal sample powder (determined humidity), wet the sample with a minimum amount of ethanol 50% (about 1.0 ml), add 7 ml of 0.2 N HCl solution, sonicate for 20 min at room temperature. Transfer the extract to a 20 ml volumetric flask, continue to extract the medicinal herbs with 8 ml of 0.2 N HCl solution. Combine the extracts, rinse, and make up to the mark with HCl 0.2 N solution. Filter through a membrane filter with a pore size not exceeding 0.22 µm.

Chromatographic system: the liquid chromatography was equipped with a PDA photometer (wavelength at 275 nm) and a C_{18} column (250×4.6 mm; 5 µm), the flow rate was 1.0 ml/min. Column temperature was maintained at 35°C.

Mobile phase: gradient conditions as mentioned in Table 3

Mobile phase A: dissolved 4.0 g sodium hexane-1-sulfonate in 800 ml water, adjusted to

pH 2.0 with ortho-phosphoric acid (5 ml), diluted to 1000 ml with water.

Mobile phase B: methanol.

Table 3. Gradient conditions

Time (minutes)	Mobile phase A (%)	Mobile phase B (%)
0	90	10
10	90	10
11	75	25
20	75	25
21	90	10
25	90	10

Procedure: inject 20 µl of each of reference solution, and test solution. The content of mimosine (µg/g) in the herbal medicine was calculated by the formula:

$$X\ (\%) = \frac{S_t \times C \times 20}{S_c \times m_t}$$

S_t: area of the peak due to mimosine in the chromatogram obtained with the test solution

S_c: area of the peak due to mimosine in the chromatogram obtained with the reference solution

m_t: mass of the herbal drug to be examined used to prepare the test solution, in grams (equivalent to dried material);

C: mimosine concentration in reference solution (µg/ml) (including mimosine content in reference standard)

3.4. Method validation

System suitability

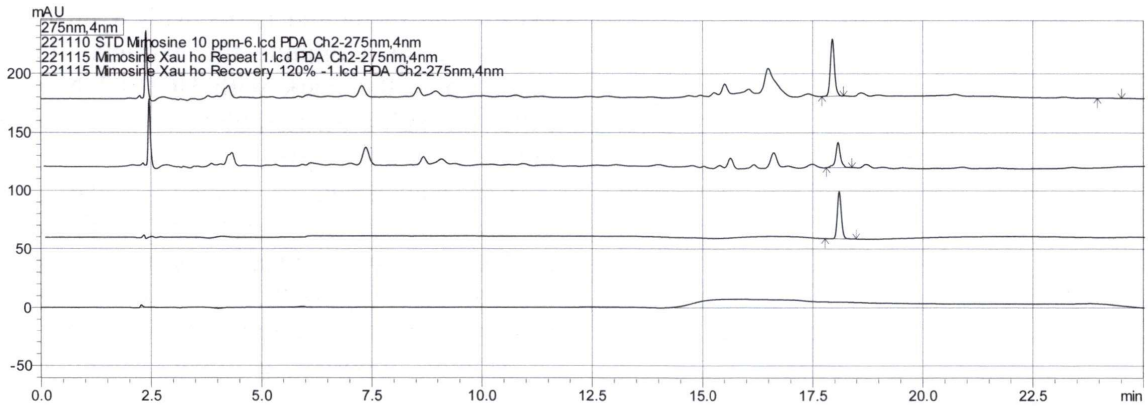
The results was presented in Table 4. Retention time, peak area of six replicates have RSD% ≤ 2%. Other parameters such as tailing factor, number of theoretical plates all meet requirements.

Table 4. System suitability parameters

No.	**t_R (min)** (RSD% ≤ 2,0)	**Area (mAU.s)** (RSD% ≤ 2,0)	**A_S** (0,8 - 1,5)	**N** (≥ 2000)
1	17.935	130688	1.059	86555
2	17.927	133584	1.067	86771
3	17.930	133554	1.065	87634
4	17.921	133901	1.062	87647
5	17.925	131770	1.065	88407
6	17.923	130417	1.051	89147
Average	17.927	132319		
RSD%	0.03	1.18		

Specificity

The results from Fig. 3 showed that in the chromatogram of the blank sample (HCl 0.2 N) no peak occurred at the retention time corresponding to the retention time of the mimosine peak in the reference sample. The chromatogram of the test sample has a peak with a retention time corresponding to the mimosine peak in the standard sample. When mimosine standard was added to the test sample, the height and area of the mimosine peak increased significantly. The UV spectrum obtained from the mimosine peak in the chromatogram of the test sample was consistent with mimosine UV spectrum in the chromatogram of the reference.



Up to down: *spiking solution, sample solution, reference solution, blank.*

Fig. 3. Chromatogram of specificity validation

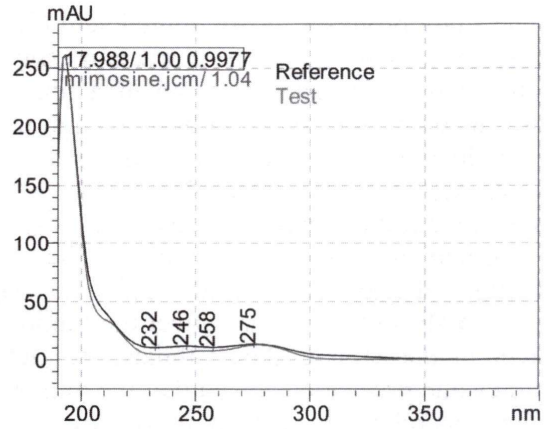


Fig. 4. UV spectrum overlay

Linear range and calibration curve:

The linear range (0.5 µg/ml - 20.0 µg/ml) validation results were presented in Fig. 5. There was a strong correlation between peak area and mimosine concentration with $R^2 = 1.0000$.

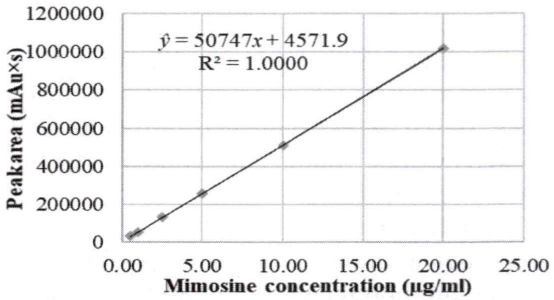


Fig. 5. Calibration curve of mimosine

Repeatability and intermediate precision

The results of the repeatability and intermediate precision study were presented in Table 5. The average content of mimosine is 125.7 µg/g and the RSD is lower than 3.0%. The procedure meets AOAC's requirements of repeatability and intermediate precision. The result also showed the stability content of mimosine in the sample.

Table 5. Precision study by the proposed procedure

No.	Mimosine content (µg/g)		On dried basis
	Day 1	Day 2	
1	124.9	127.4	
2	125.4	125.4	
3	127.3	124.7	**Average**: 125.7 (µg/g)
4	121.3	126.3	**RSD**: 1.42%
5	126.9	124.3	
6	127.8	126.0	
Average	125.6	125.7	
RSD%	1.90	0.92	

Accuracy

The mimosine standard was added in the test sample at three levels. The results were presented in Table 6. The recovery of mimosine in *Mimosa pudica* meets AOAC's requirements at three levels. RSD of mimosine's recovery from each level was lower than 3.0% [4].

Table 6. Recovery study of mimosine by the proposed procedure

Mimosine amount added (µg)	Recovery (%)	Average (%), RSD (%)
	96.01	Average = 94.01 RSD = 1.94
10.95	92.43	
	93.59	
	96.20	Average = 95.09 RSD = 1.86
21.91	96.02	
	93.06	
	96.28	Average = 97.27 RSD = 0.95
32.86	97.40	
	98.11	

Limit of detection (LOD), limit of quantitation (LOQ)

The LOD and LOQ of the method were performed by diluting the test solution to the concentration that give the minimum signal to noise (S/N) 3 and 10, respectively. The result showed that LOD is 0.1 µg/ml and LOQ is 0.25 µg/ml. Those also mean that the LOD and LOQ of the method calculated on sample weight is 5.0 µg/g and 12.5 µg/g, respectively.

3.5. Real samples analysis

Analytical results from different sources (Table 8) showed that the mimosine content was in the range of 64.5 µg/g - 363.1 µg/g, on dried basis.

Table 8. Analytical results from different sources

Sample no.	Mimosine content Avg. ± SD (µg/g, n = 3)
1	363.1 ± 2.1
2	64.5 ± 0.7
3	286.5 ± 1.1
4	107.8 ± 4.8

4. Discussion

Although *Mimosa pudica* is a commonly used medicinal herb in traditional medicine, the current Pharmacopoeia of Vietnam and other countries do not include monograph for this herb. This study has been conducted to develop a quantitation method of chemical marker in the plant from different sources to assure an appropriate quality level.

There are few publications on quantitative determination of mimosine in the herbal medicine *M. pudica* in Vietnam and around the world. The principal technique is liquid chromatography, with mass spectrometry or spectroscopy detector [4],[5],[6]. Mimosine is a highly polar compound that is poorly retained on a C_{18} reversed-phase chromatographic column, its peak usually elutes very fast, which is equivalent to the column's unretained time. Experimental results also confirmed this. The specificity of the method can be supplemented by mass spectrometry, however, for spectroscopic detector, application on a sample matrix with a complex composition such as medicinal herbs make the method less specific. Therefore, it is necessary to change the chromatographic conditions such as the mobile phase by adding an ion-pairing agent, or the stationary phase by switching to a HILIC column. In order to take advantage of the reverse phase chromatography columns available in the laboratory, the study supposes to use the ion pairing agent sodium hexane-1-sulfonate added to the mobile phase based on the structure containing both amino and carboxylic acid functional groups of mimosine. The results showed that the retention time of mimosine was significantly increased. On the other hand, this dipole compound must be converted to a fully ionized form with an acidic or basic mobile phase to have a good peak shape. The acidic mobile phase was chosen because the silica-based chromatographic column is less stable to bases than to acids. For the above reasons, the ion pairing agent used is sodium hexane-1-sulfonate, adjusted to pH 2.0 with ortho-phosphoric acid. However, in order to separate the mimosine peak from other peaks but still ensure that the chromatographic time is not too long, a gradient elution program was developed for mimosine peak with a retention time of about 17 minutes and completely separated from the adjacent peaks, in accordance with the requirements.

The study proposes to use diluted acid (0.2 N hydrochloric acid) as the extraction solvent because this compound is stable in acidic and neutral medium, less stable in basic medium, but still conducting investigations on other extraction solvents such as water, 50% ethanol [8],[9]. The sample preparation procedure also investigated the influencing factors to obtain the best extraction efficiency, avoiding going through many complicated steps that affect the mimosine content in the sample. The validated results of accuracy and precision showed that the mimosine content was relatively stability in the same batch, reflecting the appropriate extraction efficiency according to the procedure. The investigated results also showed the agreement between experiment and theory on solubility and stability of mimosine. In addition, although mimosine is poorly soluble in ethanol, the procedure used this solvent to 'moisten' the medicinal herbs before extraction because the use of 0.2 N HCl lonely created a large surface tension between the solvent and the sample. On these bases, the procedure was applied to quantify mimosine in medicinal samples of *M. pudica* from different sources. Based on experimental results as well as published works, mimosine content fluctuates much between different sources.

5. Conclusion

The quantitation procedure performed by high-pressure liquid chromatography on chemical marker mimosine met the requirements according to the ICH guidelines for system suitability, linearity, specificity, accuracy, and precision. The research has contributed to the quality control of the medicinal herbs on the market and created the basis for collection of qualified raw materials for the production of high-quality finished products of traditional medicine.

Acknowledgment: *This study was financially supported by Ho Chi Minh City Foundation for Science and Technology Development. The authors would like to thank Ho Chi Minh City Department of Science and Technology.*

Reference

1. Gupta R., Ahmad H., Sehgal S., Mishra A. (2012), *Mimosa pudica* L. (Laajvanti): An overview. *Pharmacognosy Reviews*, 6(12), 115. doi:10.4103/0973-7847.99945 **2.** Bộ Y Tế. Dược Điển Việt Nam V Tập 2. 2018. **3.** Lalitha K., Rajendra K. S. (2004), Determination of mimosine by a sensitive indirect spectrophotometric method, *Talanta*, 63(3), 635-640. doi:https://doi.org/10.1016/j.talanta.2003.12.006. **4.** Champanerkar P. A., Vaidya V. V., Shailajan S., Menon S. N. (2010), A sensitive, rapid and validated liquid chromatography - tandem mass spectrometry (LC-MS-MS) method for determination of Mimosine in *Mimosa pudica* Linn., *Natural Science*, 2(7), 713-717. doi: 10.4236/ns.2010.27088 **5.** Mohamed M. S., Athar M., Kamal Y., et al (2011), Quantitative estimation of mimosine in anti-psoriatic ayurvedic formulation containing whole plant extract of *Mimosa pudica* Linn. by validated isocratic RP-HPLC method. *Acta Chromatographica*, 23(3), 531-538. doi: 10.1556/AChrom.23.2011.3.13 **6.** Dhanya K. G., Thangavel M. (2019), Mimosine - a novel substance and evaluate the lipid

peroxidation, *Asian Journal of Pharmaceutical and Clinical Research*, 12(3), 57-60. doi:10.22159/ajpcr.2019.v12i3.22812 **7.** ICH Harmonised Guideline. Validation of Analytical Procedures: Q2(R1). 2005. **8.** O'Neil M.J., eds. The Merck Index. 13th ed. Merck & Co, NJ. 2001:mimosine. **9.** Long P. T., Đào Đ. Q. A., Đài N. T. X. (2009), Nghiên cứu trích ly và tinh sạch mimosin từ cây Mắc cỡ, *Tạp Chí Khoa Học & Công Nghệ Các Trường Đại Học Kỹ Thuật*, 71: 90-95. 10. AOAC International. Appendix K: Guidelines for Dietary Supplements and Botanicals. 2019.

Journal of Medicinal Materials, 2023, Vol. 28, No. 2 (pp. 109 - 116)

AMELIORATIVE EFFECTS OF *BACOPA MONNIERI* (LINN.) WETTST *n*-BUTANOL EXTRACT ON NEUROBEHAVIORAL DEFICITS IN TRANSGENIC DROSOPHILA EXPRESSING HUMAN AMYLOID PRECURSOR PROTEIN

Pham Thi Nguyet Hang[1, 2,]*, Phi Thi Tuyet Nhung[3], Nguyen Van Hiep[1], Tran Thi Hong Van[1], Nguyen Thi Lap[4]

[1]National Institute of Medicinal Materials, Hanoi, Vietnam; [2]University of Medicine and Pharmacy, Vietnam National University; [3]Thanh Do University, Vietnam; [4]Hanoi Pharmacy University, Vietnam

*Corresponding author: nguyethangpt@nimm.org.vn

(Received December 12th, 2022)

Summary

Ameliorative Effects of *Bacopa monnieri* (Linn.) Wettst *n*-Butanol Extract on Neurobehavioral Deficits in Transgenic Drosophila Expressing Human Amyloid Precursor Protein

Alzheimer's disease (AD) is a neurodegenerative disorder and the most common form of dementia in the group aged above 60 years. It is accompanied by neuronal disorders along with learning and memory defects, as well as locomotor impairment. Because 70% of human disease-related genes are conserved in *Drosophila melanogaster* (*D. melanogaster*), especially those associated with types of risk factors for AD, this is a powerful model to study AD and develop the therapeutic AD drugs. In this study, we evaluate the effect of n-butanol extract from *Bacopa monnieri* (BM) on behaviors in transgenic flies expressing human amyloid precursor protein (hAPP) as a model organism for AD. Behavioral experiments were conducted to evaluate the crawling ability and short-term memory of third-instar larvae as well as climbing ability of adult flies. Furthermore, the expression of hAPP was evaluated in the head of adult flies which were exposed by 4 and 6 mg/mL BM extract using Western blot method. The results showed that the n-butanol extract from BM was effective in improving the ability of third-instar larvae to crawl and short-term memory, the ability of adult flies to climb, and at the same time suppressed the hAPP expression in adult brain. The results suggest that BM extract is a candidate for the treatment of behavioral deficits in transgenic *Drosophila* expressing hAPP protein.

Keywords: *Alzheimer's disease, Drosophila model, Bacopa monnieri, Behavioral alterations, hAPP expression.*

1. Introduction

Alzheimer's disease (AD) is a disease characterized by symptoms of impaired memory and cognitive function and is the most severe form of the group of dementia diseases today. According to statistics from the World Alzheimer's Association 2015, the incidence of AD increases gradually with age, from about 5% of people under 75 years old to 40-50% of people after 85 years of age [1]. AD always comes with a huge treatment budget and a physical and emotional burden on patients and their loved ones [2, 3, 4]. Drugs for the treatment of AD include cholinesterase inhibitors, N-methyl D-aspartate (NMDA) receptor antagonists, and drugs acting on pathological amyloid. However, these drugs have many side effects [5]. Therefore, the need to research and develop new drugs derived from medicinal herbs to support and treat Alzheimer's disease is very necessary.

Bacopa monnieri (Linn) Wettst (BM) is a medicinal plant used in traditional Indian medicine as a nerve tonic, memory booster, and epilepsy treatment. In Vietnam, BM grows wild in many places and is used as a daily vegetable and to treat a number of diseases such as neurasthenia, epilepsy, loss of muscle tone, according to folk experience [6]. There are much research works in the world that have proven that BM has the effect of improving memory and cognitive impairment by models of memory impairment in mice with ethylcholine aziridinium ion, colchicine, $AlCl_3$ and in mice carrying AD gene (PSAPP mice) [7],[8],[9]. In Vietnam, BM has also been studied very carefully for its effect on preventing/improving memory impairment and its mechanism of action on a mice model of trimethyltin (TMT)-induced memory impairment and olfactory bulbectomized (OBX) model of AD [10],[11]. In addition, Nguyen Thi Thu