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Research Article
Anti-spasmolytic and Anti-diarrheal Activities of *Newbouldia laevis*, *Cola nitida* and *Acanthus montanus* Extracts on Gastrointestinal Muscles

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Abstract

Background and Objective: In developing countries, diarrhea is the leading cause of death in children. Several medicinal plants have been implicated in treatment of diarrhea. The present study was carried out to evaluate the anti-spasmolytic and anti-diarrhea properties of ethnomedicinal plants *Newbouldia laevis*, *Cola nitida* and *Acanthus montanus.* **Materials and Methods:** Forty male rats were divided into 8 groups of 5 rats each. About 1 mL castor oil was administered to the animals in each group to induce diarrhea. An hour later group 1 was administered 2 mL normal saline orally. Group 2 received 1 mg kg^{-1} Atropine (i.p). Group 3 and 4 received 400 and 800 mg kg^{-1} b.wt., of *A. montanus* leaf extract. Group 5 and 6 received 400 and 800 mg kg^{-1} of *C. nitida* leaf extract while group 7 and 8 received 400 and 800 mg kg^{-1} of *N. laevis* leaf extract. Thirty minutes later, 10 mL kg^{-1} of activated charcoal meal was administered orally. Animals were sacrificed by suffocation in chloroform chamber after 30 min. Data analysis was carried out with SPSS using one-way analysis of variance (ANOVA). **Results:** The results showed a significant ($p<0.05$) relaxation of rabbit jejunum smooth muscles in a dose dependent manner in all the three plants. In the *in vivo* study, an oral administration of *A. montanus* and *C. nitida* extracts at 400 and 800 mg kg^{-1} dose exhibited slightly lesser inhibition of movement of charcoal meal in the rat's gastrointestinal tract (GIT) than that of the standard drug atropine. In contrary, *N. laevis* provoked more inhibition than that of the standard drug atropine. Like atropine, the three extracts significantly ($p<0.05$) blocked the contractile effect of acetylcholine. The aforementioned results clearly demonstrate that *N. laevis* showed the highest activity in terms of both the *in vivo* and the *in vitro* model. **Conclusion:** The results therefore indicate that the extracts may possess possible anticholinergic properties and may prove valuable in disease management including diarrhea muscular spasms and other related diseases.

Key words: *A. montanus*, *C. nitida*, *N. laevis*, anti-diarrhea, anti-spasmolytic, charcoal meal, rabbit jejunum

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diarrhea diseases caused several millions of deaths in the world annually[1]. In developing countries, they are the most common causes of morbidity and mortality[2]. At the beginning of the 1980s, deaths caused by diarrhea were estimated at 4.6 million every year for children under the age of 5 years[3]. Infants younger than 1 year account for more than half of these deaths and the risk can be 2-3 times higher among infants who are not exclusively breast-fed[4].

The role of the muscarinic receptors in the maintenance of the normal physiological contractility of the gastrointestinal tract (GIT) is well established[5,6]. In contrast of adrenergic activity that produces relaxation of the intestine through adrenergic receptor binding, intestinal motility (cholinergic activity) is as a result of binding of muscarinic receptor[7]. It is the activities of the cholinergic and adrenergic section of the autonomic nervous system the produces the stability in GIT peristaltic physiology. The excessive activities of the cholinergic nervous system in the GIT can result to gastrointestinal cramp, ulcer, diarrhea and vomiting[8,9].

Newbouldia laevis commonly called African border tree or boundary tree is known locally as Aduruku in Hausa, Ogirisi in Igbo and Akoko in Yoruba languages of northern, eastern and western Nigeria, respectively. The plant is valued for many medicinal properties in various African tropical catalogues. Extracts from different parts of the plant have been shown to possess anti-microbial, anti-malarial, anti-oxidant, nociceptive and anti-inflammatory properties[10,11]. The aqueous and ethanol leaf extracts of this plant displayed uterine contractile effects[12]. Various preparations from the plant leaves are highly popular and acclaimed locally for its anticoagulant effects by traditionalists in Ovoko community, Nsukka Local Government Area (L.G.A), Enugu state, Nigeria. The plant is considered sacred and used as a symbolic marker for sacred spots by the Yoruba tribe of western, Nigeria. *Cola nitida*, also known as Kola nuts, is the seed pods of various evergreen trees that are native to Africa. Kola nuts are popular masticatory in West Africa and Sudan[13]. They are important in various social and religious customs and may also be used to counteract hunger and thirst. In Nigeria for instance the rate of consumption of kola nut especially by students is very high as a principal stimulant to keep awake and withstand fatigue[14].

A. montanus is popular in southern Nigeria where it is diversely called Elele-nyijuo, Agamsoso and Agameru and employed in traditional medicine[15]. In the democratic republic of Congo, the leaves are pounded in water with those of *Ananas comosus* and *Costus* spp. and used to treat urogenital infections, urethral pain, endometritis, urinary disease, cystitis and leucorrhoea[16]. The roots are also used for bathing to relieve aches and pains[17]. Documented evidence of pharmacological activities shows that the leaves of the plant possess spasmolytic[18], analgesic[19], anti-inflammatory and anti-pyretic[20] properties. On the basis of previous reports related to spasmolytic activity of these plants, we speculated whether the leaf extracts of these plants could have any possible anti-diarrheal potential. To get the answer, a study was undertaken to evaluate the anti-spasmolytic and anti-diarrheal activities using both *in vivo* charcoal meal transit model in rats and *in vitro* isolated tissue model in rabbit to observe the inhibition of movement of charcoal meal in the rat's GIT and the relaxation of rabbit jejunum smooth muscle, respectively.

MATERIALS AND METHODS

Collection of plant leaves: The plant leaves were collected in Umudike, Abia state and validated by Dr. Amosun, G. of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Nigeria.

Preparation of plant extract: The plant leaves were dried for 7 days and pulverized to powder using a homogenizer. About 50 g of the ground material was extracted in soxhlet apparatus using methanol as solvent at 45°C for 48 h. After extraction the methanol was evaporated in an electric oven at 40°C. The crude extract obtained weighed 11.20 g and represented a yield of 22.40% for *A. montanus*, 7.24 g represented a yield of 14.48% for *C. nitida* and 13.56 g yield of 27.12% for *N. laevis*, respectively.

Phytochemical screening: The phytochemical screening of the methanolic extract was carried out in order to detect the presence of secondary metabolites utilizing standard methods of analysis described by Trease and Evans[21].

Animals: Forty rats (120-150 g) and 3 rabbits (1.8-2.5 kg), acquired from the production unit of the College of Veterinary Medicine, MOUAU were used for this study. The animals were housed in aluminum cages under Specific Pathogen Free (SPF) conditions and were giving standard feed and water *ad libitum*. Before the experiments were carried out, the animals were starved for 24 h. All protocols were carried out in compliance NIH guidelines for Care and Use of Laboratory Animals (Pub. No. 85-23, Revised 1985) as reported by Akah *et al.*[22]. The study was conducted at the Physiology

Laboratory of the Department of Physiology, Pharmacology and Biochemistry, MOUAU in August, 2017 and lasted for a period of 3 weeks. Ethical approval was obtained from the Ethical Committee of the Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike and was given ethical approval number BCH/ETH/18/127.

Acute toxicity and lethal dose test (LD_{50}): It was carried out with modification according to the method of Lorke[23]. A total of 18 mice were used. They were divided into two stages: In stage one (phase 1), the animals were divided into 3 groups having three mice in each groups and the extracts were orally administered at the dose of 10, 100 and 1000 mg kg^{-1} b.wt. The stage (phase II) animals were given 160, 2900 and 5000 mg kg^{-1} b.wt., orally. The mice were observed for signs of toxicity hourly in the first 12 h and then daily for 7 days.

***In vivo* effect of the extracts on charcoal meal transit in rats:** Forty male rats were divided into 8 groups having 5 rats in each group. This study was carried out in accordance of the method of Mascolo *et al.*[24] and Rahman *et al.*[25]. The distance travelled by the charcoal meal was measured and expressed as percentage. Each animal was dissected and the full length of the small intestine was measured. The distance travelled by the charcoal meal was also measured and expressed as a percentage of the length of the intestine using the equation:

$$\text{Distance traveled by charcoal meal (\%)} = \frac{\text{Distance moved by charcoal}}{\text{Full length of intestine}} \times 100$$

Percentage inhibition for the *in vivo* study was evaluated as:

$$\text{Inhibition (\%)} = \frac{A - B}{B} \times 100$$

Where:
A = Distance moved by charcoal in control (%)
B = Distance moved by charcoal in test (%)

Preparation of intestinal smooth tissue: Preparation of intestinal smooth tissue for contraction effect of the extract was done by method of Uchendu[26]. Dose-dependent relationships were established for acetylcholine and the three extracts after regular rhythmic contractions were recorded. A minimum time of 1 min was allowed for all administration for individual tissue responses then washed 3 times with Tyrode solution. Concentration of test substances given in the text are all Final Bath Concentrations (FBC), except otherwise indicated.

Statistical analysis: Results for this study were expressed as Means±standard error of mean (SEM). Significant values were taken at $p<0.05$. Analysis was carried out using SPSS v22.

RESULTS

Gastrointestinal motility test: Atropine a standard cholinergic blocker was able to block acetylcholine-induced smooth muscle contraction giving the baseline for comparison of the three different plant extracts as shown in Fig. 1. The methanol extract of *N. laevis*, *C. nitida* and *A. montanus* showed inhibitory effects on intestinal motility (depicted by the distance moved by the charcoal) when compared with the control group with *N. laevis* showing the highest inhibitory effect in intestinal motility compared to atropine as shown in Table 1. The methanol extract of *N. laevis* lessened gastrointestinal distance traveled by the charcoal meal in the rats noticeably (98.20±4.02-31.80±1.95 cm) compared to that of the control group. Atropine (5 mg kg^{-1}) produced a marked (53.14%) decrease in the propulsion of charcoal meal through gastrointestinal tract as shown in Table 1.

Table 2 Shows that the methanol extract of *Acanthus montanus* revealed a dose-related increase in percentage inhibition of the basal amplitude of the intestinal smooth muscle. The methanol extract of *Cola nitida* also revealed a dose-related increase in percentage inhibition of basal amplitude of the intestinal smooth muscle as shown in Table 3. The percentage inhibition showed by *Cola nitida* extracts was higher at the respective doses compared to those of *Acanthus montanus*. The result in Table 4 showed that the methanol extract of *N. laevis* elicited a dose-related increase in percentage inhibition of basal amplitude of the intestinal smooth muscle. This was higher than those observed in the intestinal smooth muscle treated with *Acanthus montanus* and *Cola nitida* extracts at the respective doses At dose of 1333 µg mL^{-1} of the extract showed the best result in all the treatment even better than atropine (Fig. 1). Acetylcholine were able to provoke intestinal smooth muscle contractions in a dose related manner as shown in Table 5. Table 6 showed that noradrenaline was able to elicit dose-dependent percentage inhibition of the basal amplitude. The result of noradrenaline percentage inhibition was lower compared to *N. laevis*.

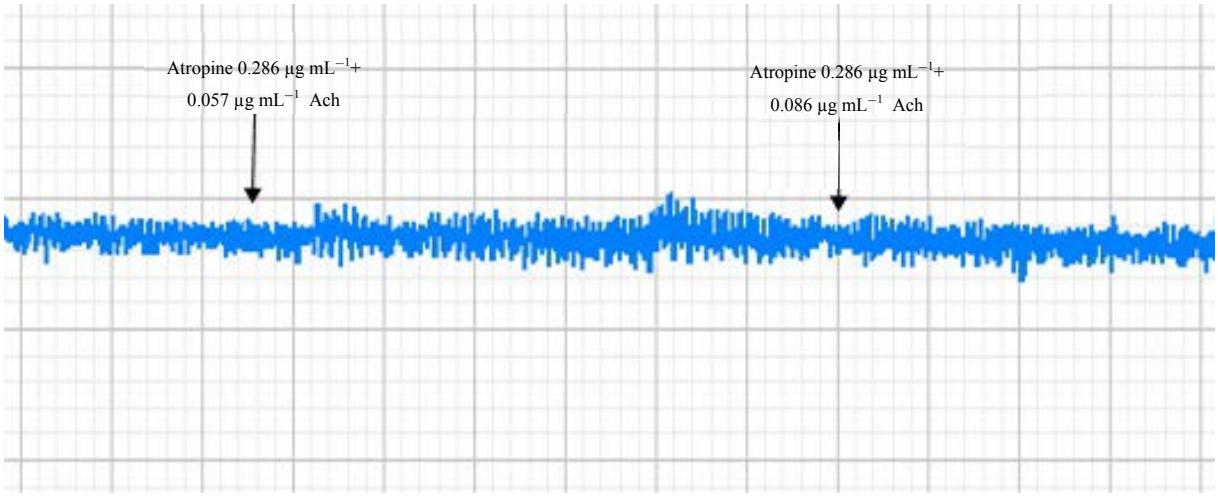

Fig. 1: *In vitro* effect of atropine on acetylcholine-induced contraction on an isolated rabbit jejunum

Table 1: Effects of the various extracts on small intestinal transit in rats (GIT Motility Test)

Parameters	Length of intestine	Distance moved by charcoal meal	Distance moved (%)	Nature of activity
Normal control	85.52±1.51	80.62±1.26	94.27	No activity
Atropine	102.55± 3.61	54.49±4.73*	53.14	Inhibitory
A. montanus leaf 200 mg kg^{-1}	105.20±2.15	75.20±5.03*	71.48	Inhibitory
A. montanus leaf 400 mg kg^{-1}	102.4±1.86	65.80±2.76*	64.26	Inhibitory
C. nitida 200 mg kg^{-1}	103.6±1.96	77.60±3.18*	74.9	Inhibitory
C. nitida 400 mg kg^{-1}	97.60±1.32	75.00±1.87*	76.84	Inhibitory
N. laevis 200 mg kg^{-1}	96.00±1.41	32.60±1.50*	33.95	Inhibitory
N. laevis 400 mg kg^{-1}	98.20±4.02	31.80±1.95*	32.38	Inhibitory

Values are expressed as Mean±SEM, *p<0.05 when compared with control group

Table 2: Effect of methanol leaf extract of *Acathus montanus* on contraction of isolated rabbit jejunum

FBS (µg mL^{-1})	Basal amplitude (mm)	Amp. in response to extract (mm)	Inhibition (%)
333.33	12±0.32	8.6±0.24	28.33
666.66	12±0.32	7.2±0.37	38.33
1333.33	15±0.37	5.2±0.37	65.33

Values are expressed as Mean±SEM

Table 3: Effect of methanol leaf extract of *Cola nitida* on contraction of isolated rabbit jejunum

FBS (µg mL^{-1})	Basal amplitude (mm)	Amp. in response to extract (mm)	Inhibition (%)
333.33	8.2±0.37	5.0±0.32	39.02
666.66	7.4±0.37	3.4±0.24	54.05
1333.33	12.4±0.25	3.6±0.24	72.72

Values are expressed as Mean±SEM

Table 4: Effect of methanol leaf extract of *N. leavis* on contraction of isolated rabbit jejunum

FBS (µg mL^{-1})	Basal amplitude (mm)	Amp in response to extract (mm)	Inhibition (%)
333.33	10.4±0.24	4.6±0.24	55.77
666.66	11.4±0.24	5.0±0.32	56.14
1333.33	12.6±0.43	3.0±0.32	76.19

Values are expressed as Mean±SEM

Table 5: *In vitro* effect of acetylcholine on isolated rabbit jejunum

FBS (µg mL^{-1})	Basal amplitude (mm)	Amp. in response to ach (mm)	Rise in amplitude (%)
0.0286	9.0±0.01	18.0±0.05	50.00
0.057	10.5±0.02	23.0±0.02	54.35
0.086	10.0±0.04	24.0±0.03	58.33

Values are expressed as Mean±SEM

Table 6: *In vitro* effect of noradrenaline on an isolated rabbit jejunum

FBS (µg mL^{-1})	Basal amplitude (mm)	Amplitude in response to NA (mm)	Inhibition (%)
0.014	10.15±0.12	6.25±0.49*	44.39
0.029	13.20±0.32	7.10±0.81*	46.07
0.057	13.10±0.23	7.12±0.15*	49.50
0.114	11.00±0.19	6.18±0.11*	43.82
0.229	12.25±0.09	4.25±0.20*	65.31

Values are expressed as Mean±SEM

DISCUSSION

In this study, the anti-spasmolytic and anti-diarrhea properties of ethnomedicinal plants *Newbouldia laevis*, *Cola nitida* and *Acanthus montanus* were screened. The incidence of diarrhea is still high and antibiotics used as anti-diarrheal drugs sometimes exasperate adverse effects

and micro-organisms tend to develop resistance toward them. Most anti-diarrheal drugs are either not available or affordable by many rural dwellers who world depend on medicinal herbs for the treatment of diarrheal conditions. This study was carried to explore more efficient and affordable anti-diarrheal agents of plant origin.

The results showed a significant ($p<0.05$) relaxation of rabbit jejunum in a dose dependent manner in all the three plants. In the *in vivo* study, an oral administration of *A. montanus* and *C. nitida* extracts at 400 and 800 mg kg^{-1} dose exhibited slightly lesser inhibition of movement of charcoal meal in the rat's gastrointestinal tract (GIT) than that of the standard drug atropine. In contrary, *N. laevis* provoked more inhibition than that of the standard drug atropine. Besides this, a similar result was found in the *in vitro* study on isolated rabbit jejunum. In particular, 666.66 μg mL^{-1} of *A. montanus* and *C. nitida* extracts produced moderate relaxation effects whereas at similar concentration *N. laevis* exhibited a comparable relaxation effect than that of noradrenaline. Like atropine, all the three extracts significantly ($p<0.05$) blocked the contractile effect of acetylcholine. The aforementioned results clearly demonstrate that *N. laevis* showed the highest activity in terms of both the *in vivo* and the *in vitro* model. These results further corroborate the usefulness of these three medicinal plants in traditional management of diarrhea among many tribes in west Africa[27-29].

The presence of selected phytochemicals such as terpenoids, flavonoids, tannins, saponins, alkaloids and glycosides in leaf extracts of *A. montanus*, *N. laevis* and *C. nitida* have been implicated in the relaxation of gastrointestinal smooth muscles[30-32]. These findings therefore suggested that the extracts may have achieved its effects by binding to the muscarinic receptors, thus, antagonizing the activity of acetylcholine and inhibiting intestinal peristaltic contractions[33]. Atta and Mouneir[34], reported that, traditionally, people use plant-derived preparations considering them to be efficacious against diarrheal disorders without any scientific basis. These experimental models were therefore employed to validate antidiarrheal efficacy of methanol extract of *A. montanus*, *C. nitida* and *N. laevis* leaves in the current study. The results of qualitative phytochemical screening of the *A. montanus*, *C. nitida* and *N. laevis* methanol extracts of leaves revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids and glycosides in all the plants extracts tested. The presence of variety of phytochemicals in the present study provides the indication that the plants extracts could be used for ameliorative potential against diarrhea and therefore, could explain their use traditionally for the treatment of wide array of illness including the management of diseases like diarrhea, asthma, incontinence, peptic ulcers, muscular spasms etc.[35]. Enteral dose of 5000 mg kg^{-1} b.wt., *A. montanus* leaf extract, *C. nitida* leaf extract and *N. laevis* leaf extract showed no death/toxicity signs. This agrees to the high safety index of the leaf extracts of the three plants and may justify the use of the plant over the years for the management of diseases. This toxicity result agrees with a pre-determined study Emmanuel *et al.*[36]. In other toxicological study by Anaduaka *et al.*[37], results revealed that dosage up to 5000 mg kg^{-1} b.wt., of ethanolic extracts of *N. laevis* are safe.

Agbor *et al.*[38] and Umer *et al.*[39] described diarrhea as the aberrant intermittent defecation of feces of low consistency which may be due to a disturbance in the movement of water and electrolytes in the intestines. Instead of the arrays of etiologies, (i) Increased electrolytes secretion (ii) Increased luminal osmolarity (iii) Unbalanced intestinal motility causing a decreased transit time and (iv) Low electrolytes absorption may be responsible for pathophysiology.

Our research showed that the overall anti-diarrheal study reveals a dose related activity apart from the group treated with *C. nitida* (Table 1). In our study, *N. laevis* leaves showed the best result with significant inhibition (%) of motility in castor oil-induced rat by 32.38 and 33.95% at the doses of 200 and 400 mg kg^{-1}, respectively, when compared with the control group, 94.24% and atropine 53.14%. The results suggest that leaves of *A. montanus*, *C. nitida* and *N. laevis* may contain anti-diarrheal components. Apart from different diseased conditions of diarrhea, specifically the hypermotility characterizes it to a good extent where the secretory component is not the causative factor[40].

It was revealed that flavonoids are responsible for the antidiarrheal activity properties[41]. Nevertheless, earlier studies also have shown that flavonoids have potentials to inhibit intestinal motility[42]. Besides, *in vivo* and *in vitro* tests have also shown that flavonoids are able to inhibit prostaglandin E2 induced intestinal secretion and spasmogens induced contraction and also inhibit release of prostaglandins and autocoids[41]. Flavonoids as the inhibitors of prostaglandins production are designed to reprieve castor oil-induced diarrhea[43]. So, the antidiarrheal activity of the methanolic extract of the leaves of these three different plants could therefore be due to the presence of flavonoids and other phytochemicals.

The extracts were able to inhibit acetylcholine-induced smooth muscle contraction in a dose dependent fashion, this inhibition may be due to interaction with the M_2, M_3 subtype

muscarinic receptor which play an important role in intestinal contractility and peristaltic innervation resulting from the parasympathetic arm of the CNS.

Eglen[44], Ehlert *et al.*[45] and Ehlert[46] established that smooth muscles of the gastrointestinal tract is rife with muscarinic receptors of both M_2 and M_3 subtypes which play an important role in intestinal contractility and peristaltic innervation resulting from parasympathetic activities. While the M_3 receptors do so by provoking phosphoinositide hydrolysis, Ca^{2+} cumulation and direct contractile response, M_2 subtype does same by inhibiting adenylate cyclase and Ca^{2+} activated K^+ channels and potentiating Ca^{2+} dependent non-selective conductance. Thus, the administered acetylcholine in the *in vivo* and *in vitro* experiments generated inositol, 1, 4, 5-triphosphate (IP3) which evoked Ca^{2+} release from intracellular storage sites in the rabbit GIT smooth muscle cells and thus eliciting contractions in the isolated tissue[26]. This underlying physiological principle is the reason for the movement of the charcoal meal along the intestines of the animals in the *in vivo* experiment since the contractions are responsible for moving intestinal contents forward[6,9]. Rang *et al.*[47] reported that atropine (1 mg kg^{-1}), inhibits the contractions induced by acetylcholine in the experiments showing competitive binding to muscarinic receptors and by so doing reduced the effects.

N. laevis, *C. nitida* and *A. montanus* methanol leaf extract exhibited a dose fashion inhibition of intestinal contractions in both study models and also significantly ($p<0.05$) blocked acetylcholine induced contractions (Fig. 1). This work agrees with existing novels which had reported that the plant exhibited antimotility effect on the gastrointestinal tract[33,48,49] and further explains its uses in folkloric medicine in the management of diarrhea.

CONCLUSION

The inhibitory effects of *A. montanus*, *N. laevis* and *C. nitida* methanol leaf extracts on intestinal motility and the balanced contraction of the jejunum are twain with its significant inhibition of acetylcholine induced contractions in the experiments. The result suggests that the extract may contain bioactive substances with anti-diarrhea and potent anticholinergic properties.

SIGNIFICANCE STATEMENT

This study discovers the activity of three ethnomedical plants *Newbouldia laevis*, *Cola nitida* and *Acanthus montanus* that can be beneficial in the treatment of Diarrhea. Diarrhea is typified by an increase in the frequency of bowel motility, unformed feces and abdominal discomfort. It is the world's third highest killer disease, contributing extensively to pediatric morbidity and mortality, especially in the malnourished. Report shows that the incidence of diarrhea is still high (about 7.1 million per year), despite the efforts of international organizations to control its occurrence. Antibiotics used as antidiarrheal drugs sometimes exasperate adverse effects and microorganisms tend to develop resistance toward them. Also, the existing antidiarrheal drugs are either not available or affordable by many rural dwellers. Many rural dwellers in the world depend largely on medicinal herbs for the treatment of diarrheal conditions because these herbs are readily available, cost effective and imperative component of traditional medicine practice. This study will help the researcher to uncover the critical areas of safe and more effective antidiarrheal agents from plant origin that many researchers were not able to explore. Thus a new theory on the therapeutic activities of the plants ethnomedical plants *Newbouldia laevis*, *Cola nitida* and *Acanthus montanus* with regards to diarrheal management may be arrived at.

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