PROTECTIVE AND CURATIVE EFFECTS OF PHUONG DONG DAI TRANG TABLETS ON AN EXPERIMENTAL MODEL OF IRRITABLE BOWEL SYNDROME

Tran Thanh Tung, Pham Thi Van Anh and Dau Thuy Duong✉
Hanoi Medical University

Phuong Dong Dai Trang tablet is a mixture of medicinal herbs including Hedychium coronarium, Coix lacryma-jobi, Dioscorea persimilis, Cynara Scolymus L., Paeonia lactiflora, and Glochidion eriocarpum. This study was carried out to evaluate the protective and curative effects of Phuong Dong Dai Trang tablets on mustard oil-induced irritable bowel syndrome model in mice. Swiss mice were divided into six groups that were given orally 0.9% sodium chloride (group 1 - 3), Duspatalin (mebeverine) 80 mg/kg b.w./day (group 4), Phuong Dong Dai Trang tablets 1080 mg/kg b.w./day (group 5) and Phuong Dong Dai Trang tablets 3240 mg/kg/b.w./day (group 6). Mice of group 3 - 6 were induced diarrhea-predominant irritable bowel syndrome by mustard oil colonic administration before or after oral treatment. The research indices included the intestinal motility measured by charcoal meal test, colon macroscopic and microscopic scores. Our findings showed that Phuong Dong Dai Trang tablets at 3240 mg/kg b.w./day reduced the intestinal motility and the stool score significantly in mice. Phuong Dong Dai Trang tablets at 1080 mg/kg b.w./day improved the stool score and tended to decrease the intestinal motility in mice.

Keywords: Phuong Dong Dai Trang tablets, mice, mustard oil, diarrhea-predominant irritable bowel syndrome.

I. INTRODUCTION

Irritable bowel syndrome (IBS) was defined as functional gastrointestinal disorders with recurrent abdominal pain associated with defecation or changes in bowel habits. Functional gastrointestinal disorders recur repeatedly without finding any structural damage or biochemical disorders.[1,2] IBS is a chronic disease which is not life-threatening, but significantly affects the patient's quality of life and requires expensive treatment costs.[2]

IBS is one of the most common gastrointestinal disorders worldwide. Its prevalence varies significantly from country to country. The overall prevalence of IBS in the world is 9.2% (according to Rome III criteria) and 3.8% (according to Rome IV criteria).[2] The prevalence of IBS is common in people under 50 years old, mostly in people at the age of 20 - 30 years old and 1.6 times higher in women than in men. It has been also demonstrated that diarrhea-predominant irritable bowel syndrome (IBS-D) is the most common subtype of IBS.[2] The incidence of IBS has increased rapidly in Asian countries. Although there have been few studies on the epidemiology of IBS in the Vietnamese population, there have been an increasing number of case series on IBS in Vietnam recently.[3]

IBS treatment requires a combination of pharmacological and non-pharmacological

Corresponding author: Dau Thuy Duong
Hanoi Medical University
Email: dauthuyduong@hmu.edu.vn
Received: 01/08/2024
Accepted: 20/08/2024

methods. Several pharmacological agents have been used to treat IBS depending on the predominant symptoms such as: antispasmodics (mebeverine, alverine), antidiarrheals (loperamide, diosmectite), antidepressants (amitriptyline, sulpiride)…[4] Due to prolongue syndrome, insufficiency or intolerance of current treatments, drug side effects, and financial burden for patients, several patients have turned to the use of complementary and alternative medicine, including hypnosis, acupuncture, and herbal medicine.[5] A number of herbal medicines have been demonstrated to have therapeutic effects in IBS.[5] With the increasing incidence of IBS and a long-standing traditional medicine in Vietnam, there is a recent trend to develop the herbal products to meet the needs of patients for a supportive treatment of IBS and improvement of the quality of life.

Phuong Dong Dai Trang tablet (PDDT) is a mixture of medicinal herbs including *Hedychium coronarium*, *Coix lacryma-jobi, Dioscorea persimilis*, *Cynara Scolymus* L., *Paeonia lactiflora*, *Glochidion eriocarpum*. The formula of PDDT is based on the traditional "Colon remedy" in Ha Giang province. This herbal mixture is prepared in a modern dosage form (tablet) to expand the accessibility, ease of administration, and the consumer preferences. Its intended indication is to treat functional gastrointestinal diseases such as IBS with diarrhea (IBS-D). There have been no study on the efficacy of this herbal mixture on IBS-D. We carried out this study with two objectives: 1) Evaluate the protective effects of PDDT on MO-induced IBS-D model in mice; 2) Evaluate the curative effects of PDDT on MO-induced IBS-D model in mice.

II. MATERIALS AND METHODS

1. Subjects

The investigational product

Phuong Dong Dai Trang tablet (PDDT) is a herbal product manufactured by Phuong Dong Pharmaceutical and Trading Company. Each tablet contains 750mg extract from 6500mg medicinal herbs including:

Hedychium coronarium	1500mg
Coix lacryma-jobi	1500mg
Dioscorea persimilis	1000mg
Cynara Scolymus L.	1000mg
Paeonia lactiflora	1000mg
Glochidion eriocarpum	500mg.

The recommended daily oral dose of PDDT to support the treatment of gastrointestinal disorders such as IBS-D in patients is 6 tablets.

Drugs/chemicals and equipments for research

- Mebeverine tablets 200mg, trade name Duspatalin, Abbott (Singapore).

- Mustard oil (MO) (94% allyl isothiocyanate), Acros Organics (Belgium).

- Activated charcoal, 90% ethanol, 3% carboxymethylcellulose (CMC), 10% formalin,

0.9% sodium chloride, distilled water.

- Surgical instruments, medical gauze, medical tape.

- Catheter 6F, syringes.

- Chemicals and machines for preparing histopathological specimens.

Experimental animals

Healthy adult *Swiss mice* (weighed 20 ± 2g) were housed and fed in standard conditions one week before and during investigation at the laboratory of the Department of Pharmacology, Hanoi Medical University.

2. Methods

The protective and curative effects of PDDT

was evaluated on mustard oil (MO)-induced IBS-D model in mice.[1]

Protective effects of PDDT on MO-induced IBS model

Mice were randomly divided into 6 groups:

- Group 1 (control group): were administered orally distilled water.
- Group 2 (ethanol control): were administered orally distilled water.
- Group 3 (model group): were administered orally distilled water.
- Group 4 (positive control): were administered orally Duspatalin 80 mg/kg b.w/day.
- Group 5 (low dose of PDDT): were administered orally PDDT 1.44 tablets/kg b.w/day (approximately 1080 mg/kg b.w./day).
- Group 6 (high dose of PDDT): were administered orally PDDT 4.32 tablets/kg b.w/day (approximately 3240 mg/kg b.w./day).

Mice were treated 7 days before IBS induction and continued until 24 days. After 7 days of treatment, mice were anesthetized by chloral hydrate and administered intracolonically to a depth of 4cm via a catheter. Two control groups (group 1 and 2) were administered intracolonically with 0.9% sodium chloride or 30% ethanol vehicle, respectively. Four other groups (group 3 to group 6) were administered intracolonically with 100 µL of 2% MO solution (diluted in 30% ethanol). After the last dose, the indices were evaluated as described in *Research indices* section.

Curative effects of PDDT on MO-induced IBS model

Mice were randomly divided into 6 groups. At the beginning of the study, mice were anesthetized by chloral hydrate and administered intracolonically to a depth of 4 cm via a catheter. Two control groups (group 1 and 2) were administered intracolonically with 0.9% sodium chloride or 30% ethanol vehicle, respectively. Four other groups (group 3 to group 6) were administered intracolonically with 100 µL of 2% MO solution (diluted in 30% ethanol). After seven days, mice were given orally for ten days as mentioned below:

- Group 1 (control group): were administered orally distilled water.
- Group 2 (ethanol control): were administered orally distilled water.
- Group 3 (model group): were administered orally distilled water.
- Group 4 (positive control): were administered orally Duspatalin 80 mg/kg b.w/day.
- Group 5 (low dose of PDDT): were administered orally PDDT 1.44 tablets/kg b.w/day (approximately 1080 mg/kg b.w./day).
- Group 6 (high dose of PDDT): were administered orally PDDT 4.32 tablets/kg b.w/day (approximately 3240 mg/kg b.w./day).

After the last dose, the indices were evaluated as described in 2.3.3. section.

Research indices

- Intestinal motility measured by charcoal meal test following the method of Dobrescu[7]: after being given 0.2ml of 10% activated charcoal (10g of activated charcoal suspended in 100ml of 3% CMC), mice were dissected to retrieve the intestines. The intestinal motility was calculated as the percentage of the intestinal length with activated charcoal per the entire length of the intestine from pylorus to cecum.
- Colon macroscopic examination: The colon weight and length, the stool and the inflammation level were scored (as shown in Table 1).

Table 1. Colon macroscopic scores[1]

Parameter	Macroscopic scores				
	0	1	2	3	4
Colon weight (% increased vs. control)	< 5%	5 - 14%	15 - 24%	25 - 35%	> 35%
Colon length (% decreased vs. control)	< 5%	5 - 14%	15 - 24%	25 - 35%	> 35%
Stool	Normal	Loose/moist	Amorphous/sticky	Diarrhea	
Inflammation	Normal	Mild inflammation, localized erythema	Moderate or more widely distributed inflammation	Severe inflammation and/or extensively distributed	Penetrating ulcers and bloody lesions

- Colon microscopic examination: A section of colon (1 to 4cm from the anus) was examined histologically. The epithelial damage, cell infiltration, and muscle damage were scored (as shown in Table 2).

Table 2. Colon microscopic scores[1]

Parameter	Microscopic scores			
	0	1	2	3
Epithelial damage	Normal	< 33% of tissue length	33 - 66% of tissue length	> 66% of tissue length
Cellular infiltration	None	1 - 2 focal areas or extent of inflammatory infiltrate ≤ 33% of tissue length	> 2 focal areas or extent of inflammatory infiltrate >33% - 66% of tissue length	Infiltrate > 66% of tissue length
Muscle damage	Normal	≤ 33% of tissue length	>33 - 66% of tissue length	> 66% of tissue length

III. RESULTS

1. Protective effects of PDDT on MO-induced IBS-D model

Table 3. Protective effects of PDDT on intestinal motility based on charcoal transit and stool scores

Group	Percentage of the intestinal length with activated charcoal	Stool scores
Group 1 (control group)	68.30 ± 6.39	0.250 ± 0.463

Group	Percentage of the intestinal length with activated charcoal	Stool scores
Group 2 (ethanol control group)	70.66 ± 8.56	0.250 ± 0.463
Group 3 (model group)	83.73 ± 8.53**	1.200 ± 1.146*
Group 4 (Duspatalin treated group)	71.65 ± 15.74 #	0.545 ± 0.688
Group 5 (1080 mg/kg PDDT-treated group)	76.98 ± 11.19	0.800 ± 0.919
Group 6 (3240 mg/kg PDDT-treated group)	67.40 ± 13.20##	0.417 ± 0.515#

**p < 0.05: compared with the control group, **p < 0.01: compared with the control group, *** p < 0.001: compared with the control group*

#p < 0.05: compared with the model group, ##p < 0.01: compared with the model group, ### p < 0.001: compared with the model group

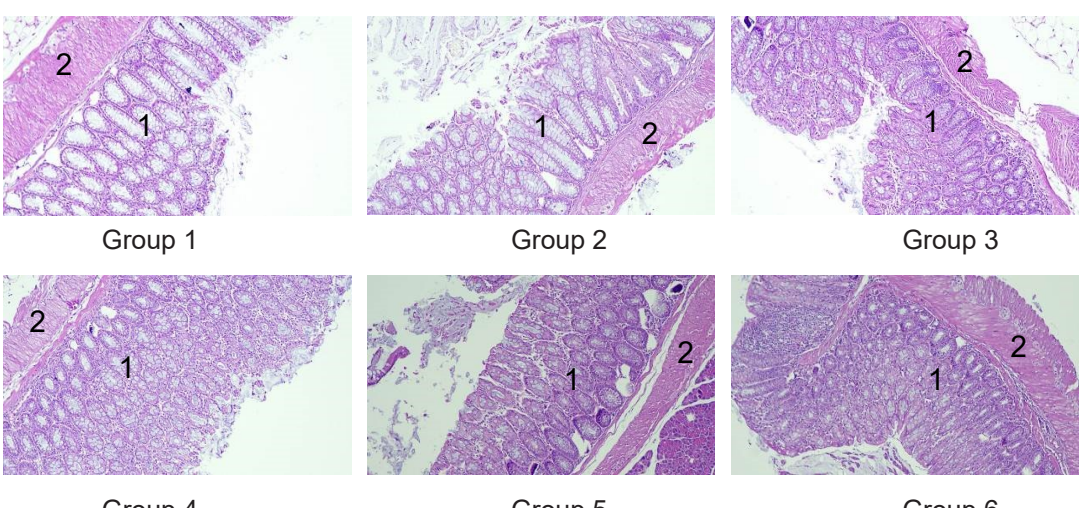
□p < 0.05: compared with Duspatalin group, ##p < 0.01: compared with Duspatalin group, ###p < 0.001: compared with Duspatalin group

As shown in Table 3:

The percentage of the intestinal length with activated charcoal and stool scores of model group increased significantly compared with the control group and ethanol group (p > 0.05 or p < 0.01).

Duspatalin and PDDT at 3240 mg/kg/day decreased significantly the percentage of the intestinal length with activated charcoal (p < 0.05 or p < 0.01). PDDT at 1080 mg/kg/day tended to decrease this index but the difference was not statically significant (p > 0.05).

Duspatalin and PDDT at 1080 mg/kg/day tended to decrease the stool score but the difference was not statically significant (p > 0.05). PDDT at 3240 mg/kg/day decreased significantly the score of stool type compared with the model group (p < 0.05).



Group 1 Group 2 Group 3

Group 4 Group 5 Group 6

Figure 1. Microscopic images of colon in protective model

1. Glandular epithelium, 2. Stroma

Our results showed that other indices including colon weight, colon length, colon microscopic scores were not statistically significant among all groups (p > 0.05). There was no difference in the microscopic images of mice in all groups. The colon samples of all groups had normal structure: the colon epithelium covered by mucus and ciliated cells, the glandular tubules contained secretions and secretory glandular epithelial cells, many round goblet cells filled with mucus, stroma scattered with slightly congested blood vessels, and muscle layers with clear structure.

2. Curative effects of PDDT on MO-induced IBS model

Table 4. Curative effects of PDDT on intestinal motility based on charcoal transit and stool scores

Group	*Percentage of the intestinal length with activated charcoal*	*Stool scores*
Group 1 (control group)	70.17 ± 9.95	0.250 ± 0.463
Group 2 (ethanol control group)	67.74 ± 5.34	0.250 ± 0.463
Group 3 (model group)	81.20 ± 4.23*	1.100 ± 0.876*
Group 4 (Duspatalin treated group)	65.83 ± 16.76 ##	0.400 ± 0.516#
Group 5 (1080 mg/kg PDDT-treated group)	74.14 ± 15.25	0.333 ± 0.492#
Group 6 (3240 mg/kg PDDT-treated group)	65.57 ± 14.75##	0.200 ± 0.422##

**p < 0.05: compared with the control group, **p < 0.01: compared with the control group, ***p < 0.001: compared with the control group*

#p < 0.05: compared with the model group, ##p < 0.01: compared with the model group, ###p < 0.001: compared with the model group

□p < 0.05: compared with Duspatalin group, ##p < 0.01: compared with Duspatalin group, ###p < 0.001: compared with Duspatalin group

As shown in Table 4:

The percentage of the intestinal length with activated charcoal and stool scores of model group increased significantly compared with the control group and ethanol group (p > 0.05).

Duspatalin and PDDT at 3240 mg/kg/day decreased significantly the percentage of the intestinal length with activated charcoal per the entire length of the intestine (p < 0.01). PDDT at 1080 mg/kg/day tended to decrease this index but the difference was not statically significant (p > 0.05).

Duspatalin and PDDT at both doses decreased significantly the stool score compared with the model group (p < 0.05)

Our results showed that other indices including colon weight, colon length, colon microscopic scores were not statically significant among all groups (p > 0.05). There was no difference in the microscopic images of mice in all groups. The colon samples of all groups had normal structure: the colon epithelium covered by mucus and ciliated cells, the glandular tubules contained secretions and secretory glandular epithelial cells, many round goblet cells filled with mucus, stroma scattered with slightly congested blood vessels, and muscle layers with clear structure. No inflammation was observed in all mice.

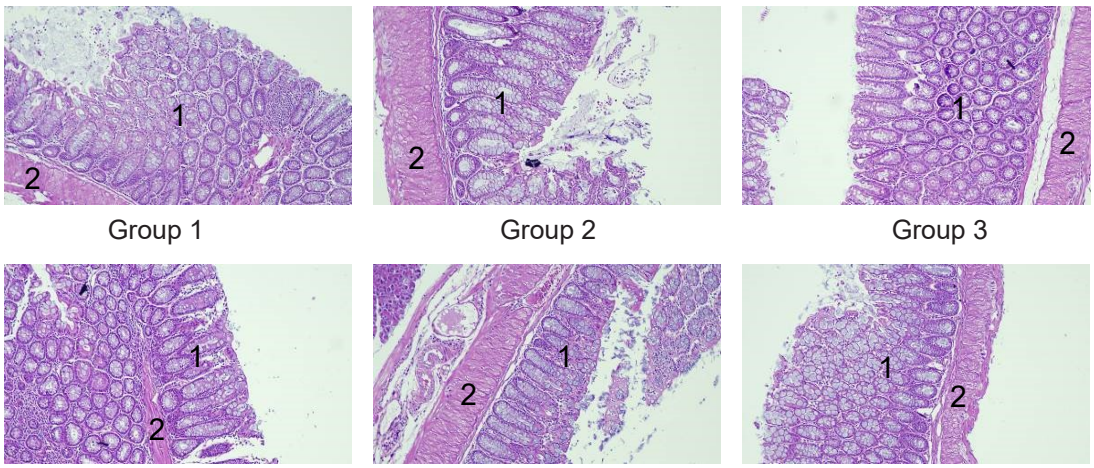


Figure 2. Microscopic images of colon in curative model

1. Glandular epithelium, 2. Stroma

IV. DISCUSSION

IBS is a disorder of the interaction between the enteric nervous system and the central nervous system with three main pathogenic mechanisms including the abnormal intestinal motility, visceral hypersensitivity, and psychosocial factors. IBS-D is the most common type of IBS that causes some symptoms such as abdominal pain, loose stool. Based on the pathogenesis of IBS-D, scientists have developed the experimental models to evaluate the efficacy of new treatment methods.[8]

There are various agents that have been used to induce IBS-D models including microorganisms, stress, and chemicals such as mustard oil (MO), trinitrobenzene. Kimball et al. investigated a model of MO-induced post-inflammatory IBS-D in mice. A single administration of MO induced a rapid, acute, and transient colitis and, in the longer term, functional changes in motility were observed when there was no gross inflammation. Therefore, it was a model of functional bowel disorders that mimic aspects of IBS-D in humans. The advantages of MO-induced IBS-D model are its ability to stimulate IBS-D, high stability, reasonable cost, and the availability of chemicals and experimental animals.[6]

In this study, MO was administered intracolonically to induce IBS-D in mice to investigate the curative and protective effects of PDDT. Our results showed that the intestinal motility and stool scores increased significantly in model group compared with the control group while no gross inflammation was observed and the colonic microscopic index was not different from the biological control group and the ethanol control group. A condition of increased intestinal motility and diarrhea appeared in mice without physical lesions in the gastrointestinal tract, similar to the manifestation of IBS-D.

Based on the induction of IBS-D model by MO in mice, we evaluated the protective and curative effects of the treatment agents. Mebeverine, an anticholinergic drug, appears to work directly on smooth muscle of the gastrointestinal tract by acting on calcium channels and muscarinic receptors, and is commonly used to treat the symptoms IBS-D.[9,10] Our results showed that mebeverine, used as a positive control drug, decreased the intestinal motility and improved

the stool score in IBS-D mice. PDDT at both doses improved the stool score. PDDT at both doses decreased the intestinal motility but the difference between the dose 1080 mg/kg/day and the model group was not significant.

The model used in this study was a post-inflammatory IBS-D model, in which the level of inflammation was closely related to post-inflammatory neurological disorders.[11] MO caused acute inflammation and increased visceral pain perception after colonic administration.[6] Several medicinal herbs in the ingredients of PDDT such as *Hedychium coronarium*, *Coix lacryma-jobi*, *Cynara Scolymus* L. have been proven to have anti-inflammatory effects. *Hedychium coronarium* and Coix extract could reduce the severity of colitis by various mechanisms. *Hedychium coronarium* reduce the expression of TLR4 and preventing the activation of the NF-κB and MAPK pathways while Coix extract inhibited the NF-κB pathway and enhancing the Nrf2 pathway.[12,13] *Cynara Scolymus* L. has been shown to contain two compounds taraxasterol and faradiol, which have the anti-inflammatory effect in experimental mice.[14] Due to this anti-inflammatory effect, PDDT could improve the MO-induced inflammatory damage of the colon, thus showed the protective effect in IBS-D mice.

Moreover, some medicinal herbs in PDDT such as *Hedychium coccineum*, *Dioscorea persimilis* have been proven to inhibit the intestinal motility and peristalsis in experimental animals.[15,16] In another study, we have demonstrated that PDDT reduced the intestinal motility in mice and reduced contractile frequency and amplitude of the rabbit's isolated intestinal segments. According to the pathogenesis of MO-induced IBS-D model, inflammatory recovery was achieved after seven days and was followed by the increased intestinal motility and diarrhea. Due to the antispasmodic effects on gastrointestinal tract, PDDT could reduce the intestinal motility and improve the stool score, thus showing both curative and protective effects in IBS-D mice.

It can be concluded that PDDT have both protective and curative effects in MO-induced IBS-D model in experimental animals. These effects might result from various mechanisms including anti-inflammatory and antispasmodic activities. In traditional medicine, herbal plants can be used alone or in combination with each other. The herbal products composed of multiple herbal ingredients might work on multiple pathophysiological targets, thus increasing the efficacy of the products. This might be another advantage of herbal medicine in the treatment of diseases. PDDT has the potential to be a herbal product to treat IBS-D in patients.

V. CONCLUSION

Our findings demonstrated that PDDT at 3240 mg/kg b.w./day decreased the intestinal motility and the stool score in IBS-D mice. PDDT at 1080 mg/kg b.w./day improved the stool score and tended to decrease the intestinal motility in IBS-D mice.

REFERENCES

1. Quigley EMM, Fried M, Gwee KA, et al. World Gastroenterology Organisation Global Guidelines Irritable Bowel Syndrome: A Global Perspective. *J Clin Gastroenterol*. 2016;50(9):704-713.

2. Oka P, Parr H, Barberio B, et al. Global prevalence of irritable bowel syndrome according to Rome III or IV criteria: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol*. 2020;5(10):908-917.

3. Quach DT, Vu KT, Vu KV. Prevalence, clinical characteristics, and management of irritable bowel syndrome in Vietnam: A scoping

review. *JGH Open*. 2021;5(11):1227-1235.

4. Qin D, Yue L, Xue B, et al. Pharmacological treatments for patients with irritable bowel syndrome: An umbrella review of systematic reviews and meta-analyses. *Medicine (Baltimore)*. 2019;98(32):e15920.

5. Bahrami HR, Hamedi S, Salari R, et al. Herbal Medicines for the Management of Irritable Bowel Syndrome: A Systematic Review. *Electron Physician*. 2016;8(8):2719-2725.

6. Edward S Kimball, Jeffrey M Palmer, Michael R D, et al. Acute colitis induction by oil of mustard results in later development of an IBS-like accelerated upper GI transit in mice. *Am J Physiol Gastrointest Liver Physiol*. 2005;288:G1266-G1273.

7. Hans Gerhard Vogel. Chapter J: Activity on the Gastrointestinal Tract. *Drug discovery and evaluation: Pharmacological Assays*. Springer Berlin, 3rd edition. 2008;1191-1322.

8. López-Gómez L, Bagüés A, Uranga JA, et al. Preclinical models of irritable bowel syndrome. *A Comprehensive Overview of Irritable Bowel Syndrome*. Academic Press. 2020:233-276.

9. Dao Van Phan. *Clinical Pharmacology*. Medical Publishing House, Hanoi. 2020.

10. Laurence L Brunton, Bruce A. Chabner. *Goodman & Gilman's The pharmacological basis of therapeutics.* McGraw-Hill, New York, 12th Edition. 2018;1323-1349.

11. Barbara G, De Giorgio R, Stanghellini V. A role for inflammation in irritable bowel syndrome. *Gut*. 2002,51:41-44.

12. Jingjing Zhang, Wei Dou, Eryun Zhang, et al. *Paeoniflorin abrogates* DSS-induced colitis via a TLR4-dependent pathway. *Am J Physiol Gastrointest Liver Physiol*. 2014;306(1):27-36.

13. Kim Min Ju, Shin Mi-Rae, Lee Jin A, et al. Improvement Effect of Sprout of *Coix lacryma-jobi* var. mayuen Stapf Water Extract on DSS-Induced Ulcerative Colitis in Mice. *The Korea Journal of Herbology*. 2020;35(6):21-28.

14. Maryem Ben Salem, Hanen Affes, Khaled Athmouni, et al. Chemicals Compositions, Antioxidant and Anti-Inflammatory Activity of *Cynara scolymus* Leaves Extracts, and Analysis of Major Bioactive Polyphenols by HPLC. *Evid Based Complement Alternat Med*. 2017;2017:4951937.

15. Shifah F, Tareq AM, Sayeed MAS, et al. Antidiarrheal, cytotoxic and thrombolytic activities of methanolic extract of *Hedychium coccineum* leaves. *J Adv Biotechnol Exp Ther*. 2020;3(1):77-83.

16. Do Huy Bich. Vietnamese medicinal plants and animals. Scientific and Technical Publishing House, Hanoi. 2016.