

Research Article

Antidiarrhoeal Activity of Chromatographic Fractions of *Stereospermum kunthianum* Cham Sandrine Petit (Bignoniaceae) Stem Bark

FP Ching^{1*}, SO Okpo², A Falodun³ and EKI Omogbai²

¹Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Yenagoa, Departments of ²Pharmacology & Toxicology, and ³Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Abstract

Purpose: The present study was undertaken in order to evaluate the antidiarrhoeal activity of three chromatographic fractions (L, S and Y) of *Stereospermum kunthianum* stem bark in mice.

Methods: Vacuum liquid/column chromatography (VLC/CC) were used to obtain three fractions (L, S and Y) of *Stereospermum kunthianum* stem bark fractions. The antidiarrhoeal activities of these fractions at doses ranging from 100 – 400 mg/kg were evaluated in diarrhoea episodes induced by castor oil in mice. The controls were given distilled water (10 ml/kg) while 10 mg/kg of morphine was used as the reference drug (positive control).

Results: Pretreatment of mice with 100, 200 and 400 mg/kg of fraction L significantly ($p < 0.05$) delayed the onset of diarrhoea compared with the distilled water-treated mice. Fraction L at the dose of 400 mg/kg significantly reduced the number of wet stools in the treated mice. Fraction S at the doses of 200 and 400 mg/kg dose-dependently and significantly delayed the onset of diarrhoea in the treated mice. At the dose of 400 mg/kg, fraction S significantly reduced the number of wet stools and total number of stools as well as the total weight of wet and total weight of stools, compared to the distilled water-treated mice. Fraction Y at the three doses significantly delayed the onset of diarrhoea in the treated mice.

Conclusion: Vacuum liquid/column chromatography (VLC/CC) derived fractions L, S and Y of *Stereospermum kunthianum* possessed antidiarrhoeal activity but to varying degrees. This study lends further credence to the ethnomedicinal use of the plant for the treatment of diarrhoea.

Keywords: Antidiarrhoeal activity, chromatographic fractions, *Stereospermum kunthianum*, stools

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*Corresponding author: **E-mail:** fidelching@yahoo.ca

INTRODUCTION

Diarrhoea is a public health problem in developing countries. Acute diarrhoea is the leading cause of morbidity and mortality amongst children in developing countries [1]. Many rural dwellers in the world depend largely on medicinal herbs for the treatment of diarrhoeal conditions because these herbs are readily available, affordable and are an indispensable component of traditional medicine practice. Additionally, the existing antidiarrhoeal drugs are either not available or are not affordable by many rural dwellers. *Stereospermum kunthianum*, Cham, Sandrine Petit (Bignoniaceae) is known as *sansami* and *golombi* amongst the Hausas and Kanuris of northern Nigeria, respectively, *umana* amongst the Tivs of the Middle Belt of Nigeria, *ayada* amongst the Yorubas of southwest Nigeria, and *alakiriti* amongst the Igbos of southeast Nigeria. The plant is found in several countries in Africa.

Stereospermum kunthianum is used in the management of diarrhoea in some rural communities in Nigeria [2]. The efficacy of its water extract in human complement system fixation *in-vitro* has been reported [3]. The antiplasmodial activity of naphthoquinones and one anthraquinone from the lipophilic extract of the root bark of the plant has also been reported [4]. We recently reported the antidiarrhoeal activity of the aqueous extract of its stem bark in *in-vivo* experimentally-induced diarrhoeal models using mice and rats [5]. Also, the analgesic activity of the aqueous extract of the stem bark has been investigated [6]. In the present study, we report the antidiarrhoeal activity of the vacuum liquid/column chromatographic fractions of *S. kunthianum* stem bark.

EXPERIMENTAL

Plant material

The fresh stem bark of *S. kunthianum* was collected in March 2006 in Ogun State, Nigeria. Botanical authentication was by a

taxonomist, Mr. Usang Felix, of the Forest Research Institute of Nigeria (FRIN), Ibadan. A voucher specimen (no. FHI 107277) was deposited in the herbarium of the same institute (FRIN) for future reference.

Preparation of plant material and extraction

The stem bark was carefully separated from the woody part, cut into small bits, shade-dried and pulverised using a grinder (Lab. mill, Christy and Norris Ltd, England). The powdered stem bark (500 g) was macerated with methanol (4 x 5 L for 48 h) at room temperature. It was filtered and the filtrate evaporated to dryness at reduced pressure using a rotary evaporator to obtain a dark brownish residue (80 g). The extract obtained was stored in a closed container in the refrigerator at 4 °C until required.

Chromatography

The extract (80 g) was subjected to vacuum liquid chromatography (VCC) over silica gel F₂₅₄ using different gradient solvent systems of n-C₆H₁₄ : CHCl₃ and CHCl₃ : CH₃OH, with 100 % CH₃OH as eluting solvents to obtain 19 fractions. These were subjected to repeated thin layer chromatography (TLC) using CH₃OH: CHCl₃ (3:1) as solvent system. Fractions with similar spot characteristics and R_f-values were bulked to obtain 3 fractions (A, 27.36 g; B, 41.40 g and C, 10.23 g). Each of these fractions (A, B and C) was further subjected to column chromatography (CC) as previously described by Pettelier *et al* [7] and Braithwaite and Smith [8]. The column was eluted with solvents of increasing polarity consisting of n-C₆H₁₄ : CHCl₃ (9:1) and CHCl₃: CH₃OH (1:4) mixtures. The column chromatography of fractions A, B and C yielded 99, 75 and 85 fractions, respectively. These fractions were subjected to thin layer chromatography (TLC) and subsequently bulked based on the R_f values to obtain 5, 6, and 4 fractions, respectively, with yields as follows: A (J, 0.21 g; K, 1.24 g; L, 9.20 g; M, 5.39 g; N, 1.17 g); B (Q, 1.08 g; R, 1.70 g;

S, 7.39 g; T, 6.82 g; V, 6.70 g; W, 6.19 g), and C(U, 0.25 g; X, 0.50 g; Y, 3.36 g; Z, 2.30g). Fractions L, S and Y were selected for antidiarrhoeal evaluation based on their relative abundance.

Animals

Swiss mice (25 - 30 g) of either sex were used. The animals were maintained under standard laboratory conditions including 12 h light and dark cycles, temperature (28 ± 1 °C), and free access to standard chow (Bendel Feeds and Flour Mill Plc, Ewu, Nigeria) and tap water. The experimental protocols were approved by institutional Committee on the Care and Use of Animals in Experiments.

Castor oil-induced diarrhoea in mice

The method employed was previously described by Izzo *et al* [9]. Swiss mice (5 per group) were administered with distilled water (10 ml/kg, p.o.), morphine (10 mg/kg, s.c.) or 100, 200, 400 mg/kg of each of the fractions L, S and Y thirty minutes before oral administration of castor oil (0.2 ml per mouse). The mice were placed singly under a glass funnel, the floor of which was lined with weighed filter paper (Whatmann No.1) and observed for 4 h. The parameters observed include the onset of diarrhoea stool (first stool that left a halo on the filter paper), number of wet stools, weight of wet stools, total number of stools and total weight of faecal output.

Statistical analysis

Data were expressed as mean \pm SEM and analyzed using the unpaired Student's t-test. Results were considered significant at $p < 0.05$ or better.

RESULTS

Pretreatment of mice with 100 and 200 mg/kg of fraction L of *S. kunthianum* stem bark significantly ($p < 0.05$) delayed the onset of diarrhoea compared with the distilled water-

treated mice (see Table 1). The dose of 400 mg/kg of the fraction was most efficacious in delaying the onset of diarrhoea ($p < 0.001$) and also significantly reduced the number of wet stools compared to distilled water-treated mice.

Fraction S of the extract at doses of 200 and 400 mg/kg significantly ($p < 0.0001$) delayed the onset of diarrhoea compared to the distilled water-treated mice (see Table 2). At the dose of 400 mg/kg, fraction S significantly ($p < 0.001$) reduced the number wet stools, total number of stools, total weight of wet stools as well as the total weight of stools, compared to the distilled water-treated mice (Table 2).

Fraction Y at the doses of 100, 200 and 400 mg/kg significantly ($p < 0.001$) delayed the onset of diarrhoea compared to the distilled water-treated mice (see Table 3).

DISCUSSION

In diarrhoea, bowel function is disturbed with consequent increase in bowel contractility, excessive intestinal secretion of water and electrolytes, and decreased intestinal reabsorption [10]. Ricinoleic acid, the active principle in castor oil, causes changes in mucosal cell layer permeability, electrolyte transport and intestinal peristalsis, leading to hypersecretory response and diarrhoea [11]. It also causes inflammatory response in the mucosa, leading to prostaglandin release, which results in an increase in the net secretion of water and electrolytes into the small intestine [12]. *Stereospermum kunthianum* stem bark fractions L, S and Y, at the tested doses, significantly inhibited and delayed the onset of diarrhoea in mice with the maximum effect observed at a dose of 400 mg/kg body weight. Fractions L and S, at a dose of 400 mg/kg, significantly reduced the number of diarrhoeal stools but fraction S, in addition, reduced the total number of stools, total weight of wet stools as well as the total weight of stools. Some of these effects were comparable to those produced

Table 1: Effect of fraction L of *Stereospermum kunthianum* stem bark on castor oil-induced diarrhoea in mice

Treatment	Onset of diarrhoea (min)	Number of wet stools	Total number of stools	Total weight of wet stools (g)	Total weight of stools (g)
Distilled water (10 ml/kg)	63.67±4.70	6.11±0.70	8.22±0.92	0.65±0.10	0.60±0.12
<i>S. kunthianum</i> (100 mg/kg)	82.40±3.05*	4.60±0.90	7.80±0.11	0.60±0.18	0.56±0.18
<i>S. kunthianum</i> (200 mg/kg)	85.20±2.68*	4.40±0.71	7.60±0.24	0.60±0.10	0.54±0.13
<i>S. kunthianum</i> (400 mg/kg)	109.40±5.30**	4.00±0.20**	6.60±0.63	0.40±0.09	0.32±0.07
Morphine (10 mg/kg)	201.83±8.90***	1.50±0.67**	2.00±1.00**	0.34±0.19	0.35±0.20

Values are mean ± SEM of at least five experiments; * $P < 0.05$, ** $p < 0.001$, *** $p < 0.0001$ significantly different from distilled water-treated animals; unpaired Student's *t*-test.

Table 2: Effect of fraction S of *Stereospermum kunthianum* stem bark on castor oil-induced diarrhoea in mice

Treatment	Onset of diarrhoea (min)	Number of wet stools	Total number of stools	Total weight of wet stools (g)	Total weight of stools (g)
Distilled water (10 ml/kg)	63.67±4.70	6.11±0.70	8.22±0.92	0.65±0.10	0.60±0.12
<i>S. kunthianum</i> (100 mg/kg)	84.00±2.45	6.00±0.91	8.00±0.98	0.58±0.14	0.58±0.14
<i>S. kunthianum</i> (200 mg/kg)	131.20±5.11***	5.60±2.04	6.20±1.85	0.54±0.18	0.54±0.18
<i>S. kunthianum</i> (400 mg/kg)	196.20±4.90***	1.20±0.80**	2.40±1.60**	0.11±0.07**	0.19±0.11**
Morphine (10 mg/kg)	201.83±8.90***	1.50±0.67**	2.00±1.00**	0.34±0.19	0.35±0.20

Values are mean ± SEM of at least five experiments; ** $p < 0.001$, *** $p < 0.0001$ significantly different from distilled water-treated animals; unpaired Student's *t*-test.

Table 3: Effect of fraction Y of *Stereospermum kunthianum* stem bark on castor oil-induced diarrhoea in mice

Treatment	Onset of diarrhoea (min)	Number of wet stools	Total number of stools	Total weight of wet stools (g)	Total weight of stools (g)
Distilled water (10 ml/kg)	63.67±4.70	6.11±0.70	8.22±0.92	0.65±0.10	0.60±0.12
<i>S. kunthianum</i> (100 mg/kg)	93.00±2.37**	4.80±0.86	5.80±0.49	0.49±0.09	0.55±0.06
<i>S. kunthianum</i> (200 mg/kg)	97.80±4.96**	3.80±1.07	5.20±0.97	0.44±0.13	0.54±0.12
<i>S. kunthianum</i> (400 mg/kg)	149.60±5.50***	3.40±1.57	3.60±1.57	0.49±0.23	0.51±0.23
Morphine (10 mg/kg)	201.83±8.90***	1.50±0.67**	2.00±1.0**	0.34±0.19	0.35±0.20

Values are mean ± SEM of at least five experiments; ** $p < 0.001$, *** $p < 0.0001$ significantly different from distilled water treated animals; unpaired Student's *t*-test.

by morphine (10 mg/kg). Castor oil-induced diarrhoea is related to the release of prostaglandin by colonic cells [13,14]. Delay in castor oil-induced diarrhoea and inhibition of intestinal fluid secretion is known to characterize non-steroidal antiinflammatory drugs [12] which are inhibitors of prostaglandin synthesis. Therefore, the antidiarrhoeal action exerted by the fractions of *Stereospermum kunthianum* in the present study may partly be due to possible inhibition of prostaglandin biosynthesis or release.

CONCLUSION

Chromatography (VLC/CC) of the methanol extract of *S. kunthianum* produced three fractions L, S and Y which possessed antidiarrhoeal activity to varying degrees, possibly suggesting that the fractions may contain different chemical constituents. The findings of the present study corroborate our earlier claims on the antidiarrhoeal activity of the aqueous stem bark of *S. kunthianum* [5]. These results further support the ethnomedicinal use of the plant in the treatment of diarrhoea.

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