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Research Paper

Antidiarrheal and antimicrobial activities of *Stachytarpheta jamaicensis* leaves

S. Sasidharan[1,3], L. Yoga Latha[1], Z. Zuraini[2], S. Suryani[2], S. Sangetha[1], L. Shirley[1]

[1]School of Biological Sciences, [2]School of Distance Education, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia, [3]Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Jalan Bedong-Semeling, Batu 3½, Bukit Air Nasi, 08100 Bedong, Kedah, Malaysia

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Correspondence to:
S. Sasidharan
E-mail: srisasidharan@yahoo.com

ABSTRACT

Objective: To evaluate the antidiarrheal and antimicrobial activity of the extract of *Stachytarpheta jamaicensis* leaves.

Materials and Methods: The methanolic extract of leaves of *S. jamaicensis* was prepared, with successive extraction in soxhlet apparatus with 300 ml of methanol for 24 h. The methanol extract of the leaves of *S. jamaicensis* (250 and 500 mg/kg) was studied for antidiarrheal activity using castor oil and magnesium sulphate-induced diarrhea models in mice. The antimicrobial activity of the extract (10 mg/ml) was determined by disk diffusion method.

Results: At the doses of 250 and 500 mg/kg, the methanol extract showed significant antidiarrheal activity ($P < 0.05$). When tested for antibacterial activity, the methanol extract displayed moderate inhibitory activity against *Escherichia coli, Staphylococcus epidermis* and *Pseudomonas aeruginosa,* with an MIC value of 5.00 mg/ml.

Conclusion: On the basis of these findings, it can be assumed that *S. jamaicensis* leaves could be a potential source for novel 'lead' discovery for antidiarrheal drug development.

KEY WORDS: Antidiarrheal activity, castor oil-induced diarrhea, magnesium sulphate-induced diarrhea, *Stachytarpheta jamaicensis*

'Diarrhea' is an alteration in the normal bowel movement and is characterized by an increase in the water content, volume or frequency of stools.[1] Diarrheal diseases are a major problem in Third World countries and are responsible for the deaths of millions of people each year.[2] Plants have long been a very important source of new drugs and many plant species have been screened to see if they contain substances with therapeutic activity. Medicinal plants are a promising source of antidiarrheal drugs.[3,4] For this reason, international organizations such as WHO have encouraged studies for treatment and prevention of diarrheal diseases using traditional medicinal practices.[5]

Stachytarpheta jamaicensis (L.) Vahl (Verbenaceae) is a weedy annual herbaceous plant that grows 60-120 cm tall. It bears small reddish-purple to deep blue flowers that grow along tall bracts. It is a native of tropical America and is acclimatized in other parts of the tropics. *S. jamaicensis* has been used in traditional medicine for many years. The leaves are used to treat dysentery and intestinal worms.[6,7] In Malaysia, a decoction of the leaves is used as a draught for ulceration of the nose and as an antiperiodic medicine in malaria.[8]

In the search for a new medicine for diarrhea, this study intended to investigate the antidiarrheal activity of the methanol extract of the leaves of *S. jamaicensis* in castor oil and magnesium sulphate-induced diarrhea models in mice. The methanol extract was also studied for its antimicrobial activity.

Materials and Methods

S. jamaicensis—sample

Fresh *S. jamaicensis* leaves were collected from Muar, Johor, Malaysia, in March 2005 and authenticated by the botanist of the School of Biological Sciences at Universiti Sains Malaysia, where the herbarium was deposited. The plant material was dried in an oven at 60°C.

Preparation of crude extract

Hundred grams of the dried leaves of *S. jamaicensis* were boiled in a soxhlet with 300 ml of methanol for 24 h. The entire extract of *S. jamaicensis* leaves was evaporated to dryness in a rotary evaporator.

Microorganisms

Test microorganisms (local clinical isolates) were obtained from the laboratory stock culture. The test microorganisms were cultured on nutrient agar slants at 37°C for 18 h. The stock culture was maintained on nutrient agar slants at 4°C.

Animals

Swiss albino mice of either sex, weighing between 25 and 35 gm, were used. The cages with the mice were placed in a room (temperature 26 ± 2°C) with controlled cycles of 12 h of light and 12 h of darkness; lights went on at 7 AM. The relative humidity was maintained at 45-55%. Water and food were provided to the animals *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee

(IAEC) of the School of Biological Sciences, Universiti Sains Malaysia. Experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care (EEC Directive of 1986; 86/609/EEC).

Antidiarrheal activity study by castor oil-induced diarrhea

The method described by Shoba and Thomas[2] was followed for this study. The animals were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhea were selected for the final experiment. The animals were divided into control, positive control and test groups, with five mice in each group. The control group received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg orally. The positive control group received loperamide at the dose of 3 mg/kg orally.[9] The test group received the methanol extract at doses of 250 and 500 mg/kg orally. Each animal was placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed every hour. Diarrhea was induced by oral administration of 0.5 ml castor oil to each mouse, 30 min after the above treatments. During an observation period of 4 h, the total number of feces and the number of diarrheic feces excreted by the animals were recorded. A numerical score based on stool consistency was assigned as follows: normal stool = 1, semisolid stool = 2 and watery stool = 3.

Antidiarrheal activity study by magnesium sulphate-induced diarrhea

A similar protocol as for castor oil-induced diarrhea was followed. Diarrhea was induced by oral administration of magnesium sulphate at the dose of 2 g/kg to the animals 30 min after administration of vehicle (1% Tween 80 in water, 10 ml/kg, p.o.) to the control group, loperamide (3 mg/kg) to the positive control group, the methanol extract at the doses of 250 and 500 mg/kg to the test group. All the administrations were carried out through the oral route.[10]

Antimicrobial activity

The antimicrobial activity of the crude extract was determined following the method described by NCCLS[11] with slight modifications.

Disk diffusion technique

The test microbes were removed aseptically with an inoculating loop and transferred to a test-tube containing 5 ml of sterile distilled water. Sufficient inoculum was added until the turbidity equaled 0.5 McFarland (10^8 cfu/ml) standard (bioMerieux, Marcy Petoile, France). One milliliter of the test-tube suspension was added to 15-20 ml of nutrient agar before setting aside the seeded agar plate (9 cm in diameter) for 15 min to allow it to solidify. Three Whatman's filter paper No. 1 disks of 6 mm diameter were used to screen for antimicrobial activity. Each sterile disk was impregnated with 20 µl of: extract (corresponding to 10 mg/ml of crude extract); chloramphenicol (30 µg/ml, as positive control) and 10% DMSO (v/v) (as negative control) before they were placed on the surface of the seeded plates. The plates were incubated at 37°C overnight and examined for zones of growth inhibition. Antimicrobial activity was tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis*.

Determination of MIC values

The minimum inhibitory concentration (MIC) of the extracts was determined for *S. aureus* and *S. typhimurium* using the twofold serial microdilution method with saline at a final concentration ranging from 10.0000 mg/ml to 0.0024 mg/ml. The tested extracts were added to sterile Mueller-Hinton broth in microtiter plates before the diluted bacterial suspension (final inoculum of 10^5 bacteria/ml) were added. Each extract was assayed in triplicate. The bacterial suspensions were used as positive control and extracts in broth were used as negative control. The MIC values were taken as the lowest concentration of the extracts in the wells of the microtiter plate that showed no turbidity after 24 h of incubation at 37°C. The turbidity of the wells in the microtiter plate were interpreted as visible growth of the microorganisms.

Statistical evaluation

The groups were compared using one-way analysis of variance (ANOVA) followed by Dunnett's test and $P < 0.05$ was considered significant.

Results

Effect on castor oil-induced diarrhea

In the mice with castor oil-induced diarrhea, the methanol extract of the leaves of *S. jamaicensis*, at doses of 250 and 500 mg/kg, reduced the total number of feces as well as of diarrheic feces in a dose-dependent manner and the results were statistically significant ($P < 0.05$) [Table 1].

Effect on magnesium sulphate-induced diarrhea

In the magnesium sulphate-induced diarrheal model, the methanol extract at the above dose levels was found to reduce the severity of diarrhea in test animals and the results were statistically significant ($P < 0.05$) [Table 2].

Antibacterial activity

In the antimicrobial activity screening, the extract inhibited the growth of *Escherichia coli, Staphylococcus epidermis* and *Pseudomonas aeruginosa* with a moderate zone of inhibition [Table 3]. The MIC values of the methanolic extracts of *S. jamaicensis* against *Escherichia coli, Staphylococcus epidermis* and *Pseudomonas aeruginosa* are shown in Table 4. The MIC values of the methanolic extracts were the same (5.00 mg ml) against *Escherichia coli, Staphylococcus epidermis* and *Pseudomonas aeruginosa.*

Discussion

In this study, methanol was used as the solvent system to prepare the crude methanolic extract. To avoid any solvent

Table 1

Effect of a methanol extract of *Stachytarpheta jamaicensis* leaves on castor oil-induced diarrhea in mice (0.5 ml to each mouse at 0 h)

Treatment	*Dose (mg/kg, p.o.)*	*Total number of feces in 4 h*[a]	*Total number of wet feces in 4 h*[a]
Control (1% tween 80 in water, 0.01 ml/g, p.o.)	-	16.20 ± 0.78	12.0 ± 0.99
Loperamide	3.0	2.80 ± 0.61[b]	0.70 ± 0.38[b]
ME	250.0	8.30 ± 0.82[b]	4.80 ± 1.00[b]
	500.0	6.20 ± 0.81[b]	3.70 ± 0.62[b]

ME - Methanol extract. [a]Values are mean ± S.E. (n = 10). [b] $P < 0.05$ *vs* control; one-way analysis of variance (ANOVA)

Table 2

Effect of methanol extract of *Stachytarpheta jamaicensis* leaves on magnesium sulphate-induced diarrhea in mice (2 g/kg at 0 h)

Treatment	*Dose (mg/kg, p.o.)*	*Total number of feces in 4 h*[a]	*Total number of wet feces in 4 h*[a]
Control (1% tween 80 in water, 0.01 ml/g, p.o.)	-	12.00 ± 1.31	9.00 ± 0.69
Loperamide	3.0	1.90 ± 0.75[b]	1.10 ± 0.34[b]
ME	250.0	7.60 ± 0.97[b]	4.10 ± 0.91[b]
	500.0	5.10 ± 0.66[b]	3.40 ± 0.68[b]

ME - Methanol extract. [a]Values are mean ± S.E. (n = 10). [b]$P < 0.05$ *vs* control; one-way analysis of variance (ANOVA)

Table 3

Antimicrobial activity (zone of inhibition and MIC[a]) of crude methanolic extract of the *Stachytarpheta jamaicensis* leaves (ME) compared to commercial antibiotic chloramphenicol

Microorganisms	*Diameter of zone of inhibition (in mm)*	
	ME (10 mg/ml /disc)	*Chloramphenicol (30 µg/disc)*
Escherichia coli	13	30
Staphylococcus aureus	-	27
Staphylococcus epidermis	10	29
Enterococcus faecalis	-	30
Pseudomonas aeruginosa	12	29

[a]Agar dilution method, mean value, n = 3. [b]The values (average of triplicate) are diameter of zone of inhibition at 10 mg/ml crude extract and 30 µg/ml chloramphenicol

effect on the experimental animals, the solvent was evaporated completely to dryness to yield a nonsticky solid mass. The methanol extracts of *S. jamaicensis* used in this study significantly ($P < 0.05$) reduced the total number of wet feces in a dose-dependent manner.

Castor oil-induced diarrhea is a secretary diarrhea since recinolic acid, the active ingredient of castor oil, induces diarrhea by a hypersecretory response.[12,13] Since the methanol extract of *S. jamaicensis* successfully inhibited the castor oil-induced diarrhea, it can be assumed that the antidiarrheal action was mediated by an antisecretory mechanism. This was also evident from the reduction of total number of wet feces in the test groups in the experiment.

Magnesium sulphate has been reported to induce diarrhea by increasing the volume of intestinal content through prevention of reabsorption of water. It has also been demonstrated that it promotes the release of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of the small intestine and thereby prevents the reabsorption of sodium chloride and water.[14] The methanol extract was also found to lessen the diarrheic condition in this model. The methanol extract increased absorption of water and electrolyte from the gastrointestinal tract.

Infectious diarrheal diseases are the second leading cause of morbidity and mortality worldwide.[15,16] In the search for newer remedies for infectious diarrhea and dysentery, this study aimed at investigating the antidiarrheal activity and antibacterial activity of *S. jamaicensis* against a number of bacterial species known to be associated with diarrhea and dysentery. The antimicrobial activity study revealed that the methanol extract of the leaves of *S. jamaicensis* possessed antibacterial activity against three pathogenic bacterial strains that cause diarrhea and dysentery. The *in vitro* antimicrobial activity of the leaf extract is less than that of chloramphenicol, which was used as the standard, with

Table 4

Determination of MIC values of extracts of *Stachytarpheta jamaicensis* against *Escherichia coli, Staphylococcus epidermis* and *Pseudomonas aeruginosa*

Concentration (mg/ml)	*Methanolic extract of Stachytarpheta jamaicensis*			*Control*	
	Escherichia coli	*Staphylococcus epidermis*	*Staphylococcus epidermis*	*Positive*	*Negative*
10.0000	-	-	-	+	-
5.0000	-	-	-	+	-
2.5000	+	+	+	+	-
1.2500	+	+	+	+	-
0.6250	+	+	+	+	-
0.3125	+	+	+	+	-
0.1563	+	+	+	+	-
0.0781	+	+	+	+	-
0.0391	+	+	+	+	-
0.0195	+	+	+	+	-
0.0098	+	+	+	+	-
0.0049	+	+	+	+	-
0.0024	+	+	+	+	

-: Absence of growth; positive control: bacterial suspensions and saline; +: presence of growth; negative control: extracts (10 mg/ml) and broth

a seemingly high MIC value. This is certainly due to the fact that a crude extract was used in this study.[17] Hence, the extract might be useful for infectious diarrheal diseases and with further detailed studies are warranted.

Conclusion

Since the methanol extract exhibited antidiarrheal activity in a number of models of diarrheic conditions in test mice along with antimicrobial activity, the extract could be useful as a nonspecific treatment for diarrhea. It is also reasonable to suppose that the methanol extract might be effective in inflammatory diarrhea, secretory diarrhea and infectious diarrhea. On the basis of these findings, it can be assumed that *S. jamaicensis* leaves could be a potential source for a novel 'lead' discovery for antidiarrheal drug development.

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