Analysis of ethanol extract of *Solanum anomalum* leaves for Antidiarrhoeal Activity

John A. Udobang[1*], Jude E. Okokon[2], Blessing D. Ukpong[2], Sifon J. Akpan[2]

[1]Department of Clinical Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, University of Uyo, Uyo, Nigeria.
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.

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*corresponding author: E-mail: johnudobang@uniuyo.edu.ng; +234-8025693590

ABSTRACT

Diarrhoea, characterized by increased frequency of stools (3 or more per 24 hrs), increased fluidity of stools and/or the presence of blood/mucus, affects people of any age, but is the third commonest disease in children in developing countries, and is responsible for about one third of all hospitalizations among children under five years. Research into drug treatment of diarrhoea, revealed that several species of *Solanum* are used in traditional medicine to treat diarrhoea. Therefore, this study aimed to investigate and possibly authenticate the antidiarrhoeal activity of ethanol extracts of *S. anomalum* leaves, as used in traditional medicine. The ethanol extract of *S. anomalum* leaves was evaluated to authenticate its antidiarrhoeal activity using rats. Ethanol extract at doses of 70, 140 and 210 mg/kg was checked for antidiarrhoeal effect using three models of small bowel transit time, castor oil induced diarrhoea, and castor oil induced accumulation of fluid. The leaf extract caused significant ($p<.001$) inhibition of small bowel transit time, castor oil induced diarrhoea, and castor oil induced fluid accumulation. In conclusion, the findings of this study show that the ethanol extract of *S. anomalum* leaves has antidiarrhoeal properties, therefore confirming its traditional use as medicine in the management of diarrhoeal disorders.

Keywords: Antidiarrhoeal, ethnomedicine, *S. anomalum*, woody plant.

INTRODUCTION

Diarrhoea is a major problem that occurs in all regions and populations, within low and middle income countries, affecting very young and old people more, and prevalent in sub-Saharan Africa and Asia. (Turyare *et al*, 2021) Globally in 2019, diarrhoea was the 8th leading cause of death among all ages, with yearly incidence of nearly 1.7 billion cases in children, resulting in death of about 525 000 children under five. (Abuzerr *et al*, 2019) *S. anomalum* is a woody plant that can reach a height of 2 metres. The various plant parts has prickles that can reach 5 mm in length. The fruits are edible, and the leaves are used as medicines. The plant can be cultivated for its fruits and is found in tropical West African countries such as Sierra Leone, Cameroon, DR Congo and Southern Nigeria. This

'childrens tomatoes' is commonly used as condiment in soups and sauces or eaten in its raw form, sometimes mixed with Parkia fruits as appetizer for those that are sick, and as laxative and digestive.(Burkill, 2000) Fruits and saps from the leaves are used orally or as enema once or twice daily to treat leprosy and gonorrhoea.(Burkill, 2000) The fruits are crushed and used to maturate inflammations on fingers or toes, (Burkill, 2000) and as juice to treat pain on ear sores.(Bukenya *et al*, 1988). There is report that the fruit possesses antidiabetic properties.(Offor *et al*, 2015). The in vivo and in vitro antiplasmodial,(Okokon *et al*, 2016, 2017a) anti-inflammatory,(Okokon 2017b) antioxidant and antiulcer,(Okokon *et al*, 2019a) anticonvulsant and antidepressant(Okokon *et al*, 2019b) and analgesic,((Okokon *et al*, 2020) activities of the leaf extract have been reported.

There are a lot of treatments available for diarrhoea, with varying potencies and side effects accompanying them. Diarrhoea remains one of the most prominent diseases treated with traditional medicines.(Maroyi, 2016). It therefore becomes expedient to search for cheaper, safer, and more effective new antidiarrhoeal medications to combat this menace. Due to paucity of information on the biological activity of the leaves, we are therefore reporting on the antidiarrhoeal activity of the plant's ethanol leaf extract.

MATERIALS AND METHODS

Plants collection

Plant leaves were taken from compounds in Uruan, Akwa Ibom State, South South Nigeria in April, 2021. Identification and authentication of the leaves was carried out by Prof. Margaret Bassey of the Department of Botany and Ecological Studies, University of Uyo. A voucher specimen was deposited in the Faculty of Pharmacy Herbarim of the University of Uyo, Uyo, Nigeria with voucher number UUH 324c

Extraction

The leaves were first washed to remove sand, then kept in a shade for two weeks for it to dry. The dried sample was pounded in a mortar until it became powder. The powder was macerated in 50 % ethanol. The liquid was filtered, and the filtrate dried in about 8-

12 hrs, and concentrated in vacuo with a vacuum pump strength of 0.15KW 1720min 60hz at 40 ˚C using a rotary evaporator. The concentrated extract was stored in a refrigerator until required.

Animals

Male and female albino Wistar rats (135-165 g) were gotten from the Animal House of the University of Uyo, and fed *ad libitum* on standard animal feeds and water. Approval was gotten from the College of Health Sciences Animal Ethics Committee in University of Uyo.

Castor oil induced diarrhoea

Diarrhoea in rats was induced by modifications of previously established methods. (Sunil *et al*, 2001, Nwafor, 2005) Thirty rats were denied feeds for 24 hrs but allowed to drink water as desired. The rats were randomly separated into five equal groups. Group 1(control) received 10 % Tween 80 (5ml/kg) through orogastric tube. Animals in groups 2, 3 and 4 received 70, 140 and 210 mg/kg, p.o. respectively of *S. anomalum* extract. Group 5 received intraperitoneal administration of 0.1 mg/kg of atropine. After 1 hr, every rat was given 2 mL of castor oil orally, then monitored for 3 hrs for fecal matter consistency and defecation frequency.

Small intestinal propulsive activity

The charcoal method (Nwafor, 2001) was used to analyse for the extract's activity on intestinal propulsive movement in unanesthetized rats. A different set of thirty rats were denied feeds for 24 hrs but allowed to drink water as desired. The rats were randomly separated into five equal groups. Group 1(control) received 10 % Tween 80 (5 ml/kg) through orogastric tube. Animals in groups 2, 3 and 4 received 70, 140 and 210 mg/kg, p.o. respectively of *S. anomalum* extract. Group 5 received intraperitoneal administration of 0.1 mg/kg of atropine. After 1 hr , every rat was given 1 mL of charcoal meal, consisting of 5 % activated charcoal that was suspended in 10 % aqueous tragacanth, orally. Thirty minutes later, the rats were sacrificed by cervical dislocation, bled, and the small bowel dissected and displayed on a clean flat surface. After careful

inspection of the small bowel, measurement of the distance moved by the charcoal meal from the pylorus, and the whole length of the small bowel from the pylorus to the ileocaecal junction was carried out. The distance moved by the charcoal meal was expressed as a percentage of the measured pylorus- ileocaecal junction length of the small bowel, as follows:

$$\frac{\text{Distance travelled by the charcoal(cm)}}{\text{Pylorus - ileocaecal junction length of the small bowel}} \times 100$$

Castor oil-induced fluid accumulation

The method of intestinal fluid accumulation (Dicarlo, 1994) was carried out with 36 rats deprived of food for 24 hrs, and allowed liberal access to water. The rats were randomly separated into six equal groups. Group 1(control) received 10 % Tween 80 (5 ml/kg) through orogastric tube. Group 2 got 2 mL/rat of castor oil, while rats in groups 3, 4 and 5 orally received 70, 140 and 210 mg/kg, respectively of the extract. Group 6 was given 3.0 mg/kg of loperamide orally. Exactly 1hr after, every rat was given 2 mL of castor oil orally. Thirty minutes later, the animals were all sacrificed by cervical dislocation and exsanguinated. The small bowels were all tied at the pyloric sphincter and ileocaecal junctions, the tied portion dissected out, and the volume of the contents measured and recorded.

RESULTS

Effect on castor oil induced diarrhoea

S. anomalum leaf extract (70, 140, 210 mg/kg) administration to rats resulted in dose dependent decreases in mean fecal output by the animal. Significant ($p<.05$) reductions at 210 mg/kg dose when compared to control group was seen (Table 1).

Effect on small intestinal propulsion in rats

Giving *S. anomalum* leaf extract (70, 140, 210 mg/kg) to rats led to a significant ($p<.05$) dose dependent inhibition of bowel propulsive activity from 0.22 to 17.91 % at the 210 mg/kg dose, in comparison with the control.(Table 2).

Castor oil-induced intestinal fluid accumulation

The extract (70, 140, 210 mg/kg) revealed a dose-dependent decrease effect on this modality. This reduction was significant

(p<.05) only at 210 mg/kg, relative to control, but did not compare to loperamide, a synthetic opiate analogue used as standard(Table 3).

Table 1: Effect on castor oil induced diarrhoea

Treatment	Dose (mg/kg)	Mean fecal matter	% Inhibition
10 % Tween 80	5 mL	6.66±0.33	-
S. anomalum	70	3.66± 0.33	45.04
	140	3.33±0.33[a]	50.0
	210	3.15±0.55[a]	52.70
Atropine	0.1	1.66±0.33	75.07

Data are expressed as mean ± SEM, level of significance is at [a]p< .05, [c]p<.001, in comparison to control, n = 6.

Table 2: Effect on small bowel propulsive activity.

Treatment	Dose (mg/kg)	Intestinal transit %	% Inhibition
10 % Tween 80	5 mL	52.34±1.20	
S. anomalum	70	81.42±4.79	0.22
	140	74.26±2.77	8.99
	210	66.98±1.76[a]	17.91
Castor oil	2 mL	81.60±3.46	-
Atropine	0.1	39.53±2.56[c]	51.55

Data are expressed as mean ± SEM, level of significance is at [a]p < .05, [c]p<.001, in comparison to control, n = 6.

Table 3: *S. anomalum* effect on bowel accumulation of fluid (intestinal enteropolation)

Treatment	Dose (mg/kg)	Mean bowel fluid volume (mL)	% Inhibition
10 % Tween 80	5 mL	5.36±0.29	-
S. anomalum	70	4.56±0.28	14.92
	140	4.46±0.68	16.79
	210	2.43±0.34[c]	54.66
Loperamide	10	3.16±0.61[a]	41.04

Data are expressed as mean ± SEM, level of significance is at [a]p < .05, [c]p<.001 in comparison to control, n = 6.

DISCUSSION

S. anomalum ethanol leaf extract displayed significant antidiarrhoeal effect in all three models. Castor oil is metabolised by intestinal lipases to a hydroxylated fatty acid called ricinoleic acid. Ricinoleic acid, the active metabolite induces diarrhoea by stimulating peristalsis in the small bowel, resulting in changes in electrolyte permeability in the small bowel mucosa. It also acts through prostaglandin EP_3 prostanoid receptors to cause release of endogenous prostaglandins.(Yoshio *et al*, 1999, Tunaru *et al*, 2012) Castor oil causes secretory and motility diarrhoea (Rouf, 2003) that can be delayed by prostaglandin synthesis inhibitors (Sunil *et al*, 2001) thereby suggesting that the extract may be causing its effect by inhibiting the synthesis of prostaglandin .

The effect of the extract on small intestine propulsive movement though significant, was not comparable to atropine, used as the standard drug. The extract could also be exerting its effect by antagonizing α_2-adrenoceptor activation or antimuscarinic activity. The effect of the extract was a significant ($p<.05$) inhibition of accumulation of fluid in the small bowel (enteropooling), that did not compare to loperamide, a synthetic opiate, which decreases the transit velocity and increases the capacity of the intestines to retain their fluids.(Vareinshang, 2004)

Report shows that medicinal plants with tannins, steroids alkaloids, flavonoids and saponins display antidiarrhoeal and antidysentric effects. (Havagiray, 2004) These constituents have been reportedly (Okokon, 2016) found in *S. anomalum* leaves and could account for the *in vivo* antidiarrhoeal effect found in this study.

CONCLUSION

This study shows that *S. anomalum* leaf extract has antidiarheal effects and its usage traditionally in treating and managing gastrointestinal disorders in traditional medicine could be justified.

DECLARATIONS

Authors' Contributions

JEO designed the work. JAU, BDU and SJU were responsible for carrying out the research. JEO was responsible for the statistical analysis and data evaluationJAU wrote up the work. All authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.

Consent For Publication

Authors have agreed to submit it in its current form for consideration for publication.

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