EFFICACY AND SAFETY OF TRANG PHUC LINH PLUS TABLETS IN PATIENTS WITH IRRITABLE BOWEL SYNDROME-DIARRHEA

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A double-blind, randomized, phase II clinical trial was conducted within a 14-week follow-up including 2 weeks of the non-drug run-in period, 8 weeks of medication, and 4 weeks of follow-up after discontinuation. The objective of the study was to evaluate the efficacy and safety of Trang Phuc Linh Plus in irritable diarrhea syndrome patients. Group I took Trang Phuc Linh Plus 3 tablets/time x 2 times/day for 8 weeks; Group II took placebo 3 tablets/ time x 2 times/day for 8 weeks. Patients will be re-examined, tested, and evaluated over the phone for symptom recurrence and adverse events (AEs). This study showed that the Trang Phuc Linh Plus tablets tended to improve symptoms in patients suffering from irritable bowel syndrome with diarrhea, suggesting its safety and tolerability.

Keyword: IBSS01, Trang Phuc Linh Plus, irritable bowel syndrome.

I. INTRODUCTION

Irritable bowel syndrome (IBS) is a type of gastrointestinal dysfunction that occurs on a regular basis without finding any structural or biochemical abnormalities.[1,2] Symptoms of IBS included abdominal pain and a prolonged change in bowel habits. IBS is a chronic disease that, while not life-threatening, has a significant impact on a patient's quality of life and requires costly treatment. It accounted for 10% to 15% of initial health examinations and 25% to 50% of gastrointestinal examinations. In a survey of 6616 individuals with colorectal and anal disorders undertaken at Bach Mai Hospital (2004), IBS was shown to be the most common, accounting for up to 83.18%.[3] IBS is classified into four types: IBS-D (diarrhea) has the greatest rate, followed by IBS-C (constipation), IBS-M (mixed), and IBS-U (unclassified).[4] The current treatment for IBS is primarily symptomatic, and no specific treatment is effective for all patients. Current antispasmodic drugs that reduce intestinal motility are strong and quick, but they also have numerous side effects and contraindications.[5] As a result, doctors are now becoming increasingly concerned about the trend of searching for and developing drugs of natural origin in order to both provide effective treatments and reduce adverse side effects for patients. Trang Phuc Linh Plus is a product that combines *Immune Gamma*, 5-*HTP*, and popular medicinal herbs such as *Atractylodes macrocephala*, *Poria cocos*, *Paeonia lactiflora*, *Phellodendron amurense* that have been used on patients as functional foods with the purpose of minimizing the stimulation that causes colon spasms, reducing symptoms such as abdominal pain, changes in bowel movements, loose stools, in addition to helping

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to balance the intestinal flora, boost resistance, regenerate the colon lining, and promote a healthy digestive system. Therefore, this study was conducted with the aim of evaluating the efficacy and safety of Trang Phuc Linh Plus tablets in patients suffering from irritable bowel syndrome with diarrhea (IBS-D).

II. METHODS

1. Study product

Trang Phuc Linh Plus (IBSS01) is distributed by Thai Minh Pharmaceutical Joint Stock Company, meeting basic standards. Each tablet contains 100 mg of Immune Gamma®, 3 mg of 5-HTP (5-hydroxytryptophan), 200 mg of *Atractylodes macrocephala*, 50 mg of *Poria cocos*, 50 mg of *Paeonia lactiflora*, and 50 mg of *Phellodendron amurense*. In humans, the recommended dose is 4-6 tablets per day divided into two doses, 30 minutes before meals and 1 hour after meals.

2. Study subjects

Inclusion criteria

- Patients 18 years of age or older who have been diagnosed with symptomatic IBS-D using Rome IV criteria based on medical histories.
- Patients over the age of 50 or those with a family history of colorectal cancer.
- Patients over the age of 65: no ischemic colitis, microscopic colitis, or other gastrointestinal disease.
- Female patients committed to using contraceptive methods during the study period.
- Patients was able to write voluntarily registrations to participate in the study, comply with the study protocol and willfully participate in the study, know how to use cell phones, and consent to stop taking loperamide 3 days prior to entering the run-in period as well as during the whole run-in period.

Non - inclusion criteria

- Patients had medical histories of diverticulitis, ischemic colitis, microscopic colitis; lactose or gluten intolerance, thyroid dysfunction, uncontrolled hypertension, insulin-dependent diabetes mellitus, hypersensitivity to any component of the study's drug or placebo.
- Patients with liver disease, kidney failure, anemia, HIV.
- Pregnant and lactating women.
- Patients had medical histories of pancreatitis, bile duct disease, cholecystitis, symptomatic gallstones, or major cardiovascular event in the last 6 months.
- Patients with clinically significant ECG abnormalities.
- Patients have entered or participated in another clinical trial within the last 4 weeks or during the study period.
- Patients with any other condition deemed unsuitable for participation in the study by the investigator.

3. Study design

Study design: A randomized, double-blind, placebo-controlled phase II study:

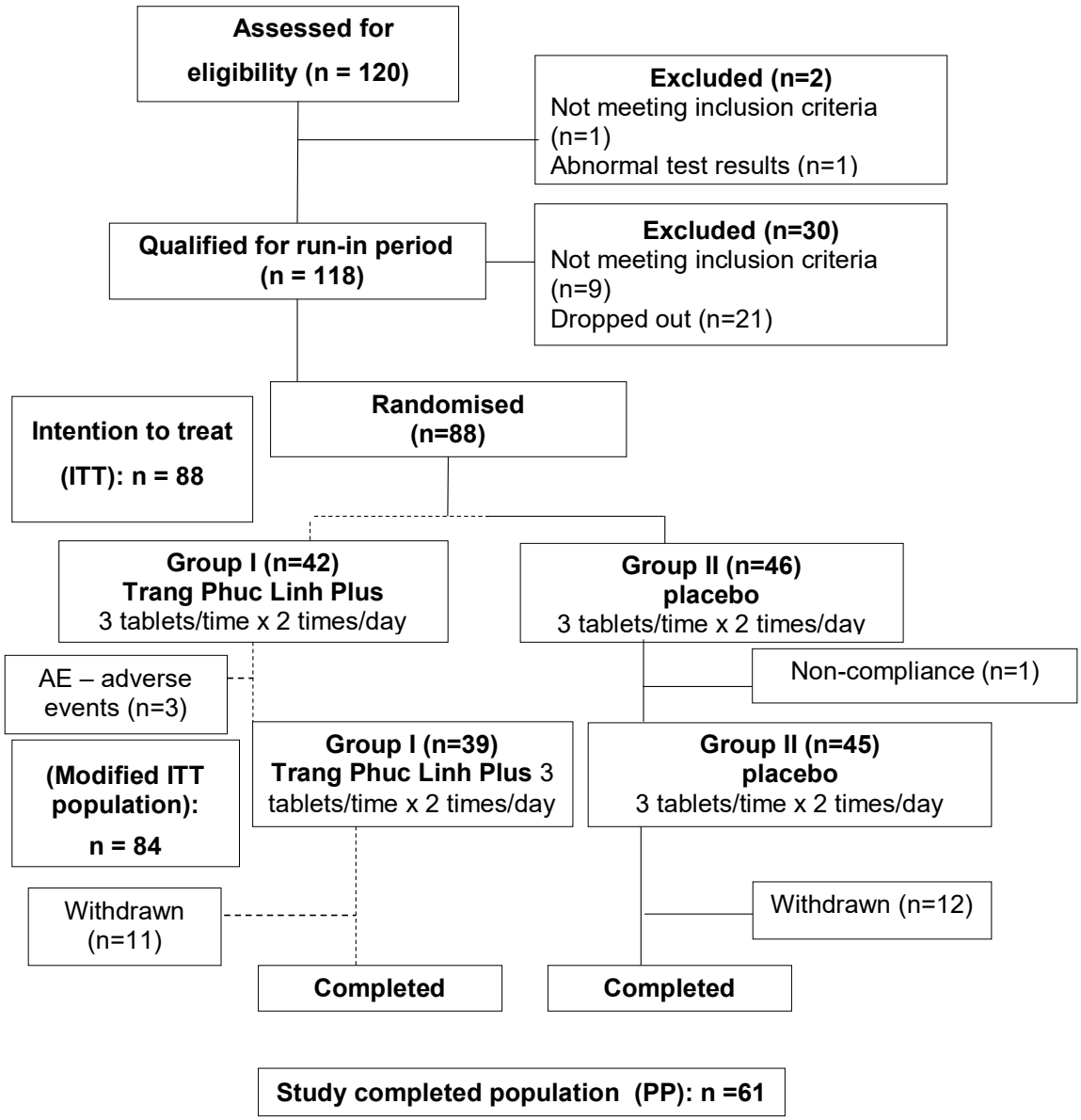
Group I: taking Trang Phuc Linh Plus 3 tablets/time x 2 times/day for 8 weeks.

Group II: taking placebo 3 tablets/time x 2 times/day for 8 weeks.

Sample size: The sample size for phase II research for drugs derived from medicinal herbs must be at least 30 people, according to Ministry of Health Circular 29/2018/TT-BYT6. The study's results were evaluated on 88 patients who were eligible for randomization after the run-in period, with 61 patients completing the study.

Time and location: Our study was conducted from November 12, 2018 to January 31, 2020 at Hanoi Medical University Hospital.

Study process:



Study indicators

- Evaluate the efficacy: based on the guidance of the Food and Drug Administration (FDA).[7]

+ Main criteria: The percentage of weekly responder for at least 50% of the treatment weeks.

Weekly responder defined as: decrease in weekly average of worst abdominal pain in past 24 hours score of at least 30% compared with baseline and decrease at least 50% in the number of days per week with at least one stool that has a consistency of Type 6 or 7 compared with baseline.

+ Sub-criteria:

The percentage of patients responding to weekly abdominal pain relief: decrease in weekly average of worst abdominal pain in past 24 hours score of at least 30% compared with

baseline.

The percentage of patients responding to improved weekly stool status: decrease at least 50% in the number of days per week with at least one stool that has a consistency of Type 6 or 7 compared with baseline.

- Evaluate the safety: the number of patients in each group who experienced at least one adverse event, the number of adverse events in each group, the rate of adverse events per patient in each group, the number of serious adverse events in each group, and clinical and laboratory parameters.

3. Statistical Analysis

Sample size (n), mean, standard deviation (SD), lowest value (min), median (median), maximum value (max) for quantitative variables, and frequency for quantitative variables were all descriptive statistics. SPSS 20.0 software was used for statistical analysis.

4. Ethical Approval

Ethical approval has been provided by the Institutional Ethics Board (IRBs) of Hanoi Medical University prior to start of the study dated 2020. The study was carried out in accordance with the protocol, current regulations, International Conference on Harmonization (ICH) of good clinical practice, the Drug Administration's guidelines, and the Helsinki Declaration.

III. RESULTS

1. Characteristics of subjects

Table 1. Characteristics of the subjects

	ITT population (n = 88)		PP population (n = 61)	
	Group I (n = 42)	**Group II (n = 46)**	**Group I (n = 28)**	**Group II (n = 33)**
Age: [year]				
Average (SD)	45.93 (11.69)	47.78 (14.01)	46.79 (10.60)	47.45 (15.37)
p_{1-2}	0.5		0.85	
18-40	16	15	11	12
41-65	25	25	16	15
≥ 66	1	6	1	6
Gender: [n(%)]				
South	26 (61.9%)	28 (60.9%)	18 (64.3%)	20 (60.6%)
Female	16 (38.1%)	18 (39.1%)	10 (35.7%)	13 (39.4%)
p_{1-2}	1.0		0.976	
Duration of the disease [month]				
Average	57.14	53.22	63.86	54.55
SD	40.70	24.87	45.73	25.12
p_{1-2}	0.58		0.32	
Pain level (VAS scale)				
< 5	9	11	6	9
≥ 5, < 8	33	35	22	24
≥ 8	0	0	0	0

The average age of the two groups of patients made no difference in the ITT (p = 0.5) or PP (p = 0.85) populations. The male/female ratio in the ITT population was 1.63 in Group I and 1.56 in Group II; in PP populations, the male/female ratio was 1.8 in Group I and 1.54 in Group II. There was no difference in the duration of the disease between the two groups. The disease primarily affects people between the ages of 41 and 65, with an average abdominal pain score of 5-8 points; no patient has pain greater than 8 points on the VAS scale. Overall, there was no significant difference between the two groups' baseline indicators.

2. Evaluation of the efficacy

Table 2. The percentage of the weekly responder[a]

	Modified ITT population (n = 84)		PP population (n = 61)	
	Group I (n = 39)	**Group II (n = 45)**	**Group I (n = 28)**	**Group II (n = 33)**
Responsive (n, %)	4 (10.3%)	4 (8.9%)	4 (14.3%)	2 (6.1%)
Non-responsive (n, %)	35 (89.7%)	41 (91.1%)	24 (85.7%)	31 (93.9%)
p_{1-2}	1		0.4	

Fisher's exact

a: the average weekly bleak of the most abdominal pain point in the last 24 hours is at least 30% compared to the rash; at least 50% reduction in the number of days of the week with at least one stool of category 6 or 7 on the Bristol Stool scale compared to the original.

The percentage of the weekly responders in the group taking the Trang Phuc Linh Plus (Group I) was higher, but there was no statistically significant difference between the two groups in the weekly responder rates (p > 0.05).

Table 3. The percentage of patients responding to weekly abdominal pain relief[b]

		Modified ITT population (n = 84)		PP population (n = 61)	
		Group I (n = 39)	**Group II (n = 45)**	**Group I (n = 28)**	**Group II (n = 33)**
Week 1	Responsive (n, %)	1 (2.56%)	6 (13.3%)	1 (3.6%)	6 (18.2%)
	Non-responsive (n, %)	38 (97.44%)	39 (86.7%)	27 (96.4%)	27 (81.8%)
	p_{1-2}	0.12**		0.11**	
Week 2	Responsive (n, %)	7 (17.9%)	9 (20.0%)	5 (17.9%)	7 (21.2%)
	Non-responsive (n, %)	32 (82.1%)	36 (80.0%)	23 (82.1%)	26 (78.8%)
	p_{1-2}	1*		0.99*	
Week 3	Responsive (n, %)	10 (25.6%)	15 (33.3%)	7 (25.0%)	11 (33.3%)
	Non-responsive (n, %)	29 (74.4%)	30 (66.67%)	21 (75.0%)	22 (66.7%)
	p_{1-2}	0.596*		0.67*	

		Modified ITT population (n = 84)		PP population (n = 61)	
		Group I (n = 39)	Group II (n = 45)	Group I (n = 28)	Group II (n = 33)
Week 4	Responsive (n, %)	17 (43.6%)	14 (31.1%)	13 (46.4%)	9 (27.3%)
	Non-responsive (n, %)	33 (56.4%)	31 (68.9%)	15 (53.6%)	24 (72.7%)
	p_{1-2}	0.339*		0.2*	
Week 5	Responsive (n, %)	19 (48.7%)	19 (42.2%)	16 (57.1%)	13 (39.4%)
	Non-responsive (n, %)	20 (51.3%)	26 (57.8%)	12 (42.9%)	20 (60.6%)
	p_{1-2}	0.71*		0.26*	
Week 6	Responsive (n, %)	18 (46.2%)	15 (33.3%)	14 (50.0%)	10 (30.3%)
	Non-responsive (n, %)	21 (53.8%)	30 (66.7%)	14 (50.0%)	23 (69.7%)
	p_{1-2}	0.33*		0.19*	
Week 7	Responsive (n, %)	21 (53.8%)	22 (48.9%)	15 (53.6%)	16 (48.5%)
	Non-responsive (n, %)	18 (46.2%)	23 (51.1%)	13 (46.4%)	17 (51.5%)
	p_{1-2}	0.82*		0.89*	
Week 8	Responsive (n, %)	21 (53.8%)	25 (55.6%)	17 (60.7%)	19 (57.6%)
	Non-responsive (n, %)	18 (46.2%)	20 (44.4%)	11 (39.3%)	14 (42.4%)
	p_{1-2}	1*		1*	

**: X^2 test, **: Fisher's exact test.*

b decreased the average weekly of the most abdominal pain point in the last 24 hours by at least 30% compared to the original.

Trang Phuc Linh Plus improved symptoms of abdominal pain at all times of the study, especially after 5 weeks and 6 weeks of treatment. After 5 weeks of treatment, 46.4% of those who used Trang Phuc Linh Plus reported an improvement in abdominal pain symptoms, compared to only 27.3% in the placebo group. After 6 weeks of treatment, the percentage of patients who respond to weekly abdominal pain relief was 53.0% while the placebo group only reached 30.3%.

Table 4. The percentage of patients responding to improved weekly stool status[c]

		Modified ITT population (n = 84)		PP population (n = 61)	
		Group I (n = 39)	Group II (n = 45)	Group I (n = 28)	Group II (n = 33)
Week 1	Responsive (n, %)	1 (2.6%)	1 (2.2%)	1 (3.6%)	1 (3.0%)
	Non-responsive (n, %)	38 (97.4%)	44 (97.8%)	27 (96.4%)	32 (97.0%)
	p_{1-2}	1**		1**	

		Modified ITT population (n = 84)		PP population (n = 61)	
		Group I (n = 39)	Group II (n = 45)	Group I (n = 28)	Group II (n = 33)
Week 2	Responsive (n, %)	1 (2.6%)	4 (8.9%)	1 (3.6%)	4 (12.1%)
	Non-responsive (n, %)	38 (97.4%)	41 (91.1%)	27 (96.4%)	29 (87.9%)
	p_{1-2}	0.34**		0.36**	
Week 3	Responsive (n, %)	3 (7.7%)	4 (8.9%)	3 (10.7%)	3 (9.1%)
	Non-responsive (n, %)	36 (92.3%)	41 (91.1%)	25 (89.3%)	30 (90.9%)
	p_{1-2}	1**		1**	
Week 4	Responsive (n, %)	4 (10.3%)	4 (8.9%)	4 (14.3%)	2 (6.1%)
	Non-responsive (n, %)	35 (89.7%)	41 (91.1%)	24 (85.7%)	31 (93.9%)
	p_{1-2}	1**		0.4**	
Week 5	Responsive (n, %)	7 (17.9%)	7 (15.6%)	7 (25.0%)	6 (18.2%)
	Non-responsive (n, %)	32 (82.1%)	38 (84.4%)	21 (75.0%)	27 (81.8%)
	p_{1-2}	1*		0.74*	
Week 6	Responsive (n, %)	9 (23.1%)	9 (20.0%)	8 (28.6%)	6 (18.2%)
	Non-responsive (n, %)	30 (76.9%)	36 (80.0%)	20 (71.4%)	27 (81.8%)
	p_{1-2}	0.94*		0.51*	
Week 7	Responsive (n, %)	10 (25.6%)	10 (22.2%)	9 (32.1%)	4 (12.1%)
	Non-responsive (n, %)	29 (74.4%)	35 (77.8%)	19 (67.9%)	29 (87.9%)
	p_{1-2}	0.91*		0.07**	
Week 8	Responsive (n, %)	9 (23.1%)	13 (28.9%)	9 (32.1%)	8 (24.2%)
	Non-responsive (n, %)	30 (76.9%)	32 (71.1%)	19 (67.9%)	25 (75.8%)
	p_{1-2}	0.72*		0.69*	

**: X^2 test , **: Fisher's exact test.*

c reduced at least 50% of the number of days of the week with at least one stool in category 6 or 7 on the Bristol Stool scale compared to the original.

The results showed that in both the ITT and PP populations, the proportion of patients responding to improved weekly stool status was higher in the Trang Phuc Linh Plus group from week 4 onwards than in the remaining weeks. At weeks 7 and 8, the Trang Phuc Linh Plus group had the highest response rate, which was significantly higher than the placebo group. At some point in time, the percentage of patients responding to improved weekly stool status was significantly higher in the Trang Phuc Linh Plus group than in the placebo group (weeks 4, 6, and 7), but the difference was not statistically significant.

3. Safety Evaluation

Table 5. Number of study subjects who record adverse events

	ITT population (n = 88)	
	Group I (n = 42)	**Group II (n = 46)**
The patient experiences at least one adverse event (n,%)	4 (9.5%)	2 (4.4%)
Patients with serious adverse events (n,%)	0 (0%)	0 (0%)
Number of adverse events (n)	4	6
The incidence of adverse events per patient (%)	0.09	0.13

Group I took the Trang Phuc Linh Plus (IBSS01) and had 4 patients that experienced adverse events, while group II took placebo and had 2 patients experienced adverse events. There were no patients who had serious adverse events in both groups.

IV. DISCUSSION

This randomized, double-blind, placebo-controlled clinical trial aims to evaluate the efficacy and safety of Trang Phuc Linh Plus which is a functional food consisting of the herbs: *atractylodes macrocephala, poria cocos, paeonia lactiflora, phellodendron amurense,* and fortified *with lactobacillus fermentum lysate (ImmuneGamma ®)* and *5-hydroxytryptophan (5-HT)*. *Immune Gamma* is extracted from the cell wall of *Lactobacillus fermentum,* composed of muramyl peptides with the main effect of enhancing immunity.[8] In 2011, a study by Nicholas and colleagues conducted in 99 athletes found that L. fermentum significantly reduced symptoms of gastrointestinal infections compared to the placebo group.[9] *5-HT* is an intermediate in the conversion of tryptophan to serotonin - a neurotransmitter secreted under the action of excitatory impulses in the intestinal lumen, helping to regulate intestinal motility and secretion.[10,11] The mechanism of action of IBSS01 on IBS-D has been considered in several studies, including a percentage reduction in the length of the gut with activated charcoal compared with the length of the intestine from the pylorus to the cecum in rats, which is similar to *Mebeverin* at a dose of 80 mg/kg; frequency and amplitude reduction of intestinal peristalsis in rabbits isolated in Tyrod environment; fluid volume and ions reduction in frog intestinal fluid according to the method of Norio Ogata and Tatsuya Baba. A study by Nguyen Trong Thong (2017) determining the pharmacological activities and mode of action of this product in animal models of IBS showed that at a dose of 654 mg/kg/day, Trang Phuc Linh Plus reduces significantly intestinal motility compared with control animals, even when the dose was increased to 1962 mg/kg/day, IBSS01 reduced intestinal motility more strongly than *Duspatalin* 80 mg/kg/day (*Mebeverine Hydrochloride*, an antispasmodic drug relieving pain and discomfort associated with gastrointestinal spasms).[12] Our study showed that, in terms of effectiveness, IBSS01 tended to increase response rates at the main outcome (proportion of patients achieving weekly response in at least 50% of treatment weeks)

and some secondary outcome (proportion of patients achieving weekly abdominal pain relief response; percentage of patients achieving weekly stool improvement response) compared with controls, similar to those used drugs of potential benefit in the treatment of IBS-D such as *alosetron, eluxadoline, and rifaximin.*[13] In a study published in 2021 by Zheng et al., herbal medicines (atractylodes macrocephala, poria cocos, paeonia lactiflora, and phellodendron amurense) were associated to remission rates of these symptoms in patients with IBS-D, especially abdominal pain ($p < 0.0001$).[14] Regarding safety, our study recorded adverse events in both Trang Phuc Linh Plus (IBSS01) and placebo groups, with no statistically significant difference in the number of adverse events ($p > 0.05$). The group using Trang Phuc Linh Plus (IBSS01) reported a low rate of adverse events per patient (0.09%) including epigastric pain and increased diarrhea. These above events were assessed as mild, moderate and did not require treatment, the patients recovered on their own without any sequelae. The placebo group reported 6 adverse events occurred in 2 patients including epigastric pain and increased diarrhea (in patient IBS012); 5 events occurred in patient IBS013 including 1 time of bowel movement with little bright red blood (patient had internal hemorrhoids), 1 time of epigastric pain and 3 times of increased diarrhea (watery stools) (however, diarrhea did not increase more than at the start of the study). All events were assessed as unrelated to the study product. In addition, the study also recorded some changes in the results of biochemical and hematological tests outside the normal range and some changes related to urinalysis, electrocardiogram, and electrolytes. However, these changes were assessed as having no clinical significance and no indications for medical intervention.

V. CONCLUSION

In patients with irritable bowel syndrome with diarrhea (IBS-D), Trang Phuc Linh Plus (IBSS01) tended to improve symptoms. The drug was well tolerated and shown a high level of safety comparable to placebo.

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