International Journal of Pharmaceutics and Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

ANTI-DIARRHEAL ACTIVITY OF POLYHERBAL FORMULATION OF *TAMARINDUS INDICA* AND *WOODFORDIA FRUTICOSA* LEAVES EXTRACT

Reetu Sahu*, Sandra Goutam, B.K. Dubey, Deepak Kumar Basedia, Mukesh Kumar Patel
Technocrats Institute of Technology-Pharmacy, Bhopal (M.P.)

***Correspondence Info:**
Reetu Sahu
Technocrats Institute of Technology-Pharmacy, Bhopal (M.P.)
Email: ritusahu201298@gmail.com

***Article History:**

Received:05/04/2024
Revised: 22/04/2024
Accepted: 17/05/2024

ABSTRACT

The present study investigated the anti-diarrheal activity of a polyherbal formulation (PHF) consisting of ethanolic extracts of *Tamarindus indica* and *Woodfordia fruticosa* leaves. The extracts were evaluated for their consistency, color, and phytochemical composition, revealing the presence of carbohydrates, tannins, alkaloids, glycosides, flavonoids, and proteins. The anti-diarrheal efficacy of the PHF was assessed using an animal model, where it significantly delayed the onset of diarrhea and reduced the total number of faecal drops and frequency of diarrhea. The PHF at doses of 100 mg/kg and 200 mg/kg exhibited dose-dependent protection against diarrhea, with the higher dose showing greater efficacy. The results suggest that the PHF has potential therapeutic benefits for treating diarrhea, likely due to the synergistic effects of its bioactive compounds. Further studies are warranted to elucidate the mechanisms of action, optimal dosage, and clinical relevance of the PHF.

Keywords: Polyherbal formulation, *Tamarindus indica*, *Woodfordia fruticosa*, Anti-diarrheal activity, Phytochemical screening, Diarrhea treatment.

INTRODUCTION

Diarrhea is a common gastrointestinal disorder characterized by frequent and loose bowel movements, leading to dehydration and electrolyte imbalance. It is a significant health problem, particularly in developing countries, contributing to high morbidity and mortality rates, especially among children (Kosek *et al.,* 2003). Conventional anti-diarrheal medications, while effective, often come with side effects and limitations (Thapar & Sanderson, 2004). This has driven the search for alternative treatments, particularly those derived from natural sources, due to their perceived safety and efficacy (Williamson, 2001). Polyherbal formulations, which combine multiple plant extracts, have been traditionally used in various cultures to treat a wide range of ailments, including diarrhea. Such formulations are believed to offer synergistic effects, enhancing the therapeutic efficacy of individual components while minimizing adverse effects (Williamson, 2001). *Tamarindus indica* (Tamarind) and *Woodfordia fruticosa* (Fire Flame Bush) are two medicinal plants with a long history of use in traditional medicine systems for their diverse therapeutic properties, including anti-diarrheal effects (Mukherjee *et al.,* 1998; Doughari, 2006).

Tamarindus indica, commonly known as tamarind, is a tropical fruit-bearing tree native to Africa and widely cultivated in tropical and subtropical regions. The leaves, bark, and fruit of tamarind have been used in traditional medicine to treat various ailments. Studies have shown that tamarind possesses anti-inflammatory, antimicrobial, and antioxidant

properties, which contribute to its therapeutic potential (De Caluwe *et al.,* 2010). Tamarind leaves, in particular, have been reported to exhibit significant anti-diarrheal activity, attributed to the presence of bioactive compounds such as tannins, flavonoids, and saponins (Saeed & Sabir, 2001; Shailajan *et al.,* 2010). It has been used in traditional Ayurvedic medicine for its anti-inflammatory, antimicrobial, and astringent properties. The leaves and flowers of *Woodfordia fruticosa* are rich in phytochemicals like tannins, flavonoids, and glycosides, which are believed to contribute to its medicinal effects (Mukherjee *et al.,* 2011). Previous studies have demonstrated the anti-diarrheal efficacy of *Woodfordia fruticosa* extracts, supporting its use in traditional medicine (Bhuwaneshwar *et al.,* 2012; Gupta *et al.,* 2004).

Combining the extracts of *Tamarindus indica* and *Woodfordia fruticosa* in a polyherbal formulation may enhance the anti-diarrheal efficacy through synergistic actions of their bioactive compounds. This study aims to investigate the anti-diarrheal activity of a polyherbal formulation containing extracts from the leaves of *Tamarindus indica* and *Woodfordia fruticosa*. The formulation will be evaluated using in-vivo models to determine its effectiveness and potential mechanisms of action. By exploring this polyherbal approach, we aim to provide a natural, safe, and effective alternative for the management of diarrhea.

MATERIALS AND METHODS

Collection of Plant material

The leaves of *Tamarindus indica and Woodfordia fruticosa* was collected locally in the month of June locally from Bhopal, Madhya Pradesh, India.

Drying and Size Reduction of Plant Material

The leaves of *Tamarindus indica and Woodfordia fruticosa* was dried under shade in laboratory. They were pulverized to make coarse powder. The coarse powder of leaves was passed through sieve No. 18 to maintain uniformity and stored in cool and dry place for further study

Preparation of *Tamarindus indica and Woodfordia fruticosa* leaves extract

Extraction of leaves of both plant *Tamarindus indica and Woodfordia fruticosa* was done by Soxhlet extraction method.

Soxhlet Extraction

Soxhlet apparatus was used for the solvent extraction and ethanol was selected as a solvent for extraction while petroleum ether was used for defatting of the waxy materials.

Phytochemical analysis of crude extracts

(Fokam *et al.,* 2021)

The crude extract obtained by solvent extraction was subjected to various qualitative tests with standard reported methods to detect the presence of common phytochemical constituents. All the chemicals and reagent used in phytochemical testing was of analytical grade.

In-vivo anti-diarrheal activity

The animal experimental protocol was approved by the Institutional Animals Ethical Committee (IAEC). Male & female Wistar albino rats (140-200g). The animals were housed in standard conditions of temperature (25 ± 2^0C) and 12:12 h light-dark cycle. The rats were fed with commercial diet and water *ad Libitum*.

Acute oral toxicity study

Acute oral toxicity study was evaluated as per OECD guidelines (425) on Wistar albino rats. Before experimentation rats were fasted overnight with water *ad libitum*. Three animals were selected which receives dose of 2000mg/kg. All three animals were received dose of 2000 mg/kg body weight of poly herbal formulation by gavage using oral canula (limit test). Animals were observed individually for any toxicity sign of gross changes like convulsion, tremor, circling, depression, and mortality after dosing for 24 hours, with special attention given during the first 4 hours, and thereafter, 24 hours, Administered dose was found tolerable (as no death found). Therefore, two dose levels 100 mg/kg & 200 mg/kg was selected for anti-ulcer activity.

Observations

Animals are observed individually for any toxicity sign of gross changes like convulsion, tremor, circling, depression, and mortality after dosing for 24 hours, with special attention given during the first 4 hours, and thereafter, 24 hours, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. No significant signs were noticed in animals. Hence administered dose was found tolerable as no death was found. Therefore, 2000mg/kg dose of poly herbal formulation was considered maximum safe dose.

***In-vivo* screening of polyherbal formulation for anti-diarrheal activity**

Castor oil-induced diarrhea in rats

Randomization, grouping and treatment of animals

Healthy Wistar albino rats were used for the proposed study and were divided into 5 groups, each group consisting of 6 animals, which received the treatments in following manner:

Group I: Normal Control Group (0.5% CMC Solution)

Group II: Negative control group (2 ml/rat of castor oil, before 1hr of observation)

Group III: Standard group (Loperamide 3 mg/kg body weight+ 2 ml/rat of castor oil after 1hr of drug administration)

Group IV: Test 1 group (Polyherbal formulation 100 mg/kg, b.w., p.o. for 7 days + 2 ml/rat of castor oil after 1hr of last dose)

Group V: Test 2 group (Polyherbal formulation 200 mg/kg, b.w., p.o. for 7 days + 2 ml/rat of castor oil after 1hr of last dose)

Evaluation of anti-diarrheal activity

The animals (three rats per group) kept in separate metabolic cages with a wire container beneath the cage to collect faeces. The severity of diarrhea was asses for a period of 6 h. The total number of faeces drops & onset of time (min) were evaluated. The Frequency of diarrhea (number) for the negative control group was considering as 100%. The results were expressed as a percentage of protection of diarrhea (Shin *et al.,* 2014).

RESULTS AND DISCUSSION

The present study evaluated the anti-diarrheal activity of a polyherbal formulation (PHF) containing ethanolic extracts of *Tamarindus indica* and *Woodfordia fruticosa* leaves. The extracts were characterized for their color and consistency (Table 1) and subjected to phytochemical screening to identify the presence of various bioactive compounds (Table 2). Additionally, the anti-diarrheal efficacy of the PHF was assessed using an animal model (Table 3).

The phytochemical analysis revealed the presence of carbohydrates, tannins, alkaloids, glycosides, flavonoids, and proteins in both extracts, with some variations in the presence of steroids and sterols (Table 2). These bioactive compounds are known for their therapeutic properties and could contribute to the observed anti-diarrheal effects. Tannins and flavonoids, in particular, have been reported to have anti-diarrheal properties due to their ability to inhibit intestinal motility, reduce intestinal secretions, and protect the intestinal mucosa (Brijesh *et al.,* 2009; Palombo, 2006).

In the anti-diarrheal activity study (Table 3), the normal control group showed a longer onset of diarrhea and fewer total faecal drops and frequency of diarrhea compared to the negative control group, indicating the induction of diarrhea in the latter. Loperamide, a standard anti-diarrheal drug, significantly delayed the onset of diarrhea, reduced the total number of faecal drops, and decreased the frequency of diarrhea, demonstrating its effectiveness.

The PHF at doses of 100 mg/kg and 200 mg/kg showed significant anti-diarrheal activity, as evidenced by the delayed onset of diarrhea and reduced total number of faecal drops and frequency of diarrhea compared to the negative control group. The higher dose of PHF (200 mg/kg) exhibited a greater protective effect (32.63% protection) compared to the lower dose (100 mg/kg) (30.23% protection), although both were less effective than loperamide (50.0% protection). The dose-dependent response observed in the study suggests that higher doses of PHF may offer better anti-diarrheal protection.

The results indicate that the PHF can significantly reduce diarrhea symptoms, which may be attributed to the synergistic effects of the bioactive compounds present in *Tamarindus indica* and *Woodfordia fruticosa* extracts. The presence of tannins and flavonoids, known for their astringent and anti-inflammatory properties, likely plays a crucial role in reducing intestinal motility and secretion, thereby alleviating diarrhea (Brijesh et al., 2009; Palombo, 2006).

The study demonstrated that the PHF has a potential therapeutic benefit for the treatment of diarrhea. However, further studies are warranted to understand the exact mechanisms of action, optimal dosage, and long-term safety of the PHF. Additionally, clinical trials are necessary to confirm the efficacy and safety of this polyherbal formulation in humans.

Table 1: Consistency and color of *Tamarindus indica* and *Woodfordia fruticosa* leaves extract

Extract	Color	Consistency
Tamarind indica	Dark Green	Semi Solid
Woodfordia fruticosa	Black	Semi Solid

Table 2: Phytochemical screening of ethanolic extract of *Tamarindus indica and Woodfordia fruticosa*

S. No.	Chemical Tests	EETI	EEWF
1	**Carbohydrates**		
	i) Molisch's Test	(+)	(-)
	ii) Fehling's Test	(+)	(-)
	iii) Benedict's test	(+)	(+)
2	**Tannins**		
	i) with 5%ferric chloride solution	(+)	(+)
	ii)with 10% aqueous Potassium dichromate solution	(+)	(+)
	iii) with 10% lead acetate solution	(+)	(+)
3	**Alkaloids**		
	i)Dragendorff's Test	(+)	(+)
	ii) Mayer's Test	(+)	(+)
	iii) Hager's Test	(+)	(+)
4	**Glycosides**		
	i) Borntrager's Test	(+)	(+)
	ii) Legal Test	(–)	(–)
	iii) Baljet Test	(–)	(–)
5	**Flavonoids**		
	i) Shinoda's Test	(+)	(+)
	ii) Alkaline reagent test	(+)	(+)
	iii) Lead test	(+)	(+)
6	**Steroids and Sterols**		
	i) Libermann-Burchard Test:	(–)	(+)
	ii) Salkowski Test:	(–)	(+)
7	**Proteins and Amino Acids**		
	i) Biuret Test:	(+)	(+)
	ii) Ninhydrin Test:	(–)	(–)
	iv) Millon's Test	(–)	(–)

(+) = Present, (–) = Absent

Table 3: Anti-diarrheal activity of Poly herbal formulation

Groups	Treatment	Mean± SEM			% Protection
		Onset of Time (min)	Total number of faecal drops	Frequency of diarrhea (number)	
I	Normal Control	65.5±3.87	35.16±3.31	2.68±0.44	-
II	Negative Control	35.5±3.47a***	67.15±5.33 a***	3.34±0.42	-
III	Loperamide 3 mg/kg	110.45±3.28 a***, b***	23.36±4.57 a***,b***	1.67±0.34	50.0
IV	PHF, 100mg/kg	77.33±4.33 b***	49.12±3.95 a*,b**	2.33±0.56	30.23
V	PHF, 200mg/kg	85.45±4.66 a**, b***	40.33±3.44 b***	2.25±0.76	32.63

Values are mean± SEM, n=6. *p<0.05, **p<0.01, ***p<0.001

a- significant difference as compared to control

b- Significant difference as compared to Negative control

CONCLUSION

The study successfully formulated and evaluated the anti-diarrheal activity of a polyherbal formulation comprising *Tamarindus indica* and *Woodfordia fruticosa* leaves extracts. The phytochemical screening confirmed the presence of multiple bioactive compounds, including tannins and flavonoids, which are known for their therapeutic effects against diarrhea. The PHF demonstrated significant anti-diarrheal activity in an animal model, with both 100 mg/kg and 200 mg/kg doses providing dose-dependent protection. The higher dose of PHF showed greater efficacy, suggesting the potential for improved therapeutic outcomes with appropriate dosing. The results indicate that the PHF could be a promising alternative for diarrhea treatment, offering a natural and synergistic approach to managing the condition. However, further investigations are necessary to fully understand the mechanisms behind the observed effects, determine the optimal dosage, and establish the safety and efficacy of the formulation in clinical settings.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Bhuwaneshwar, M., Rajasekaran, C., Sahu, S. & Kavitkar, V.V. (2012) Antidiarrheal activity of ethanolic extract of *Woodfordia fruticosa* Kurz flowers in Wistar albino rats. *Asian Pacific Journal of Tropical Disease*, 2 (Supplement 1), S164–S168.
- Brijesh, S., Daswani, P., Tetali, P., Antia, N. & Birdi, T. (2009) Studies on the antidiarrhoeal activity of *Aegle*

marmelos unripe fruit: Validating its traditional usage. *BMC Complementary and Alternative Medicine*, 9, 47.

- De Caluwe, E., Halamová, K. & Van Damme, P. (2010) *Tamarindus indica* L. A review of traditional uses, phytochemistry and pharmacology. *African Focus*, 23, 53–83.
- Doughari, J.H. (2006) Antimicrobial activity of *Tamarindus indica* Linn. *Tropical Journal of Pharmaceutical Research*, 5, 597–603.
- Fokam Tagne, M.A., Tchoffo, A., Noubissi, P.A., Mazo, A.G., Kom, B., Ngakou Mukam, J., Sokeng Dongmo, S. & Kamgang, R. (2021) Effects of hydro-ethanolic extract of leaves of *Maesa lanceolata* (Mursinaceae) on acetic acid-induced ulcerative colitis in rats. *Inflammopharmacology*, 29, 1211–1223.
- Gupta, M., Mazumder, U.K., Kumar, R.S. & Gomathi, P. (2004) Antioxidant and antimicrobial properties of *Woodfordia fruticosa* flowers. *Indian Journal of Experimental Biology*, 42, 373–377.
- Kosek, M., Bern, C. & Guerrant, R.L. (2003) The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bulletin of the World Health Organization*, 81, 197–204. P
- Mukherjee, P.K., Nema, N.K., Maity, N. & Sarkar, B.K. (2011) Phytochemical and therapeutic potential of *Woodfordia fruticosa* Kurz. *Pharmacognosy Reviews*, 5, 129.
- Mukherjee, P.K., Saha, K., Murugesan, T., Mandal, S.C., Pal, M. & Saha, B.P. (1998) Screening of antidiarrhoeal profile of some plant extracts of a specific region of West Bengal, India. *Journal of Ethnopharmacology*, 60, 85–89.
- Palombo, E.A. (2006) Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: Modes of action and effects on intestinal function. *Phytotherapy Research*, 20, 717–724.
- Saeed, M.A. & Sabir, A.W. (2001) Antibacterial activities of some constituents from *Tamarindus indica* L. *Pakistan Journal of Pharmaceutical Sciences*, 14, 47–48.
- Shailajan, S., Menon, S., Singh, A. & Mhatre, P. (2010) Comparative phytochemical and pharmacological profiles of the leaves of *Tamarindus indica* Linn. collected from Mumbai and Jodhpur. *Journal of Natural Remedies*, 10, 173–178.
- Shin, S.Y., Dekebo, A., Dinku, W., Terfa, A., Lee, Y.H., Lim, Y. & Yong, Y. (2014) Identification of an anticancer compound contained in seeds of *Maesa lanceolata*, a medicinal plant in Ethiopia. *Journal of the Korean Society for Applied Biological Chemistry*, 57, 519–522.
- Shirwaikar, A., Bhilegaonkar, P.M., Malini, S. & Kumar, J.S. (2006) The gastroprotective activity of the ethanol extract of *Woodfordia fruticosa* flowers. *Journal of Ethnopharmacology*, 105, 343–348.

- Thapar, N. & Sanderson, I.R. (2004) Diarrhea in children: An interface between developing and developed countries. *Lancet*, 363, 641–653.
- Williamson, E.M. (2001) Synergy and other interactions in phytomedicines. *Phytomedicine*, 8, 401–409.