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Anti-Diarrheal Evaluation of *Medinilla septentrionalis*

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ABSTRACT- *M. septentrionalis* is shrub, widely distributed in Nui Ba National park, Lam Dong, Vietnam. Although there is no scientific publication about the *M. septentrionalis* as medicinal plant, the plant has been used for diarrhea treatment by ethnic minorities there. In this study, *M. septentrionalis* ethanolic extract was used to evaluate antibacterial activity, toxicity and anti-diarrheal activity. The results showed that *M. septentrionalis* ethanolic extract had highly antibacterial activity, particularly for diarrhea relating bacteria such as *Salmonella* sp., *Shigella* sp., *Vibrio* sp., and *Escherichia coli*. *M. septentrionalis* ethanolic extract also effectively prevented enteropooling, reduced either time of charcoal transit in the small intestine or defecation in castor oil-induced mice at 63 mg kg^{-1} body weight. In addition, no significant toxicity signs and mortality were observed in mice after treating the plant extract up to doses of 10000 mg kg^{-1} body weight. The preliminary phytochemical screening of *M. septentrionalis* ethanolic extract has shown the presence of carbohydrates, saponins, cardiac glycosides, flavonoids, phenolic compounds, tannins and steroids. These results strongly demonstrated that *M. septentrionalis* ethanolic extract possessed highly anti-bacterial and anti-diarrheal properties. The results in this study contributed a validation data for the used of *M. septentrionalis* in diarrheal treatment.

Key-words: Antibacterial activity, Anti-diarrheal activity, Ethanolic extract, *Medinilla septentrionalis*

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INTRODUCTION

Diarrhea is one of the popular diseases which leading causes of death among children under five globally, especially in the developing countries. Every year, there are more than 5-8 million deaths all over the world [1]. Diarrhea is caused by many reasons including bacterial infections such as *Salmonella enteritidis, S. typhii, Shigella flexneri, Escherichia coli, Vibrio cholerae,* and *Clostridium difficile* or chemicals such as castor oil or magnesium sulfate [2,3]. These agents cause the influx of water and ions to the lumen and thus increase the intestinal motility, thereby using watery stool [4].

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To treat diarrhea, some medicine are used such as diphenoxylate, loperamide. However, the usage of those medicines may cause some side effects as vomit, intestine obstruction and constipation [5]. For this reason, recently there has been great interest in herbal remedies for diarrhea treatment with no side effects.

M. septentrionalis (Melastomataceae) is shrub belonging to family Melastomataceae and widely distributed at Bi-doup- Nui Ba National Park, Lam Dong province, Vietnam. The ethnobotany information revealed that local people use its leaves and young shoot to treat diarrhea. Since there is no report on *M. septentrionalis* as an anti-diarrheal herb, the present study is conducted to evaluate its bioactivity, toxicity and anti-diarrheal activity.

MATERIALS AND METHODS

Plant identification

M. septentrionalis (W.W. Sm) H.L. Li obtained from Bi-Doup-Nui Ba National Park, Lam Dong Province, Vietnam was identified by the Department of Ecology and Evolutionary Biology, Faculty of Biology- Biotechnology, University of Science, Vietnam.

Plant identification was carried out by comparison of the

Morphology of reproductive (flowers, fruits) and vegetative organs (leaves, stems, and rhizomes) of specimens with those described in taxonomy references and with those in digital herbaria: Missouri Botanic Garden, Royal Botanic Gardens-Kew, Berlin-Dahlem Botanical Garden. The voucher specimen was deposited at the Herbarium of University of Science, Ho Chi Minh City Vietnam National University.

Preparation of *M. septentrionalis* ethanolic extract (MsEE)

M. septentrionalis was collected from BiDoup-Nui Ba National Park, Lam Dong Province, Vietnam. Young shoots and leaves were dried under sunlight and powdered and prepared as modified procedure of Milosevic *et al.* [6]. The sample was extracted with ethanol 70% by immersion method at room temperature for 48 hours. The extract was filtered and evaporated at 40^0C using a rotary evaporator to constant weight. The yield of the extract was 21.41% (w/w). The extract was then dissolved in 1% dimethyl sulfoxide (DMSO) and stored in sterilized bottle in 4^0C.

Preparation of indicator bacteria and animals

Two groups of indicator bacteria that included 19 strains of pathogenic bacteria (4 strains of *Salmonella* sp.; 3 strains of *Shigella* sp.; 4 strains of *E. coli*; 3 strains of *Vibrio* sp. and 5 strains of other pathogenic bacteria) and 7 strains of useful bacteria (4 strains of *Lactobacillus* sp. and 3 strains of *Bacillus* sp.) were used to evaluate antibacterial activity of *M. septentrionalis* ethanolic extract. Albino mice (25–30 g) were used to evaluate anti-diarrheal activity of *M. septentrionalis* ethanolic extract (MsEE). All the animals were housed in glass cages in laboratory conditions at least 1 week before performing experiment.

Antibacterial testing using well diffusion agar method

M. septentrionalis ethanolic extract was evaluated the antibacterial activity by well diffusion agar method [7]. Indicator bacteria were enriched at temperature 37^0C for 24 hours. 100 µl of diluted bacteria (10^6 cfu ml^{-1}) solution was spread on agar medium until drying. Then, wells (6 mm diameter) were made in each of plate by using sterile metal cylinders. 100 µl of the *M. septentrionalis* ethanolic extract (100 mg ml^{-1}) was added into the wells. Control experiment comprised inoculums with 1% DMSO. Plates were incubated at 37.0 ± 0.1^0C for 24 hours. The diameter of the inhibition zone (mm) was measured. Each experiment was triplicated and collected data were subjected to statistical analysis.

Acute toxicity study

The acute toxicity of *M. septentrionalis* ethanolic extract was determined in mice. Mice were fasted for 18 hours and randomly divided into five groups (6 mice per group). Different doses of plant extract (2500, 5000, 7500, and 10000 mg kg^{-1}) were separately orally administered to the mice. The fifth groups of animals as control was administered DMSO 1% (2 ml kg^{-1} body). All of animals were observed over a period of 5 days for the deaths and signs of acute toxicity.

Castor oil-induced diarrhea in mice

Mice were fasted for 10–12 hours, then divided into six groups (6 mice per group). Group of control animals was administered DMSO 1% (2 ml kg^{-1} body). The second group received standard drug, loperamide (3 mg kg^{-1} body) orally as a suspension. The plant extract was administered orally at the doses 63, 125, 250, 500, 1000 mg kg^{-1} body for the five remain groups, separately. After 30 min of treatment, the animals of each group were received 0.4 ml castor oil orally. Then, they were housed separately in a cage over clean filter paper. Diarrhea episodes were observed for a period of 4 hours.

During this period, the first defecation time, the defecation animal number in each group and the cumulative wet fecal mass was recorded. The percent of diarrheal inhibition (PI) was defined as a formula:

PI (%) = [Mean of defecation (control group – treated group)/ mean of defecation of control group)] x 100

The results of treated groups were compared with control group to evaluate diarrheal treatment effectiveness of the *M. septentrionalis* ethanolic extract.

Castor oil-induced enteropooling test

Mice were treated with castor oil and plant extract as mentioned above. After 1 hour of castor oil administration, all animals were sacrificed by an overdose of diethyl ether. The small intestine (from the pylorus to the caecum) was dissected out and weighed. Its content was collected into cylinder and volume measured. The empty intestine was weighed again then percentage reduction of intestinal secretion (volume) was calculated.

Small intestine transit test in mice

Mice were fasted and treated with plant extract as mentioned above. 30 minutes after plant extract administrated, the mice were administered 0.4 ml castor oil, followed by 0.2 ml charcoal meal (3% charcoal suspension in carboxymethyl cellulose (CMC) 0.5% (w/v). Then, each animal was housed separately in cage. After 1 hour, all animals were sacrificed by overdose of diethyl ether then the small intestine (from the pylorus to the caecum) was dissected. Charcoal meal moving distance was measured and then expressed as a percentage of the distance from the pylorus to the caecum.

Preliminary phytochemical analysis

The *M. septentrionalis* ethanolic extract was chemically tested for the presence of different constituents, including carbohydrates, alkaloids, saponins, cardiac glycosides, anthraquinone glycosides, flavonoids, phenolic

compounds, tannin, steroids and amino acids by using standard methods [8].

STATISTICAL ANALYSIS

Values were expressed as mean±standard deviation. Mean values were evaluated by Analysis of Variance. Duncan test was used to determine the statistical significance ($P < 0.05$).

RESULTS AND DISCUSSION

Identification of *M. septentrionalis*

M. septentrionalis is shrub of 1–5(–7) meter tall with many brown branches erect or scrambling. The leaves have a petiole of 0.4–1 mm long; leaf blade is lanceolate or ovate-lanceolate in shape, 7–10×2–3 cm, papery; its apex is long-acuminate, its base is obtuse to surrounded and its margin is sparsely serrulate just around in the apex area; five veins are found from the bottom, in which the secondary veins (2) are on each side of mid-vein. From 3 to five flowers, 2.5–5 cm, are found in small terminal cymose panicles and in lateral cymes. The receptacle is in hypanthium cup-shaped, 4–4.5 mm, sparsely ciliate papules. Calyx lobes have four, in green color and inconspicuous. Petals have four, in light pink or purplish red, triangular-ovate, 8–10 mm. Their stamens are eight, equal or nearly equal: four longer (outer) stamens and four shorter (inner) stamens; the connectives are slightly elongated. The ovary is ovoid. Fruit is a berry, globose-ovoid, 6–7×4–5 mm (Fig. 1). It blooms during June to September and produces fruit from February to May.

The plant is found in dense forests, forest margins and damp shady areas of Vietnam, China, Myanmar and Northern Thailand.

Fig. 1 *Medinilla septentrionalis* plant leaves

Antibacterial activity of *M. septentrionalis*

The ethanolic extract of *M. septentrionalis* showed significant inhibitory activity to several different diarrhea-related bacteria strains, including *Salmonella* sp., *Shigella* sp., *Vibrio* sp. and *E. coli* (Table 1). Interestingly, our experiment also revealed that *M. septentrionalis* ethanolic extract inhibited against an ampicillin resistant *S. enteritidis* strain with 13.33±1.04 mm diameter of inhibition zone at 100 mg ml^{-1} used concentration. Besides, the MsEE strongly inhibited other pathogens such as *L. monocytogenes, L. innocua, S. aureus, E. feacalis* and *P. aeruginosa* (Table 1). Interestingly, MsEE did not inhibit 4 strains of *Lactobacillus* sp. and 3 strains of *Bacillus* sp., which known to commonly present in human small intestine and played an important role in the digestion process. The pathogenic indicator bacteria were used such as *S. typhii* caused typhoid fever; *S. enteritidis, S. flexneri* caused diarrhea; *S. sonnei, S. boydii* caused dysentery with dangerous symptoms; *E. coli* was responsible for the diarrhea; *Vibrio* spp. caused a number of serious diseases in human such as cholera disease (*V. cholerae*), gastroenteristic (*V. parahaemolyticus*). Those bacteria were strongly inhibited by *M. septentrionalis* ethanolic extract. Those results strongly demonstrated that MsEE had high potential of antibacterial activity.

Anti-diarrheal activity of *M. septentrionalis*

In this study, defecation in castor oil-induced diarrhea mice, which were treated with MsEE, was significantly reduced. While, loperamide (3 mg kg^{-1}) inhibited 80.85% of mice defecation, the crude extract of *M. septentrionalis* inhibited 41.49% of mice defecation at 63 mg kg^{-1} used concentration. The MsEE defecation inhibitory activity was dose-dependence, when it was clearly increasing in high MsEE dose of treatment (Table 2). In castor oil-induced diarrheal mouse model, anti-diarrheal activity may be attributed to an anti-electrolyte permeability action and intestine transit. In our experiment, castor oil-induced enteropooling was observed in all experimental mice and it was strongly weakened by MsEE treatment at 63 mg kg^{-1} dose. The MsEE had shown a dose-dependent effect in reduction in intestinal weight and volume (Table 3). Besides, MsEE also strongly reduced the charcoal meal transit rate in castor oil-induced mice (Table 4).

The result was shown that the MsEE had the highest anti-diarrheal activity. The cause of diarrhea is characterized by excessive secretion water and electrolytes into the intestine lumen, exudation of protein and fluid from the mucosa and altered intestinal motility, resulting in rapid transit time and an increase wet feces. Castor oil stimulates secretion of fluid and electrolytes and increases the intestinal transit [9]. These results showed that MsEE was clearly effective to treat diarrhea in castor oil-induced mice model by reducing wet feces, inhibiting secretion fluid and gastrointestinal propulsion. Compared to other studies, the anti-diarrheal potential of *M. septentrionalis* is roughly equivalent to other herbs such as *Moringa oleifera* [1,5], *Vinca major* [10], *Alangium salviifolium* [11], *Lepidium sativum* [12], *Dillenica indica* [13].

Table 1 Antibacterial activity of *M. septentrionalis* ethanolic extract

Indicator bacteria	Diameter of inhibition zone (mm)	Indicator bacteria	Diameter of inhibition zone (mm)
S. dublin	14.33±0.76	*E. coli* O157*:H7*	13.33±0.76
S. enteritidis	13.33±1.04	*E. coli* 0208	13.83±0.29
S. typhii	14.50±0.00	*E. Coli*	13.33±0.76
S. typhimurium	13.83±1.04	ETEC	14.67±0.58
S. boydii	14.67±0.58	*V. alginolyticus*	14.00±1.00
S. flexneri	14.50±0.58	*V. cholerae*	12.50±0.87
S. sonnei	13.67±0.29	*V. parahaemolyticus*	12.17±0.29
L. innocua	14.17±0.29	*S. aureus*	14.83±0.28
L. monocytogenes	13.83±0.76	*E. feacalis*	13.83±0.76
P. aeruginosa	13.83±0.29	–	–
B. subtilis	NA	*L. lactis* LB1	NA
B. subtilis BS1	NA	*L. plantarum* LB2	NA
B. licheniformis BS2	NA	*L. plantarum* SC01	NA

NA: No Activity

Table 2 Defecation inhibitory activity of *M. septentrionalis* ethanolic extract

Treatment	Onset of diarrhea (min)	Animals with diarrhea	No. of faeces in 4 h (g)	Inhibition of defecation (%)
Castor oil + DMSO 1% (2 ml kg^{-1})	85.33 ± 11.54^{a}	6/6	0.78 ± 0.12^{a}	–
Castor oil + Loperamide (3 mg kg^{-1})	220.50 ± 7.78^{d}	2/6	0.15 ± 0.07^{e}	80.85
Castor oil + MsEE (1000 mg kg^{-1})	206.50 ± 6.36^{cd}	2/6	0.23 ± 0.03^{de}	71.28
Castor oil + MsEE (500 mg kg^{-1})	182.00 ± 10.15^{c}	3/6	0.27 ± 0.06^{cde}	65.96
Castor oil + MsEE (250 mg kg^{-1})	146.50 ± 9.15^{b}	4/6	0.35 ± 0.06^{bcd}	55.32
Castor oil + MsEE (125 mg kg^{-1})	144.80 ± 4.87^{b}	5/6	0.43 ± 0.16^{bc}	45.11
Castor oil + MsEE (63 mg kg^{-1})	136.00 ± 11.26^{b}	6/6	0.46 ± 0.11^{b}	41.49

The value are mean ± SEM, n = 6; ^{a,b,c,d,e} p<0,05 when compared with control group (ANOVA followed by Duncan test)

Table 3 Enteropooling reduction in *M. septentrionalis* ethanolic extract treated mice

Treatment	Weight intestinal content (g)	Inhibition of intestinal content (%)
Castor oil + DMSO 1% (2 ml kg^{-1})	1.12 ± 0.18^{a}	–
Castor oil + Loperamide (3 mg kg^{-1})	0.25 ± 0.09^{c}	76.87
Castor oil + MsEE (1000 mg kg^{-1})	0.35 ± 0.19^{bc}	68.66
Castor oil + MsEE (500 mg kg^{-1})	0.42 ± 0.21^{bc}	62.69
Castor oil + MsEE (250 mg kg^{-1})	0.45 ± 0.08^{bc}	59.70
Castor oil + MsEE (125 mg kg^{-1})	0.50 ± 0.37^{bc}	55.22
Castor oil + MsEE (63 mg kg^{-1})	0.70 ± 0.18^{b}	40.30

The value are mean ± SEM, n = 6; ^{a,b,c} p<0,05 when compared with control group (ANOVA followed by Duncan test)

Table 4 Effect of *M. septentrionalis* ethanolic extract on small intestine transit in mice

Treatment	Mean intestine length (cm)	Mean distance travel by charcoal (cm)	Movement (%)	Inhibition (%)
Castor oil + DMSO 1% (2 ml kg^{-1})	43.58±5.04	35.08±1.39	81.28±8.63[a]	18.72±8.63
Castor oil + Loperamide (3 mg kg^{-1})	39.25±6.21	13.50±2.12	34.91±4.99[de]	65.09±4.99
Castor oil + MsEE (1000 mg kg^{-1})	51.00±8.83	17.92±12.14	35.39±6.72[d]	64.61±6.72
Castor oil + MsEE (500 mg kg^{-1})	47.25±4.98	20.67±4.87	43.47±9.81[cd]	56.53±9.81
Castor oil + MsEE (250 mg kg^{-1})	47.17±8.29	25.08±6.97	52.63±4.95[bcd]	47.37±4.95
Castor oil + MsEE (125 mg kg^{-1})	42.25±4.27	24.17±2.48	57.96±11.27[bc]	42.04±11.27
Castor oil + MsEE (63 mg kg^{-1})	40.00±6.19	28.58±5.77	71.40±11.31[ab]	28.60±11.31

The value are mean ± SEM, n = 6; $^{b,c,d,e}p<0,05$ when compared with control group (ANOVA followed by Duncan test)

Acute animal study

In acute toxicity study, mice were treated by MsEE at several different high doses (2500, 5000, 7500 and 10000 mg kg^{-1}). Our observation on all treated mice demonstrated that during the 5 days after MsEE oral administration no significant toxicity signs and mortality were obtained. The treated mice were holding normal physiological condition, body temperature.

Preliminary phytochemical analysis

The preliminary phytochemical screening of *M. septentrionalis* ethanolic extract showed the presence of carbohydrates, saponins, cardiac glycosides, flavonoids, phenolic compounds, tannins and steroids (Table 5).

The data on indicator bacteria and castor oil-induced mouse model strongly contributed evidence of *M. septentrionalis* antibacterial and anti-diarrheal activity. Coincidently, the preliminary phytochemical screening data provided a sight of mechanism on study of *M. septentrionalis* activity as an anti-diarrheal herb. It is well known that flavonoid; phenolic compound and tanin are antibacterial compounds [14,15]. Our data also demonstrated that *M. septentrionalis* ethanolic extract contains those mentioned compounds. Taken together, it suggested that the antibacterial activity of the plant might due to the flavonoid, tannin and phenolic compounds. Furthermore, both tannin and flavonoid can precipitate protein of the electrolyte and reduce small intestine transit and intestinal secretion [16,17], the compounds may play function in *M. septentrionalis* anti-diarrheal activity.

Table 5 Phytochemical screening of *M. septentrionalis* ethanolic extract

Chemical test	Results	Chemical test	Results
Test for carbohydrate		**Test for saponin**	
Molisch's test	+	Foam test	+
Fehling's test	+	**Test for amino acid**	
Barfoed's test	+	Ninhydrin test	–
Test for flavonoid		**Test for phenolic compound**	
Alkaline reagent test	+	Lead acetate test	+
Shinoda's test	+	Gelatin test	+
Ferric chloride test	+		
Test for tannin		**Test for steroids**	
Ferric chloride test	+	Salkowski's test	+
Lead acetate test	+	Libermann Burchard test	+
Test for alkaloid		**Test for anthraquinone glycosides**	
Mayer's test	–	Borntrager's test	–
Dragendorff's test	–	**Test for cardiac glycosides**	
Hager's test	–	Legal's test	+
Wagner's test	–	Keller Killiani's test	+

(+) positive; (–) negative

CONCLUSIONS

M. septentrionalis are used to treat diarrhea at ethnic minorities in Vietnam. In our study, diarrhea- related bacteria were strongly inhibited by *M. septentrionalis* ethanolic extract. The data of assay on castor oil-induced mouse model strongly contributed evidence of *M. septentrionalis* anti-diarrheal activity. Coincidently, the preliminary phytochemical screening data provided a sight of mechanism on study of *M. septentrionalis* activity as an anti-diarrheal herb. Since *M. septentrionalis* has not been reported as anti-diarrheal herb yet, the present study strongly demonstrated that *M. septentrionalis* is effective in the treatment of diarrhea either by its antibacterial or anti-diarrheal activity.

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