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Assessment of Pharmacological Properties of *Mimosa diplotricha* Leaf Extract

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Abstract

This present study was designed to explore the different pharmacological activities of methanol extracts of *Mimosa diplotricha* leaves (MDME) and its chloroform (MDCH), n-hexane (MDNH), pet ether (MDPE), and aqueous (MDAQ) fractions. The antidiarrheal activity was investigated by the castor oil-induced diarrhea method. The thrombolytic activity was done using *in vitro* clot lysis model. The anthelmintic activity was conducted on the earthworm *Pheretima posthuma* and the antimicrobial activity was evaluated by the disc diffusion method. In the castor oil-induced model, the MDME (200 mg/kg) exhibited significant (p<0.001) inhibition in the total number of defecations within the testing period compared to standard loperamide (p<0.001). During the clot lysis procedure, MDME and MDCH (20 mg/ml) exhibited significant (p<0.001) clot lysis (%) compared with standard streptokinase. In the anthelmintic procedure, MDME and MDAQ (50 mg/ml) showed significant anthelmintic activity in a concentration-dependent manner, while the standard drug albendazole (15 mg/ml) provided significant anthelmintic activity. In the disc diffusion method, MDME provides no antimicrobial activity. So, our study revealed that the plant extracts have potential antidiarrheal, thrombolytic, and anthelmintic activities.

Keywords: *Mimosa diplotricha*, antidiarrheal, thrombolytic, anthelmintic, and antimicrobial.

Introduction

Diarrhea is the prominent reason for mortality of children sparked by the prompt movement of fecal substances through the intestine which results in reduced absorption of water, electrolytes, and nutritional content producing excessive frequent defecation[1]. Gastrointestinal infection is the common reason for diarrhea[2]. Intestinal transit inhibitors, enkephalinase inhibitors, 5-HT_3-receptor antagonists, calcium-sensing receptor ligands, pro-absorptive, antisecretory, and intraluminal agents are presently available on market for diarrhea treatment, but all of the prevailing drugs exhibited numerous adverse effects like bronchospasm induction, drowsiness, severe constipation, abdominal pain, irregular heartbeat, vomiting, and intestinal obstruction[3]. Medicinal plants are a potential resource for antidiarrheal drugs[4]. Recently thrombus formation has been a terrible problem in blood circulation consolidating a mechanism in the human body to repair blood vessels that are injured[5]. Tissue plasminogen activator (t-PA), alteplase, reteplase, desmoteplase, tenecteplase, anistreplase, urokinase, streptokinase, SK-plasminogen activating complex are currently existing synthetic thrombolytic drugs in the market[6]. Traditional thrombolytic agents still have significant limitations[7]. Helminthiasis is one of the life-threatening parasitic diseases worldwide, inflicting substantial production failures in livestock[8]. Commonly used anthelmintics agents are subjected to resistance and slowing in vaccine development[9]. There is a great scientific interest in the progression of innovative anthelmintic agents from natural sources[10]. Microbial infections endure posing a life-threatening risk to public health throughout the world[11]. The situation becomes more complicated by multidrug-

resistant (MDR) pathogens resulting in enhanced morbidity and mortality[12]. Therefore, an exploration for safe and more efficient therapeutic agents is required in terms of cost-effectiveness, efficiency, and safety. Natural resources are mostly considered a reliable source for the exploration of anticipated efficient and safe medications[13]. To improve the standard of life and sustain good health, the utilization of herbal medicines has been tremendously expanding over the last decades as a substitutive approach[14]. Medicinal plants have been treated as medicine for human diseases since the inauguration of civilization[15]. It is anticipated that about 30% of pharmaceuticals are produced from plant derivatives. A comprehensive investigation of adverse effects and the development of a sound correlation between plants and biomarkers are indispensable to ensure the quality of herbal medicines[16]. The researchers have become successful in uncovering several bioactive phytochemical compounds proven to expose numerous physiological actions from medicinal plants with the improvement of scientific research[17]. About 80% of the global population utilizes herbs and other traditional medicines for their primary healthcare, according to a WHO report[18]. The *Mimosa* genus belongs to Fabaceae family, which comprises about 400 species of shrubs and herbs distributed worldwide. This genus revealed numerous pharmacological activities[19]. *Mimosa diplotricha* is a fast-growing leguminous woody subshrub indigenous to the Americas and is usually well-known as the giant false sensitive plant, giant sensitive plant, or nila grass[20]. This leguminous vine is located all over the subtropics and tropics of Asia, South America, North America, Africa, and Australia[21]. A qualitative phytochemical screening indicated the existence of alkaloids, carbohydrates, saponins & phytosterols in high concentration, phenols &flavonoids in moderate concentration, proteins, lipids & glycosides in low concentration, while tannins, gum& mucilages, fixed oils, volatile oil & terpenes were found to be absent[22]. Six new meroterpenoids named diplomero terpenoids A-F, two novel chalcone-lignoids named diplochalcolins A, diplochalcolins B, and four 5-deoxyflavones named diplotrin A, diplotrin B, diplotrin C, and diplotasin D were recently isolated compounds from this plant[23]. The plant was reported to possess several medicinal properties like antidiarrheal, anti-inflammatory, antioxidant[24], insecticidal[25], anticancer[26], antimicrobial, and antinociceptive activity[22]. Now considering the potentiality of this plant, the present investigation was conducted on evaluating the antidiarrheal, thrombolytic, anthelmintic, and antimicrobial properties of the methanol extract of *M. diplotricha* leaf and its different fractions.

Materials and Methods

Solvents and chemicals

Solvents and chemicals used in most experiments are analytical and laboratory grade. Methanol (99.93%), Chloroform (99.8%), Petroleum ether (ACS grade), Carbon tetrachloride (99.9%), n-hexane (95%), DMSO, Tween-80, Albendazole (10 mg/mL, Eskayef Pharmaceuticals Ltd), Loperamide (3 mg/kg, Square Pharmaceuticals Ltd), Streptokinase (30000 I.U., Popular Pharmaceuticals Ltd), Tetracycline (Incepta Pharmaceuticals Ltd), and Castor oil were used.

Plant sample collection and authentication

The leaves of the M. diplotricha were collected from the hilly region of Rangamati, Chittagong, Bangladesh, in January 2018. Then it was identified and authenticated by Taxonomist, Department of Botany, University of Chittagong, under an accession number; ACCU 2018/07.

Preparation of plant extract

Leaves of *M. diplotricha* were cleaned properly and then air-dried for several days prior to final grinding. Then the dried leaves were grounded using a high-speed grinding machine in the Phytochemistry Research Lab, Department of Pharmacy, University of Chittagong. About 800 g of the grounded material was taken in a round-bottomed, clean flask and soaked in 4 L of pure methanol. After 15-20 days of intermittent shaking and stirring, the entire mixture was filtered. Filtrate found

through Whatman No.1 filter paper was concentrated using a rotary under reduced temperature and pressure. The weight of the extract yielded was 28 g. The yield extract represented % yield by resorting to simple mathematical expressions[27].

Solvent-solvent partitioning

Solvent-solvent partitioning was conducted utilizing the protocol which was proposed by Kupchan[28] and modified by Van Wagenen[29]. The solvents used for the partitioning are chloroform, n-hexane, carbon tetrachloride, and ethyl acetate, respectively. All four fractions were vaporized to dryness and used for the investigation.

Experimental Animals

Swiss albino, young male mice (20-25 g) were purchased from the animal house of the Pharmacy department, Jahangirnagar University, Dhaka, Bangladesh and housed comfortably in a group of six in a single clean plastic cage. They are familiarized at a temperature of 25 ± 2 °C with a relative humidity of 60-70% for 14 days and operated with a 12 h day and night schedule before the commencement of experimentation. The mice were afforded water ad libitum and standard pellet diets (Ingredients include Wheat flour, Skim milk powder, Roasted Bengal gram flour, Salt mixture, Casein, Refined groundnut oil, and Vitamin mixture) throughout the study.

Experimental design

To assess the antidiarrheal property, thirty experimental animals were randomly selected and divided into six groups denoted as Group-I (control group), Group-II (standard group), Group-III, Group-IV, Group-V, and Group-VI, each group consisting of 5 mice, i.e., n = 5, and kept in a pre-cleaned animal cage. Group (I): Normal control (Normal saline with 1% Tween-80; 0.10 ml/10 mg of body weight); Group (II): Positive control (Loperamide; 3 mg/kg); Group (III): treated with 200 mg/kg methanol leaves extract of *M. diplotricha;* Group (IV), Group (V), and Group (VI) were treated with 200 mg/kg chloroform, n-hexane, and pet-ether soluble fraction of methanol extract respectively.

Acute Toxicity Studies

Acute toxicity studies were performed according to guidelines prescribed by the Organization for Economic Co-operation and Development (OECD). The LD_{50} for each of the extracts was ascertained and one-tenth of the lethal dose (LD_{50}) was considered the effectual therapeutic dose for exploring different pharmacological activities. The LD_{50} was calculated using the following equation[30], $LD50 = LD100 - \sum(a \times b)/n$.

Evaluation of antidiarrheal activity

Castor-Oil induced diarrhea

The antidiarrheal activity was evaluated by using the castor oil-induced method which is described by Shoba and Thomas[31]. In this process, a pure grade of castor oil of 1 ml was given to each rodent after the administration of crude plant extract and its related fractions. Each of the rodents was observed for four hours and the stool was recorded.

Evaluation of thrombolytic activity

In-vitro clot lysis method

Blood Specimen

The venous blood of 5 mL was drawn from 10 healthy subjects with which a history of oral contraception or anticoagulant was absent in them. The informed consent was filled up by every person participating willingly in this research process[32]. The blood was collected by an expert lab technologist from the Pharmacy Department, University of Chittagong.

Study design

The method followed was exactly described by Prasad *et al*[33]. The collected blood was distributed in different Eppendorf tubes (500 µL/tube), and they were incubated at 37 °C for 45 minutes to form the clot. Now the plant extracts of different concentrations, standard and normal control were introduced into only clot-

containing tubes. Then these tubes were again incubated for the next 90 minutes to observe the clot lysis occurred.

Evaluation of anthelmintic activity

The *in vitro* anthelmintic property was conducted by following the method described by Ajaiyeoba[34]. The adult earthworm "*Pheretima posthuma*", was used in this procedure. They were observed to look out for any physical changes (paralysis and ultimately death) after the treatment with testing materials. Albendazole used as standard drug (10 mg/mL).

Evaluation of antimicrobial activity

Disc diffusion method

The antimicrobial activity was conducted by using the disc diffusion method[35]. *Staphylococcus aureus*, *Escherichia coli*, and two unknown bacteria strains were used. The tetracycline disc (100 Ug/disc), and the blank disc residual solvents were used as a positive and negative control, respectively. Assay plates were incubated at 37 ℃ for 24 hours. The diameter of the zone of inhibition surrounding each disc was recorded. All these methods have been summarized in figure 1.

Statistical Analysis

The results were represented as mean ± SEM. Statistical comparisons of *M. diplotricha* leaf extract were performed using one-way ANOVA, Dunnett's test. Significance was considered at $^{***}p<0.001$, $^{**}p<0.01$, and $^{*}p<0.05$ compared with the control. In addition, SPSS tools are utilized for the analysis of all data. For the estimation of IC_{50} values, linear regression equations were used through the utilization of Microsoft Excel 2010.

Results and Discussion

Evaluation of antidiarrheal activity

At a dose of 200 mg/kg of body weight, the methanol extract, MDME showed a significant reduction in the total number of defecation in 4 hours (59.09% of inhibition, $p<0.001$). The standard drug loperamide showed 63.64% inhibition ($p<0.001$). Among other fractions, the MDNH fraction exhibited 53.03%

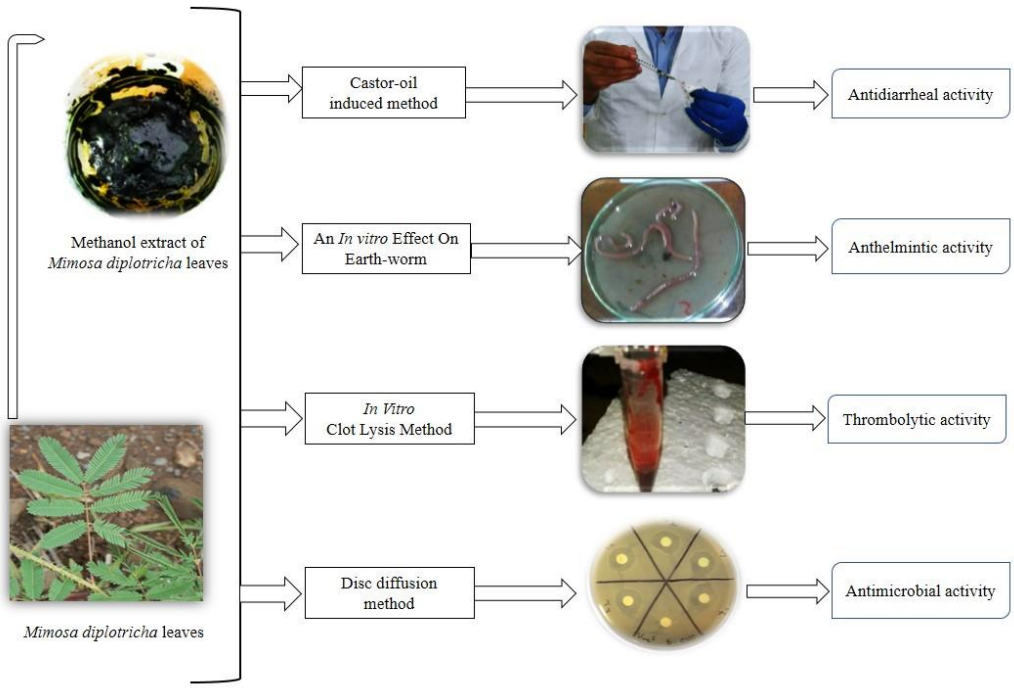

Figure 1: Graphical representation of methanol extract of *M. diplotricha* leaves on antidiarrheal, thrombolytic, anthelmintic, and antimicrobial activity.

Table 1: Antidiarrheal activity of investigated methanol extract and its different fractions of *M. diplotricha* leaf.

Animal Group	Dose mg/kg	Mean ± SEM (% of Inhibition)				
		1st hr.	2nd hr.	3rd hr.	4th hr.	Total
Control	-	3.40 ± 0.51	4.40 ± 0.24	3.20 ± 0.37	2.20 ± 0.58	13.20 ± 0.86
Standard	50	0.40 ± 0.24 88.24***	1.20 ± 0.20 72.73***	1.60 ± 0.24 50**	1.60 ± 0.51 27.27	4.80 ± 0.37 63.64***
MDME	200	1.00 ± 0.32 70.59***	2.20 ± 0.20 50***	1.40 ± 0.24 56.25**	0.80 ± 0.20 63.64	5.40 ± 0.51 59.09***
MDCH	200	1.40 ± 0.24 58.82**	2.40 ± 0.40 45.45***	1.60 ± 0.40 50**	1.20 ± 0.37 45.45	6.60 ± 0.51 50***
MDNH	200	1.00 ± 0.32 70.59***	2.60 ± 0.24 40.91**	1.80 ± 0.20 43.75*	0.80 ± 0.20 63.64	6.20 ± 0.37 53.03***
MDPE	200	0.80 ± 0.37 76.47***	2.60 ± 0.40 40.91**	2.20 ± 0.37 31.25	1.40 ± 0.24 36.36	7.0 ± 0.84 46.97***

MDME = Methanol Extract, MDCH = Chloroform Fraction, MDNH = n-hexane Fraction, MDPE = Pet Ether Fraction. Results were expressed as mean ± SEM. (n= 5). Results were significant as $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ compared with control.

inhibition ($p<0.001$). The results of the percentage inhibition of defecation in these investigated extracts are shown in Table 1. The catalytic impact of lipases on castor oil in the small intestine liberates ricinoleic acid, which induces irritating and inflammatory effects on the intestinal mucosa, leading to the release of prostaglandins in the castor oil-induced technique (Figure 2). This condition causes an increase in mucosal cell permeability and changes in electrolyte transport, resulting in a hyper-secretory response (decreased Na+ and K+ absorption), inducing peristaltic activity and diarrhea. Prostaglandin synthesis inhibitors have been shown to postpone castor oil-induced diarrhea[30,36]. The extract MDME and different fractions of *M. diplotricha* leaf may suppress prostaglandin production. Tannins, alkaloids, saponins, flavonoids, sterols, and/or triterpenes and reducing sugars are responsible for medicinal plants' antidysenteric and antidiarrheal activities[30,37]. *M. diplotricha* methanol extract contains alkaloids, saponins, flavonoids, sterols, and reducing sugars that may contribute to this effect.

Evaluation of thrombolytic activity

The results in Table 2 showed that all the investigated extracts produced significant ($p<0.001$) clot lysis (%) in a concentration-dependent manner. Here, both MDME and MDCH showed 38.94% and 36.31% clot lysis at concentrations of 20 mg/ml, respectively. The standard antithrombotic drug streptokinase (30000 I.U.) provides 60.92% clot lysis.

Table 2: Thrombolytic effect of methanol extract and its fraction of *M. diplotricha*

Sample	% of Clot Lysis (Mean ± SEM)
Control	6.04 ± 0.77
Streptokinase (30,000 I.U.)	60.92 ± 3.17***
MDME 20 mg/ml	38.94 ± 2.52***
MDME 10 mg/ml	27.07 ± 2.05***
MDME 5 mg/ml	19.44 ± 1.65***
MDCH 20 mg/ml	36.31 ± 2.06***
MDCH 10 mg/ml	29.81 ± 3.17***
MDCH 5 mg/ml	16.99 ± 1.47**

MDME = Methanol Extract, MDCH = Chloroform Fraction. Results were expressed as mean ± SEM. (n= 10). Results were significant as $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ compared with control.

As the thrombolytic effect of those extracts was comparable to Streptokinase, they may have acted by activating tissue plasminogen activator (tPA), a serine protease that converts plasminogen (Pg) to plasmin and can activate the destruction of extracellular matrix proteins or clots by breaking down the fibrinogen and fibrin within a clot (Figure 3) and thereby exerting thrombolysis[38,39]. Although the contribution of alkaloids, saponins, tannins, terpenoids, glycosides, and flavonoids as thrombolytic agents is uncertain, their presence in extracts may contribute to thrombolysis via platelet aggregation[33, 39, 40]. The presence of alkaloids, saponins, glycosides, and flavonoids in *M. diplotricha* methanol extract may be responsible for the thrombolytic effect[22].

Evaluation of anthelmintic activity

The results conferred in Table 3 showed that all extracts of *M. diplotricha* leaves exhibited an anthelmintic effect in a concentration-dependent manner. Among these extracts, MDME and MDAQ showed significant activity at the highest dose (50 mg/mL) while the standard drug albendazole showed significant activity at a concentration of 15 mg/mL. Phytochemical screening of *M. diplotricha* extracts showed the presence of tannins as well as other phytoconstituents within it[22]. And it has been reported that the anthelmintic action can be produced in presence of tannins as they bind with free protein in the host's GI tract[41,42] or glycoprotein present on cuticles of the parasite (worm) and thus kills the worm[43] (Figure 2). So, the observation made suggests that the active compound of MDME and MDAQ responsible for the action should be identified and further investigated.

Evaluation of antimicrobial activity

The results exhibited in Table 4 indicate that this plant did not show any antimicrobial action.

In this study, MDME showed no zone of inhibition around the test sample disc (Figure 3). Other species of the genus Mimosa like *M. pudica* have been found to

Table 3: Time for paralysis and death of *Pheretima posthuma* for methanol extract of *M. diplotricha* and its aqueous fraction.

Sample	Concentration (mg/ml)	Time is taken for paralysis (min) (Mean ± SEM)	Time is taken for death (min) (Mean ± SEM)
Control	-	115.4 ± 4.08	185.8 ± 6.16
Standard (Albendazole)	15	19.6 ± 2.20***	37 ± 2.21***
MDME-Sample 01	10	55 ± 3.05***	79.2 ± 3.68***
MDME -Sample 02	20	38.4 ± 2.16***	67.6 ± 2.66***
MDME -Sample 03	30	41.2 ± 3.51***	63.8 ± 2.63***
MDME -Sample 04	40	29.4 ± 1.86***	58.6 ± 2.25***
MDME -Sample 05	50	17.8 ± 1.28***	40.2 ± 1.53***
MDCH-Sample 01	10	61.4 ± 2.66***	87.8 ± 2.18***
MDCH -Sample 02	20	49.8 ± 1.56***	72.8 ± 1.60***
MDCH -Sample 03	30	31.4 ± 2.09***	61.2 ± 1.91***
MDCH -Sample 04	40	37.2 ± 2.33***	67.6 ± 2.50***
MDCH -Sample 05	50	22.6 ± 1.86***	50.2 ± 3.00***
MDAQ-Sample 01	10	56.8 ± 0.92***	83.4 ± 2.46***
MDAQ -Sample 02	20	58.2 ± 2.46***	93.4 ± 3.87***
MDAQ -Sample 03	30	43.2 ± 0.86***	70.4 ± 2.27***
MDAQ -Sample 04	40	35.4 ± 3.85***	61.2 ± 3.31***
MDAQ -Sample 05	50	24.6 ± 1.44***	47.6 ± 1.57***

MDME = Methanol Extract, MDCH = Chloroform Fraction, MDNH = n-hexane Fraction, MDPE = Pet Ether Fraction. Results were expressed as mean ± SEM. (n= 5). Results were significant as $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ compared with control.

Table 4: Antimicrobial activity of *M. diplotricha* at different concentrations.

Test Organism	Diameter of zone of inhibition (mm)				
	MDME- Stock	MDME- 1:10	MDME-1:100	MDME- 1:1000	Standard (Tetracycline)
Staphylococcus aureus	-	-	-	-	22
Escherichia coli	-	-	-	-	26
Unknown bacteria L_8	-	-	-	-	Whole plate
Unknown bacteria L_9	-	-	--	-	Whole plate

"– "means no zone of inhibition, ME= Methanol Extract of *M. diplotricha* leaf

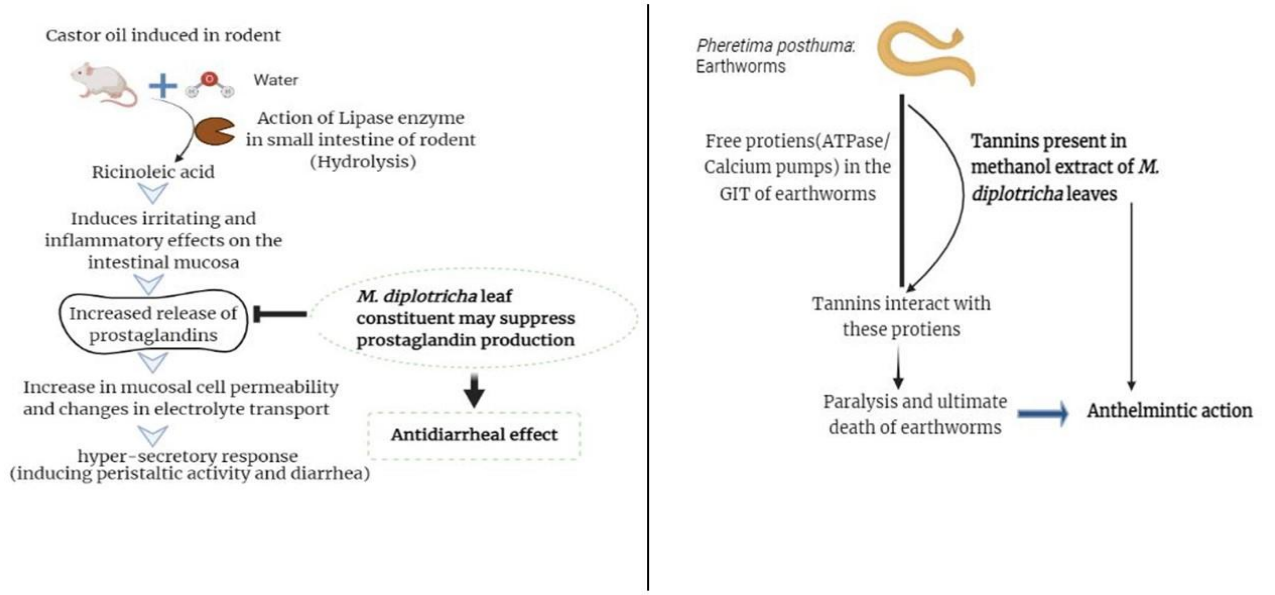

Figure 2: Graphical representation of the mechanism of methanol extract of *M. diplotricha* leaves on antidiarrheal, anthelmintic activity

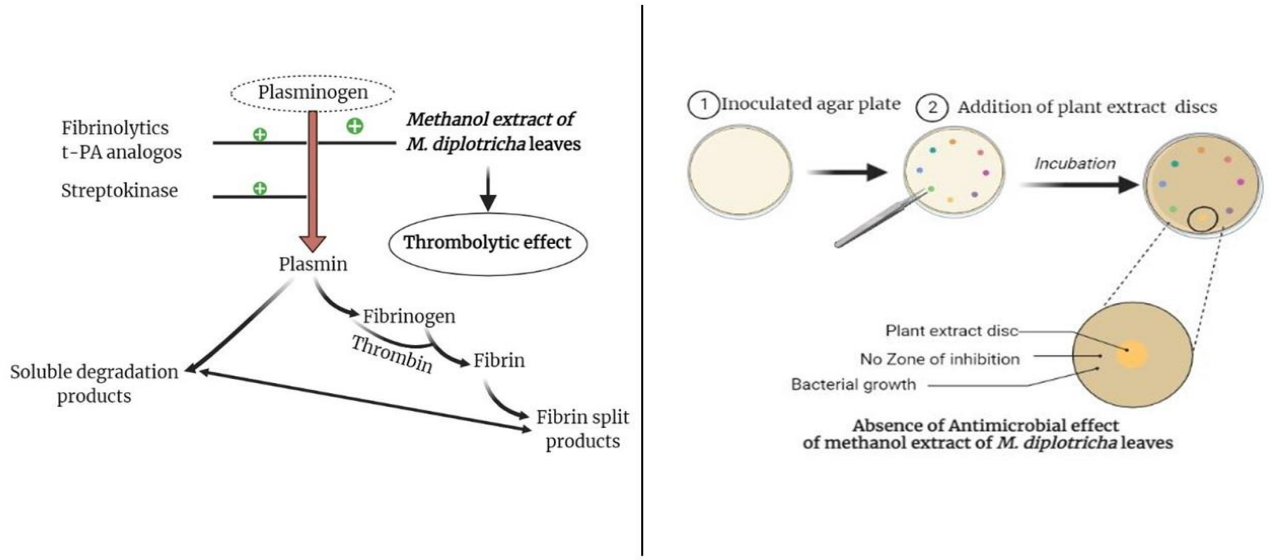

Figure 3: Graphical representation of the mechanism of methanol extract of *M. diplotricha* leaves on thrombolytic and antimicrobial activity

reveal antimicrobial action[44] (Gandhiraja *et al*., 2009). So, further studies are required to establish its therapeutic value. An overview of the mechanism of methanol extract of *M. diplotricha* leaves on the above activities has been summarized in both Figures 2 and 3.

Limitations of the study

This study was limited to leaf extract of *M. diplotricha.* The same study needs to be conducted on other parts (stems, roots, etc.) of this plant is suggested.

Conclusion

The current research concluded that *M. diplotricha* leaves extract possesses potential antidiarrheal, thrombolytic, and anthelmintic properties. Future researchers may find it to be a significant addition to future plant-based drug development techniques.

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