

Antidiarrheal Activity of Some Selected Nigerian Plants Used in Traditional Medicine

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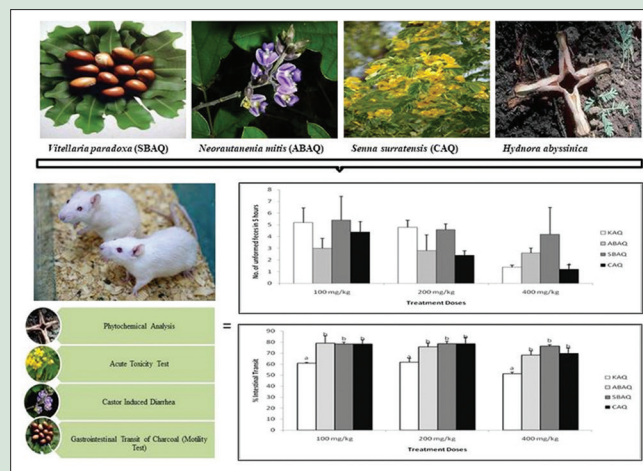
ABSTRACT

Context: Herbal preparations of the various parts of *Vitellaria paradoxa*, *Neorautanenia mitis*, *Senna surattensis*, and *Hydnora abyssinica* have been used in the Nigerian traditional medical practice to treat the symptoms of diarrhea in humans and animals. **Aims:** This study aims to validate claims of the traditional use of these plants in the treatment of diarrhea and provide a scientific basis for further studies. **Materials and Methods:** The median lethal dose (LD₅₀) values of the extracts were obtained using the Limit test. Castor oil induced diarrhea and intestinal transit (motility) models in albino rats were used to determine the antidiarrheal activity. Graded doses of the extracts were administered to 3 test groups, while the positive control groups were given standard drugs (atropine and loperamide) and the negative control groups received distilled water *per os*. **Results:** The LD₅₀ was considered higher than 2000 mg/Kg for all the extracts. In the castor oil-induced diarrhea model, the highest percentage inhibition of defecation was observed in the test groups treated with the extracts of *H. abyssinica* (82%) followed by *S. surattensis* (81%), *N. mitis* (66%), and *V. paradoxa* (32%). *H. abyssinica* extract significantly decreased the intestinal transit of charcoal meal compared to the other extracts. **Conclusion:** The antidiarrheal activities of all the extracts give credence to their traditional use. *H. abyssinica* comparatively had the best antidiarrheal activity and has the potential as an antidiarrheal agent. Thus, the need for further studies of this extract to investigate active fractions, isolate and characterize active compounds, and determine their activities and safety.

Key words: Antidiarrheal activity, *in vivo*, Nigerian plants, phytochemicals, traditional medicine

SUMMARY

- The study validates the *in vivo* safety and antidiarrheal properties of aqueous crude extracts of *Hydnora abyssinica*, *Neorautanenia mitis*, *Vitellaria paradoxa*, and *Senna surattensis* used in Nigeria traditional medicine. Results indicate that the extract of *H. abyssinica* is comparatively a more potent antidiarrheal agent.



Abbreviations Used: CAQ extract: Crude aqueous extract of *Senna surattensis*; ABAQ extract: Crude aqueous extract of *Neorautanenia mitis*; SBAQ extract: Aqueous crude extracts of *Vitellaria paradoxa*; KAQ extract: Aqueous crude extract of *Hydnora abyssinica*; UJ: University of Jos, Nigeria; LD₅₀: Median lethal dose; AUCC: Animal Use and Care Committee.

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INTRODUCTION

Plants have served as the major source of medicines to humans and animals from ancient times and are still important today.^[1] Centuries ago, medical practice depended largely on medicines from plants to proffer cure to many illnesses; however, a decline in the use of plant-based medicines was experienced especially in the Western countries owing to the development of more specific orthodox/synthetic drugs. In contrast, developing countries of the World continue to access this rich knowledge of herbal practice for their healthcare needs. This however is not without its associated challenges, such as unreliable evidence of safety, standardization, efficacy, and the ever-changing production practices.^[2] In spite of these challenges, the use of herbal medicine still thrives in many industrialized and nonindustrialized countries of the world. For example, the Ayurvedic medicine of India, Kampo medicine used in Japan, traditional Chinese medicine, and the Unani medicine in the Middle East and South Asia are practiced by a large population of

people.^[3,4] In Africa, about 80% of the people in rural communities rely on traditional medicine for their primary healthcare needs and that of their animals. Approximately, 85% of traditional medicine involves the use of plant extracts.^[5,6]

Diarrhea is a global health problem, with many causes ranging from viral, bacterial, parasitic, fungal, and other non-infectious causes such

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as indigestion and malnutrition.^[7] Diarrhea is a common symptom in many diseases, having a high mortality rate in children under the age of 5 years and causing about 4–5 million deaths annually.^[8] Scientific reports suggest that diarrhea accounts for 1 in 9 child deaths globally, making diarrhea one of the leading cause of deaths in children.^[8,9] This condition is very important in most rural communities, including nations like Nigeria. It has been reported that this condition kills 2195 children daily, more than AIDS malaria and measles combined.^[10]

The above statistical information is quite alarming and worrisome. Hence, the need for continuous research into finding new alternative drugs that will effectively curb the menace of diarrhea. Our study therefore seeks to evaluate medicinal plants with claims of antidiarrheal activity from folklore and explore their potential scientifically in the search for newer, effective and more specific treatments for diarrhea. In addition, the World Health Organization have endorsed the use of herbal formulations in managing diarrhea.^[11]

In this paper we report our study of four medicinal plants; *Vitellaria paradoxa*, *Neorautanenien mitis*, *Senna surattensis*, and *Hydnora abyssinica*. These are well known among traditional medical practitioners and rural livestock farmers in Plateau State Nigeria for their efficacy in the treatment of many diseases, including diarrhea (personal communication). *V. paradoxa* is also reported as useful in the treatment of symptoms of diarrhea among livestock farmers.^[12,13]

Neorautanenien mitis (A. Rich) Verdc. belongs to the Fabaceae family, with other species including *N. Edulis*, *N. pseudopachyrrhiza*, *N. amboensis*, and *N. ficifolia*. It is mostly found in Central, South, and West Africa.^[14] In Nigeria, the plant is found in the middle belt or central regions. Local names include Abargora (Hausa, Nigeria), Karamin karara (Hausa, Nigeria), Igitembatenibe or Amakubwe (Rwanda).^[14,15] It is traditionally used as a fish poison, insecticide, and anticonvulsant and is used to treat conditions such as syphilis, female frigidity, skin infection, dysmenorrhea, neuropsychiatric disorders, and other painful conditions.^[16,17] Empirical studies conducted on this plant include acaricidal and insecticidal activities, cytotoxicity, antimicrobial and antinociceptive activities. Mosquitocidal activities against larvae of *Anopheles gambiae* and *Culex quinquefasciatus* have also been documented.^[17,18] The preliminary phytochemical studies on the crude extract revealed the presence of flavonoid, saponin, glycoside, tannins, and alkaloids.^[17] However, within the limits of our literature review, no antidiarrheal studies are reported.

Vitellaria paradoxa C.F. Gaertn is from the family Sapotaceae. This plant is indigenous to Africa, growing naturally in the wild in the open and parkland savannahs as well as the Southern Sahel. It is a small- to medium-sized branched deciduous tree which bears fruit when it is 10–15 years of age. It is commonly found in Nigeria, Togo, Mali, Cameroun, Cote d'Ivoire, Ghana, Guinea, Uganda, Senegal, Burkina Faso, and Sudan. The popular tree has the following common names: Shea butter tree (English), Kareje (Fulfulde, Nigeria), Kadanya (Hausa, Nigeria), Okwuma (Igbo, Nigeria), Ikini (Taroh, Nigeria), and Mmameng (Cham, Nigeria).^[19] The fruits of *V. paradoxa* are eaten for their nutritional and medicinal values. The oil, extracted from the seed, known as shea butter is used as a skin and hair moisturizer and for the treatment of other skin conditions such as acne, chapped lips, dry skin, burns, scars, stretch marks, wrinkles, arthritis, and rheumatism.^[19] Shea butter is also used in the formulations of cosmetics, pharmaceuticals, chocolates, and biodiesel.^[20] The roots, seeds, fruits, and stem bark are used for the treatment of enteric infections such as diarrhea, dysentery, helminthiasis and other gastrointestinal tract infections, skin disease, and wound infection.^[21] Pharmacological evaluation carried out on this plant include antimicrobial,^[22] antidiarrheal,^[23] anti-inflammatory, and anti-arthritis activities.^[24] In a qualitative phytochemical analysis of the

ethanolic extract of the stem bark, carbohydrates, alkaloids, saponins, tannins, and cardiac glycosides were present.^[19]

Senna surattensis (Burm. f.) H. Irwin and Barneby belongs to the Fabaceae family. It is a small tree with about 18 cm leaves and 6–10 pairs of leaflets, and its origins are obscure. It was speculated that *S. surattensis* was introduced into Nigeria and then escaped into the wild. The plant is sighted by roadsides, in pastures and wastelands. Some common names include Golden or Bush Senna (English) and Dorowan bature (Hausa, Nigeria). It is used for ornamental purposes but the leaves are eaten in some parts of the world and it is an important ingredient in traditional and folklore medicine. The plant possesses anticancer, anti-inflammatory, antioxidant, and antimicrobial activities. A phytochemical analysis revealed the presence of alkaloids, anthraquinones, flavonoids, carbohydrates, proteins, steroids, terpenoids, cardiac glycosides, and phlobatanins.^[25] Within the limit of our search, we find no antidiarrheal studies conducted on this plant.

Hydnora abyssinica A. Braun is from the Hydnoraceae family. It is a parasitic plant mostly found attached to the roots of Acacia trees.^[26,27] It lacks chlorophyll, and the body parts consist of only roots and flowers. It is widely spread over many African countries including Somalia, Sudan, Angola, Namibia, Ethiopia, Kenya, and Nigeria. In Sudan, it is used in the treatment of bacterial infections, dysentery, cholera, and tonsillitis, while in Kenya, it is used for throat ache, stomach ache, dysentery, and retained placenta in animals. In Nigeria, it is used in the treatment of diarrhea (personal communication). The antibacterial activity and cytotoxicity of the chemical constituents of these plants were studied by Yagi *et al.*^[27] The phytochemical analysis of the root extracts revealed the presence of alkaloids, tannins, phenols, steroids, and flavonoids.^[28]

The herbal preparations of these four plants are normally done by drying and grinding the medicinal plant parts into a powder. The plant powder is soaked in cold water (maceration) for a period and thereafter decanted and the soluble portion is administered to the patient to treat the symptoms of diarrhea (personal communication). Therefore, we carried out our experiments using the aqueous extracts from these plants prepared as described above to validate their potency as antidiarrheal medications. We have not found any scientific reports of the antidiarrheal activity of the aqueous extracts of these four plants, and for the first time, we report herein a comparison of the antidiarrheal activities of these plants using the castor oil-induced diarrhea and motility test models in albino rats. Furthermore, these models in albino rats allow for easy experimental replication of diarrhea, which affects both humans and animals alike.

MATERIALS AND METHODS

Collection and identification of plants

The selected plants were collected from Kabwir in Kanke Local Government Area of Plateau State, Nigeria in July 2016. Mr. Otuwose Agyeno of the Department of Plant Science and Technology University of Jos, Nigeria identified the plants. Plant samples were deposited in the herbarium, and voucher/reference numbers were allocated as follows: *V. paradoxa* (UJ16000245), *N. mitis* (UJ16000246), *S. surattensis* (UJ16000248), and *H. abyssinica* (UJ16000248).

Experimental animals

Young adults (12 weeks old) Wistar albino rats of both sexes were obtained from the animal house of the National Institute for Trypanosomiasis Research Vom, Nigeria. The rats were kept in metal cages lined with wood shavings and were allowed to adjust to the laboratory environment for a period of 2 weeks before the commencement of the experiments. The rats were housed in spacious cages (6 rats per cage size 17" × 13") before the experimental groupings. They were fed on pelletized rodent feed from Dagom Farms of the National Veterinary Research Institute (NVRI) Vom and water was provided *ad libitum*.

Ethics approval and consent to participate

Ethics approval for animal use was obtained from the Animal Use and Care Committee of the NVRI (Approval number–NVRI/EC/20/010). No consent approval was required for this study.

Extraction

Hydnora abyssinica

The roots were cut into smaller pieces and dried in a hot air oven at 45°C for 5 days then ground into powder. To 571 g of the pulverized roots was added 3 L of distilled water and kept in the refrigerator and allowed to extract for a period of 24 h, after which it was filtered and the filtrate dried in a hot air oven at 50°C for 2 days to obtain the dried aqueous extract as brick red solid weighing 64.0 g with a percentage yield of 11.2%.^[29,30] The dried aqueous extract of *H. abyssinica* was labeled KAQ, coined from the common Hausa name, Kaushe.

Neorautanenia mitis

Tubers of *N. mitis* were cut into smaller pieces and sun dried for 3 days and further dried in a hot air oven at 45°C for 1 day. The dried material was then pulverized, and 1000 g of the powder was extracted with 4 L of distilled water and kept in the refrigerator for 24 h, after which it was filtered and the filtrate dried in a hot air oven at 50°C for 2 days to obtain the dried aqueous extract as dark brown solid weighing 65.2 g with a percentage yield of 6.5%.^[29,30] The dried *N. mitis* aqueous extract was labeled ABAQ, coined from the Hausa local name Abargora.

Vitellaria paradoxa

The stem bark was cut into smaller pieces, dried in a hot air oven at 45°C for 4 days and then pulverized. To a portion of 1000 g of powdered plant material, 4 L of distilled water was added and the mixture was kept in the refrigerator for 24 h, after which it was filtered and the filtrate dried in a hot air oven at 50°C for 3 days to obtain the dried aqueous extract as light brown solid weighing 35.0 g with a percentage yield of 3.5%.^[29,30] The dried aqueous extract was labeled SBAQ, coined from the English common name Shea butter.

Senna surattensis

The leaves of the plant were dried in a hot air oven at 45°C for 2 days, after which they were pulverized. To 285 g of the plant powder was added 2 L of distilled water and kept in the refrigerator and allowed to extract for a period of 24 h, after which it was filtered and the filtrate dried in the hot air oven at 50°C for 3 days to obtain the dried aqueous extract as dark green solid weighing 17.0 g with a percentage yield of 6.0%.^[29,30] The dried aqueous extract of *S. surattensis* was labeled CAQ, coined from the name Cassia synonym for the genus Senna.

Preliminary phytochemical analysis

All of the aqueous extracts were subjected to a qualitative phytochemical analysis to indicate the presence or absence of specific classes of secondary metabolites, using standard methods as described by Sasidharan *et al.*^[31]

Acute toxicity studies (limit test)

The acute toxicity studies were carried out using the limit test in Wister albino rats, to determine the median lethal dose (LD₅₀) of the aqueous extracts, using a standard protocol described by the Organization for Economic Co-operation and Development (OECD).^[32] Briefly, three albino rats kept in separate cages were used for the testing of each extract. The first rat was given a limited dose of 2000 mg/kg of the extract orally and observed for signs of toxicity and ultimate death. If the first rat survived, the procedure was repeated with the second rat, and then the third rat if the second rat survived.

Determination of antidiarrheal activity

The four dried aqueous extracts were used for the antidiarrheal experiments, which was carried out at the Drug Development Section in the Department of Biochemistry, NVRI Vom, Nigeria.

Castor oil-induced diarrhea

For each of the aqueous extracts, 25 rats of both sexes weighing between 130 and 200 g were used for the experiments, which were conducted during the daytime. The rats were fasted for 12 h before the commencement of the experiment, having access to water only. The rats were randomly allocated into five groups of five rats each. Groups 1, 2, and 3 were given graded doses (100 mg/kg, 200 mg/kg, and 400 mg/kg) of the dried extract reconstituted in distilled water. The graded doses for the extracts were considered based on the result obtained from the toxicity studies; the doses were within safe limits for experimental purpose. Group 4 were given distilled water, while Group 5 was given loperamide. Groups 4 and 5 are the control groups. The rats were then housed singly in a perforated cage lined with white blotting paper. One h after the above treatment, all the rats in the groups were given 1 mL of castor oil orally. The rats were observed for 5 h for watery (wet) or unformed feces. The watery feces from each rat were counted hourly for up to 5 h. At the end of the experiment, the group mean feces was obtained, and the percentage of protection was calculated using the formula:^[29,33]

$$\frac{\text{Mean of unformed faeces of water control} - \text{mean of unformed faeces of treatment}}{\text{Mean of unformed faeces of water control}} \times 100$$

Gastrointestinal transit of charcoal (motility test)

For each of the aqueous extracts, 25 rats of both sexes weighing between 130 and 200 g were used for the experiments, which were conducted during the day time. The animals were fasted for 16 h before the commencement of the experiment but allowed access to water. They were randomly divided into five groups of five rats each. Groups 1, 2, and 3 were treated orally with graded doses (100 mg/kg, 200 mg/kg, and 400 mg/kg) of the dried aqueous extracts reconstituted in distilled water. The graded doses for the extracts were considered based on the result obtained from the toxicity studies; the doses were within safe limits for experimental purpose. Group 4 were treated with distilled water, while Group 5 was treated with atropine sulfate. Groups 4 and 5 are the control groups. 30 min after extract and drug administrations, 1 mL of 5% activated charcoal suspension in 10% aqueous solution of acacia gum powder was given orally to each rat. After 30 min of administering the activated charcoal, the rats were humanely sacrificed and the abdomen was opened to access the intestine. The distance travelled by the charcoal meal from pylorus was measured and expressed as a percentage of the total length of intestine from pylorus to the cecum to calculate the percentage intestinal transit of activated charcoal.^[34]

$$\text{Percentage of intestinal transit} = \frac{\text{Meal travel from pylorus to cecum}}{\text{Total length of small intestine from pylorus to cecum}} \times 100$$

Statistical analysis

The data were presented as mean \pm standard error of mean ($n = 5$). Difference between means of different treatments was determined by analysis of variance using the SPSS version 23 IBM® SPSS® (NY, USA). $P < 0.05$ was considered as statistically significant.

RESULTS

The physical appearance and percentage yields obtained from the extraction procedure of all the four plants are summarized in Table 1.

Preliminary phytochemical screening

Qualitative phytochemical analysis showed the aqueous extracts of *H. abyssinica* (KAQ), *N. mitis* (AB AQ), *V. paradoxa* (SBAQ), and *S. surattensis* (CAQ) contained tannins, cardiac glycosides, steroids, and alkaloids. In addition, flavonoids were detected in all these extracts except for KAQ. Saponins were detected in KAQ, ABAQ, and CAQ but not detected in SBAQ. Anthraquinones were not detected in any of the four extracts [Table 2].

Median lethal dose (LD₅₀)

No mortality was recorded in the rats treated with the limit dose of 2000 mg/kg of all the plants extracts [Table 3]. This indicates that none of the Plant has LD₅₀ ≤ 2000 mg/kg.

Gastrointestinal transit of activated charcoal in rats treated with the aqueous crude extracts

Aqueous crude extract of *Hydnora abyssinica*

There was significant decrease in the intestinal transit of activated charcoal administered to rats treated with all doses of KAQ extract when compared to rats treated with distilled water [Table 4]. The rats treated with the highest dose of the extract decreased the transit to an extent comparable with the standard drug (atropine sulfate 5 mg/kg). This is an indication that the plant may have antidiarrheal activity.

Crude aqueous extract of *Neorautanenia mitis*

Administration of ABAQ extract did not affect the gastrointestinal transit of activated charcoal administered to rats treated with the extract when compared with the control rats treated with distilled water [Table 4]. A significant ($P < 0.05$) decrease was observed in rats treated with the standard drug (atropine sulfate 5 mg/kg) when compared to the control rats.

Aqueous crude extracts of *Vitellaria paradoxa*

Treatment with SBAQ did not result in significant decrease in the distance travelled by charcoal meal in the gastrointestinal tract of rats when compared to the control [Table 4]. The standard drug on the other hand significantly ($P < 0.05$) decreased the distance travelled by the charcoal meal.

Crude aqueous extract of *Senna surattensis*

The gastrointestinal transit of charcoal in rats treated with CAQ extract was not significantly different from the control rats. However, treatment with atropine significantly decreased intestinal transit of activated charcoal [Table 4].

Comparison of the effects of the aqueous crude extracts on intestinal transit of activated charcoal in rats

Comparison of the four extracts showed that dose-for-dose, KAQ extract significantly decreased the intestinal transit of activated charcoal when compared to the other extracts [Figure 1]. This is an indication that the KAQ extract was more effective in inhibiting gastrointestinal tract motility. Hence, this may have greater potential for antidiarrheal activity.

Inhibition of defecation after administration of the aqueous crude extracts in albino rats

Aqueous crude extract of *Hydnora abyssinica*

In the castor oil-induced diarrhea model, the KAQ extract significantly decreased fecal output in rats treated with the extract when compared with the control rats treated with distilled water [Table 5]. The inhibition of defecation in rats treated with 400 mg/kg (82%) was comparable to that of rats treated with the standard drug (loperamide 10 mg/kg). This is a further indication of the antidiarrheal potential of the KAQ extract.

Crude aqueous extract of *Neorautanenia mitis*

The fecal output of rats treated with the ABAQ extract was significantly lower than that of rats treated with distilled water. The percentage inhibition was marginally dose dependent with 100 mg/kg, 200 mg/kg, and 400 mg/kg showing 61%, 64%, and 66% inhibition of fecal output, respectively [Table 5].

Aqueous crude extracts of *Vitellaria paradoxa*

The mean defecation of rats treated with the SBAQ extract was significantly lower than that of rats treated with distilled water. The highest dose administered (400 mg/kg) inhibited fecal output by 32% while the lowest dose (100 mg/kg) inhibited fecal output by 13% [Table 5].

Crude aqueous extract of *Senna surattensis*

The fecal output of rats treated with various doses of the CAQ extract was significantly lower than that of rats treated with distilled water. The highest percentage inhibition of 81% was observed in rats treated

Table 1: Physical appearance and percentage yield of extracts from *Hydnora abyssinica*, *Neorautanenia mitis*, *Vitellaria paradoxa*, and *Senna surattensis*

Extract	Appearance	Amount of dry plant material (g)	Amount of extract (g)	Percentage yield (%)
KAQ	Brick red solid	571	64.0	11.2
AB AQ	Dark brown solid	1000	65.2	6.5
SBAQ	Light brown solid	1000	35.0	3.5
CAQ	Dark green solid	285	17.0	6.0

KAQ: Aqueous crude extract of *Hydnora abyssinica*; ABAQ: Crude aqueous extract of *Neorautanenia mitis*; SBAQ: Aqueous crude extracts of *Vitellaria paradoxa*; CAQ: Crude aqueous extract of *Senna surattensis*

Table 2: Phytochemical composition of *Hydnora abyssinica*, *Neorautanenia mitis*, *Vitellaria paradoxa*, and *Senna surattensis* aqueous extracts

Extract	Phytochemical composition						
	Tannins	Cardiac glycosides	Steroids	Alkaloid	Flavonoid	Anthraquinones	Saponins
KAQ	+	+	+	+	–	–	+
AB AQ	+	+	+	+	+	–	+
SBAQ	+	+	+	+	+	–	–
CAQ	+	+	+	+	+	–	+

+: Detected; –: Not detected; KAQ: Aqueous crude extract of *Hydnora abyssinica*; ABAQ: Crude aqueous extract of *Neorautanenia mitis*; SBAQ: Aqueous crude extracts of *Vitellaria paradoxa*; CAQ: Crude aqueous extract of *Senna surattensis*

with 400 mg/kg of the extract [Table 5]. This shows that the extract has antidiarrheal potential.

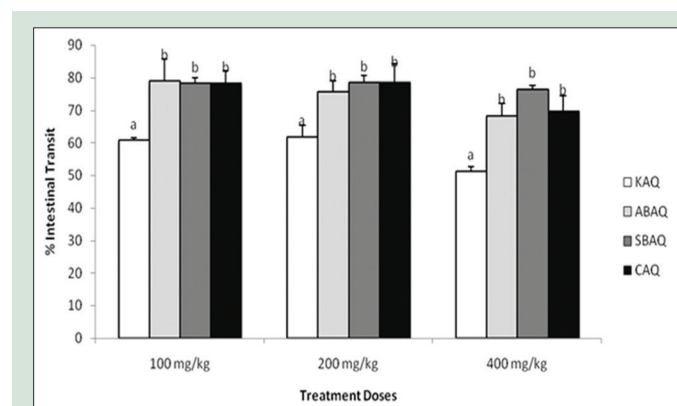


Figure 1: Comparison of intestinal transit of charcoal meal in rats treated with the different aqueous extracts. KAQ: *Hydnora abyssinica*; ABAQ: *Neorautanenia mitis*; SBAQ: *Vitellaria paradoxa*; CAQ: *Senna surattensis*. Bars with different letter superscripts in the same cluster are significantly different

Table 3: Median lethal dose of *Hydnora abyssinica*, *Neorautanenia mitis*, *Senna surattensis*, and *Vitellaria paradoxa* aqueous extracts

Extract	Number of rats	Dose (mg/kg)	Mortality	LD ₅₀ (mg/kg)
KAQ	3	2000	0/3	>2000
ABAQ	3	2000	0/3	>2000
SBAQ	3	2000	0/3	>2000
CAQ	3	2000	0/3	>2000

KAQ: Aqueous crude extract of *Hydnora abyssinica*; ABAQ: Crude aqueous extract of *Neorautanenia mitis*; SBAQ: Aqueous crude extracts of *Vitellaria paradoxa*; CAQ: Crude aqueous extract of *Senna surattensis*

Table 4: Percentage intestinal transit of charcoal meal in rats treated with *Hydnora abyssinica* extract, *Neorautanenia mitis* extract, *Vitellaria paradoxa* extract, and *Senna surattensis* extract

Treatment	Total length of intestine (cm)	Distance travelled by charcoal meal (cm)	Percentage intestinal transit
KAQ extract			
Water	103.28±3.87	75.30±1.30	73.36±3.26
100 mg/kg	114.56±4.07	69.66±3.05	60.75±0.76*
200 mg/kg	102.88±4.24	63.38±4.00	61.87±3.58*
400 mg/kg	117.44±5.60	52.48±8.27	44.80±6.58*
Atropine 5 mg/kg	123.68±3.00	50.26±4.14	44.79±4.24*
ABAQ extract			
Water	103.28±3.87	75.30±1.30	73.36±3.26
100 mg/kg	102.28±2.96	80.80±6.27	78.90±6.71
200 mg/kg	110.00±2.59	86.84±3.82	78.44±2.28
400 mg/kg	108.40±3.03	82.80±2.44	68.19±3.92
Atropine 5 mg/kg	123.68±3.00	50.26±4.14	40.43±2.32*
SBAQ extract			
Water	104.20±4.12	72.20±6.55	74.77±4.77
100 mg/kg	101.60±4.98	76.60±5.03	78.16±1.90
200 mg/kg	110.00±2.59	86.40±3.83	78.44±2.28
400 mg/kg	108.40±3.03	82.80±2.44	76.41±1.25
Atropine 5 mg/kg	108.60±2.01	57.16±4.42	40.43±2.32*
CAQ extract			
Water	104.20±4.12	72.20±6.55	74.77±4.77
100 mg/kg	93.20±2.78	72.20±6.55	78.17±3.91
200 mg/kg	99.80±4.04	77.40±3.36	78.40±5.78
400 mg/kg	96.80±2.82	67.00±2.88	69.71±4.75
Atropine 5 mg/kg	108.60±2.0	57.16±4.42	40.43±2.32*

Values are expressed as mean±SEM (n=5). *P<0.05 significantly different from distilled water treated group. SEM: Standard error of mean; KAQ: Aqueous crude extract of *Hydnora abyssinica*; ABAQ: Crude aqueous extract of *Neorautanenia mitis*; SBAQ: Aqueous crude extracts of *Vitellaria paradoxa*; CAQ: Crude aqueous extract of *Senna surattensis*

Comparison of inhibition of defecation in rats treated with the aqueous crude extracts

A dose for dose comparison of the extracts showed no significant difference in the output of unformed feces of the extract-treated rats. The only exception was the significant reduction observed in defecation of rats treated with CAQ extract (400 mg/kg) when compared with SBAQ extract (400 mg/kg) [Figure 2].

When the percentage inhibition of defecation of the extracts is compared, the highest inhibition of 82% was observed in rats treated with 400 mg/kg of KAQ followed by rats treated with 400 mg/kg of CAQ (81%). The lowest inhibition at a dose of 400 mg/kg (32%) was observed in rats treated with SBAQ. At the dose of 100 mg/kg, ABAQ exhibited the highest inhibition (61%). However, on administration of higher doses (200 mg/kg and 400 mg/kg), the increase in inhibition was marginal (64% and 66%, respectively). On the other hand, inhibition of defecation in rats treated with KAQ and CAQ increased substantially with increase in dose from 200 mg/kg to 400 mg/kg [Table 6].

DISCUSSION

Phytochemicals are responsible for the biological activities of medicinal plants, and their presence in any plant extract is an indication of activity. The tannins, cardiac glycosides, steroids, alkaloids, and saponins present in *H. abyssinica* (KAQ) agrees with the findings of Wintola and Afolayan.^[28] In addition, Saadabi and Ayoub^[35] reported the presence of these phytochemical types with the exception of cardiac glycosides and saponins. The phytochemicals detected in *V. paradoxa* (SBAQ) were similar to those reported by El-Mahmood *et al.*^[22] when various organic solvents were used for extraction. Kabila *et al.*^[36] in their review on *Cassia* (synonym for *Senna*) species reported the presence of alkaloids, flavonoids, saponins, and tannins in either the leaf, leaf/flower, and/or aerial parts of the plant *Cassia surattensis* using various organic extraction solvents (methanol, ethanol, ethyl acetate, hexane, and chloroform).

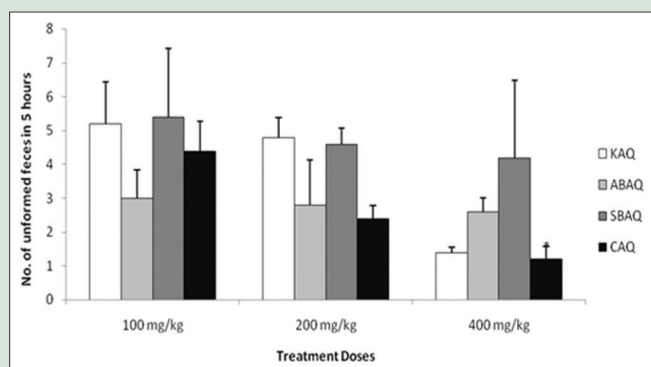


Figure 2: Comparison of inhibition of defecation in rats treated with the different aqueous extracts. KAQ: *Hydnora abyssinica*; ABAQ: *Neorautanenia mitis*; SBAQ: *Vitellaria paradoxa*; CAQ: *Senna surattensis*. *Significantly different from SBAQ in the same cluster

There are no reports of these phytochemicals within the aqueous extract. Alkaloids, tannins, flavonoids, and phenolic phytochemical class types present in these current four plants extracts have been shown to have antidiarrheal activities.^[33]

The median lethal dose (LD_{50}) of more than 2000 mg/kg was observed in all the extracts, and the absence of mortality in this study is an indication that the plants are relatively safe. According to OECD, a substance with an LD_{50} >2000 mg/kg is considered relatively safe for experimental purposes.^[32]

In the gastrointestinal transit of activated charcoal model, the significant ($P < 0.05$) decrease at all doses of the aqueous extract of *H. abyssinica* when compared to those administered distilled water is an indication of a possible antidiarrheal activity. Diarrhea is defined as the increase in frequency and decrease in consistency of feces. Any substance that can decrease the intestinal motility as seen with the extract of *H. abyssinica* will potentially decrease the rate of stooling. The decrease in motility while increasing the gastric emptying time will cause greater absorption of fluid from the fecal content thereby increasing the consistency of the feces. The decrease was observed to be dose dependent.

When the extracts of the four plants were compared on a dose-to-dose basis, the significant reduction in intestinal motility of rats treated with the extract of *H. abyssinica*, as compared with the extracts from *N. mitis*, *V. paradoxa*, and *S. surattensis*, is an indication of its greater ability to inhibit intestinal motility. This is the first study that compared the effect of these extracts on intestinal motility.

In the castor oil-induced diarrhea study, the dose-dependent inhibition of defecation observed with the *H. abyssinica* extract is a further indication of the antidiarrheal potential of this plant. The significant ($P < 0.05$) dose-dependent reduction in the number of feces excreted by rats treated with extracts of *N. mitis*, *V. paradoxa*, and *S. surattensis* when compared to the control rats treated with distilled water showed that the extracts possess antidiarrheal activity. The antidiarrheal activity of these extracts is mediated in part by a mechanism other than the inhibition of gastrointestinal tract (GIT) motility since the extracts did not inhibit intestinal motility in the transit of activated charcoal meal test.

The inability of the extracts to significantly decrease intestinal motility and ability to significantly inhibit defecation is similar to that observed in albino rats treated with aqueous and ethanol stem bark extracts of *Khaya senegalensis*.^[33] The extract of black tea (*Camellia sinensis*), which is used in the management of diarrhea, has also been shown to increase upper gastrointestinal tract motility but decreased intestinal fluid accumulation thereby alleviating diarrhea.^[37] Diarrhea can be caused by increased

Table 5: Percentage inhibition of defecation of rats treated with *Hydnora abyssinica* extract, *Neorautanenia mitis* extract, *Vitellaria paradoxa* extract, and *Senna surattensis* extract

Treatment	Mean defecation in 5 h	Percentage inhibition of defecation
KAQ extract		
Castor oil + water	7.8±1.32	-
Castor oil + 100 mg/kg	5.2±1.24	28
Castor oil + 200 mg/kg	4.8±0.58*	38
Castor oil + 400 mg/kg	1.4±0.15*	82
Castor oil + water	0.0±0.00*	100
ABAQ extract		
Castor oil + water	7.8±1.32	-
Castor oil + 100 mg/kg	3.0±0.84*	61
Castor oil + 200 mg/kg	2.8±1.32*	64
Castor oil + 400 mg/kg	2.6±0.40*	66
Castor oil + loperamide 10 mg/kg	0.0±0.00*	100
SBAQ extract		
Castor oil + water	6.2±0.68	-
Castor oil + 100 mg/kg	5.4±2.02	13
Castor oil + 200 mg/kg	4.6±0.46*	26
Castor oil + 400 mg/kg	4.2±2.28*	32
Castor oil + loperamide 10 mg/kg	0.0±0.00*	100
CAQ extract		
Castor oil + water	6.2±0.68	-
Castor oil + 100 mg/kg	4.4±0.86*	29
Castor oil + 200 mg/kg	2.4±0.38*	61
Castor oil + 400 mg/kg	1.2±0.38*	81
Castor oil + loperamide 10 mg/kg	0.0±0.00*	100

Values are expressed as mean±SEM ($n=5$). * $P<0.05$ significantly different from distilled water treated group. SEM: Standard error of mean, KAQ: Aqueous crude extract of *Hydnora abyssinica*; ABAQ: Crude aqueous extract of *Neorautanenia mitis*; SBAQ: Aqueous crude extracts of *Vitellaria paradoxa*; CAQ: Crude aqueous extract of *Senna surattensis*

Table 6: Comparison of inhibition of defecation in rats treated with *Hydnora abyssinica*, *Neorautanenia mitis*, *Vitellaria paradoxa*, and *Senna surattensis* extracts

Treatment (mg/kg)	Percentage inhibition of defecation in rats treated with the extracts			
	KAQ	ABAQ	SBAQ	CAQ
100	28	61	13	29
200	38	64	26	61
400	82	66	32	81

KAQ: Aqueous crude extract of *Hydnora abyssinica*; ABAQ: Crude aqueous extract of *Neorautanenia mitis*; SBAQ: Aqueous crude extracts of *Vitellaria paradoxa*; CAQ: Crude aqueous extract of *Senna surattensis*

intestinal motility or increased fluid accumulation in the gastrointestinal tract. In the castor oil-induced diarrhea, castor oil when administered orally is broken down to ricinoleic acid. The acid is a gastrointestinal tract irritant that causes inflammation and prostaglandin release.^[38] It also alters the permeability of the intestinal mucosa to electrolytes and water hence causing diarrhea.^[39]

All of the extracts contained tannins, which may be responsible for the protection against castor oil-induced diarrhea. Tannins are thought to decrease the permeability of the intestinal mucosa to water and electrolytes because of their astringent property.^[38]

CONCLUSION

A preliminary qualitative phytochemical analysis of the aqueous extracts from *H. abyssinica*, *N. mitis*, *V. paradoxa*, and *S. surattensis* revealed the presence of secondary metabolite classes that have been shown to be

useful in the management of diarrhea. *H. abyssinica* extract appeared to be the most active in the reduction of both intestinal motility and castor oil-induced diarrhea. *N. mitis*, *V. paradoxa*, and *S. surattensis* extracts on the other hand only inhibited defecation in the castor oil-induced diarrhea model. Thus, the studies give credence to the traditional use of these plants to treat the symptoms of diarrhea in humans and livestock. Further studies are necessary to identify the specific phytochemicals in these extracts and to determine their antidiarrheal activities and safety.

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Conflicts of interest

There are no conflicts of interest.

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