IJMPR 2021, 5(5), 78-83

International Journal of Modern Pharmaceutical Research

www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article

SJIF Impact Factor: 5.273

EVALUATION OF THE ANTIDIARRHEA EFFECT OF METHANOL LEAF EXTRACT AND FRACTIONS OF *ALCHORNEA LAXIFLORA (*BENTH.)

IKE Chibueze Jeremiah[1], Nwankwo Azubuike A.[2], IFEBI Hope Morris Nchekwube*[3], Elebeke Kaosisonna Chris, Nnedum Chinenye Henrietta

[1]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
[2]Department of Physiology, College of medical science, Abia State University, Nigeria.
[3]Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Received on: 30/08/2021
Revised on: 20/09/2021
Accepted on: 10/10/2021

***Corresponding Author**
IFEBI Hope Morris Nchekwube
Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

ABSTRACT

Introduction: *Alchornea laxiflora* is a shrub which is widely distributed through Africa. It is one of the African medicinal plants used in treatment of some illments. The leave methanol extract and fractions of the plant were evaluated for antidiarrhea activity as part of effect to authenticate its oral claims scientifically. **Method:** Pulverized leaves (400 g) were cold macerated in methanol (1 liter) to obtain the crude extract. The extract was fractionated using liquid-liquid partitioning to obtain *n*-hexane, ethyl acetate and *n*-butanol fractions. The crude extract and its fractions were screened for phytochemical constituents using standard procedures. Acute toxicity (LD_{50}) of the crude extract was determined using Lorke's method.Standard procedures were used to determine the antidiarrhea activity. Adult albino wister mice was used for the study. The mice were starved for 18h having access to drinking water only. The test groups were treated with 50, 100 and 250 mg/kg of the crude extract and fractions while the negeitive controls and positive controls were given distilled water (10 ml/kg) and loperamide (2 mg/kg.) respectively. Thirty minutes after the treatment, diarrhea was induced by oral administration of 0.5 ml castor oil. The time of onset of diarrhea, number and weight of wet stool were noted. Gastrointestinal motility and enteropooling studies were also determined using standard procedures. **Results:** Phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids and cardiac glycosides. The LD_{50} was calculated to be $\geq$ 5000 mg/kg. The crude extract and n-hexane fraction (100 and 250 mg/kg.) significantly ($p<0.05$) prolonged the onset of diarrhea in mice delaying the onset of time of diarrhea. also indicated significant ($P < .05$) antimotility activity in comparison with the control. **Conclusion:** Results obtained from this study proved that crude methanol extract and fractions of *Alchornea laxiflora* possess antidiarrhea activity.

KEYWORDS: *Alchornea laxiflora*, phytochemicals, enteropooling, gastrointestinal motility, antidiarrhea.

INTRODUCTION

Diarrhea can be defined as an alteration in the normal bowel movement, characterized by a situation in which an adult daily stools exceeds 300 g and contains 60 – 95 % water. Diarrhea can cause severe dehydration that can lead to death. Diarrhoea remains the second leading cause of mortality among children under five years of age next to respiratory infections and kills more young children than AIDS, malaria, and measles combined.[1,2] These diseases predominantly affect developing countries and of all child deaths from diarrhoea, 78% occur in the African and South-East Asian regions. The WHO estimation revealed that diarrhea causes 4 5 million deaths annually throughout the world. 80% of these deaths are reported in developing countries including Nigeria. In Nigeria, diarrheal infection remains the number one killer disease among children under 5 years, while 7 to12 month old babies remains the most susceptible.[3] Even if sufficient drugs are available for treating diarrhea, the majority of the existing drugs suffer from adverse effects like the induction of bronchospasm, vomiting, intestinal obstruction, constipation, and dependency. Because of this, there is a necessity of strengthening research into medicinal plants to investigate alternative drugs from natural products.[4] There are many medicinal plants that possess antidiarrheal activity with lesser side effects than the conventional drugs. According to World Health Organization, medicinal plants would be the best source

to obtain a variety of drugs. Therefore such plants should be investigated to better understand its safety and efficacy.[5] Tannins, alkaloids, flavonoids and terpenoids are the major constituents that are primarily responsible for antidiarrheal activity of these medicinal plants.[6] However, the safety and therapeutic potentials of some of these medicinal plants have not been validated yet. Among them, *Alchornea laxiflora* is one of the popular medicinal plants being used in the traditional medicine for treatment of diarrhea. *A. laxiflora* possesses numerous therapeutic benefits and has been used in the treatment of various disease conditions such as HIV/AIDS, malaria, diabetes, sickle-cell anemia, mental disorders and microbial infections.[7] Despite its traditional claims, the efficacy of the leaves of *A. laxiflora* against diarrhea is not yet scientifically validated. Therefore, this study was aimed to evaluate its antidiarrheal activity against castor oil–induced diarrhea in mice.

MATERIALS AND METHODS

Drugs and chemicals

Drugs used in the study include; Castor Oil (Bell Sons & Co., England), Loperamide (Janssen, Germany), activated charcoal (Acuro Organics Ltd, New Delhi, India), other chemicals and reagents used for the study were of analytical grade and procured from approved organizations.

Experimental animals

Adult Swiss albino mice (20-30 g) was also used for the study and was obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Science, Nnamdi Azikiwe University. The animals were fed with palletized feed (UAC feed, Nigeria) and had access to water *ad libitum.* Housing of the animals was done in standard cages in the Animal House of the Department of Pharmacology and Toxicology. They were allowed free access to food and water. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals (Pub. No. 85 – 23 Revised 2011)

Plant collection and identification

Plant material: *A. laxiflora* leaves was collected from Agulu in Anaocha local Government area Anambra state Nigeria in November 2019. It was authenticated by a trained taxonomist, Mr Felix Nwafor of Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka, Enugu State, Nigeria. Voucher specimens (No. PCG 474/A/063) were deposited at the herbarium of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University Awka for future reference.

Method of Extraction

Five hundred grams (1000 g) of the pulverized *A. laxiflora* leaves was macerated in two litres of methanol over a period of 48 hours. The mixture was sieved using porcelain cloth. It was further filtered with no .l Whattman filter paper. The filtrate was concentrated using rotary evaporator. It was further dried in a water bath at a temperature of 40° C to obtain the ethanol extract. The extract was then stored in a refrigerator at 4°C until further use.

Fractionation (Liquid-liquid chromatography)

The methanol extract (100 g) was subjected to liquid-liquid containing water (200 mL in 100 g of extract) were subjected to liquid-liquid partition successively with 1000 ml n-hexane, 1000 ml ethyl acetate and 500 ml n-butanol in increasing order of polarity. The fractions were filtered with Whatman no 1 filter paper and concentrated *in vacuo* using rotary evaporator at 40 °C to obtain the n-hexane fraction (HF), ethylacetate fraction (EF) and butanol fraction (BF). The fractions were stored in refrigerator between 0-4°C until futher use.

Phytochemical analysis

The phytochemical screening was carried out on the crude extract and fractions of *A. laxiflora* leaves according to standard methods to identify the classes of bioactive compounds present [8, 9].

Acute toxicity studies

Acute toxicity analysis of the extracts was performed using Lorke's method [10]. This method has two phases (Phase 1 and Phase 2).

Phase 1: Nine adult albino mice were weighed, marked and randomized into three groups of three mice each. Each group of animals were administered different doses (10, 100 and 1000 mg/kg) of the extracts. The mice were observed for 24 hours for signs of toxicity as well as mortality.

Phase 2: Four mice were weighed, marked and randomized into four groups of one mouse each. Dose selection was based on result obtained in Phase 1. Observation for 24 hours for obvious signs of toxicity and death was recorded accordingly. The LD_{50} was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality.

Anti-Diarrhea Activity

Anti-diarrhea activity of *A. laxiflora* was carried out using animal model [11]. A total of 55 mice were used. They were grouped into eleven groups of five mice per group. The animals were fast for 18hour having access to drinking water only. They were treated as follows:
Group 1 (negative control) received 10ml/kg distilled water P.O
Group 2 (positive control) received 2mg/kg loperamide P.O
Group 3 received 50mg/kg crude extract P.O
Group 4 received 100mg/kg crude extract P.O

Group 5 received 250mg/kg crude extract P.O
Group 6 received 100mg/kg n-hexane fraction P.O
Group 7 received 250mg/kg n-hexane fraction P.O
Group 8received 100mg/kg ethylacetate fration P.O
Group 9received 250mg/kg ethylacetate fration P.O
Group 10 received 100mg/kg butanol fraction P.O
Group 11 received 2500mg/kg butanol fraction P.O

Thirty (30) minute post treatment diarrhea was induced by single oral administration of 0.5 ml of castor oil. The animals were observed for onset of diarrhea and the time noted. The number and weight of wet stool was noted 1, 2, 3 and 4 hours post diarrhea induction. The groups and reference group was compared with the control group for significant difference.

Gastro-Intestinal Motility Study

Gastro-intestinal study was done as was described by Aye-Than *et al*[12] using charcoal meal. Fifty-five (55) adult albino mice of both sex was used, and they were grouped into eleven (11) groups of five mice each. The animals were fasted for 24hrs prior to the experimental day, having excess to drinking water only. The animals were treated and immediately given 0.5ml of 10% deactivated charcoal in mucilage of tragacanth as follows:

Group 1 received 10ml/kg distilled water + 0.5ml charcoal meal P.O
Group 2 received 10mg/kg atropine I.P + 0.5ml charcoal meal P.O
Group 3 received 50mg/kg crude extract + 0.5ml charcoal meal P.O
Group 4 received 100mg/kg crude extract + 0.5ml charcoal meal P.O
Group 5received 250mg/kg crude extract + 0.5ml charcoal meal P.O
Group 6 received 100mg/kg n-hex fraction + O.5ml charcoal meal P.O
Group 7 received 250mg/kg n-hex fraction + O.5ml charcoal meal P.O
Group 8 received 250mg/kg ethylacetate fraction + 0.5 charcoal meal P.O
Group 9 received 250mg/kg ethylacetate fraction + 0.5 charcoal meal P.O
Group 10 received 250mg/kg butanol fraction + O.5ml charcoal meal P.O
Group 11 received 250mg/kg butanol fraction + O.5ml charcoal meal P.O

Fifteen (15) minute post-treatment, the animals were sacrificed by cervical dislocation, their abdomen cut open and the intestine layed out. The mesenteries were cut to free the intestine. The distance travelled by the charcoal plug along the intestine was measured against the total length of the intestine from the pyloric region to the caecum and was expressed as percentage of the total length of the intestine.

Enteropooling Study

The enteropooling effect of *Alchornea laxiflora* was studied using mice model.[12] A total forty mice was used. They were grouped into eight groups of five mice per group. The animals were fasted for 24hrs prior the experimental day.

Group 1 received 10mg/kg distilled water P.O
Group 2 received 2mg/kg Loperamide P.O
Group 3 received 50mg/kg crude extract P.O
Group 4 received 100mg/kg crude extract P.O
Group 5 received 250mg/kg crude extract P.O
Group 6 received 100mg/kg n-hexance fraction P.O
Group 7 received 250mg/kg n-hexance fraction P.O
Group 8 received 100mg/kg ethylacetate fraction P.O
Group 9 received 250mg/kg ethylacetate fraction P.O
Group 10 received 100mg/kg butanol fraction P.O
Group 11 received 250mg/kg butanol fraction P.O

One hour post- treatments, the animals were given 0.5ml castor oil orally. 1hour after the castor oil treatment, the mice were sacrificed by cervical dislocation. The abdomen of each mouse was open and the whole length of the intestine, from the pylorus to the caecum, was ligated, dissected and carefully removed. The small intestines were weighed and the intestinal contents were collected by milking into a graduated tube to measure the volume. The weight of empty intestine was also taken using eletronic weighing balances.

Statistical analysis

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Dunnett Multiple Comparisons Test. The values are expressed as mean ± standard mean error (SME). $P < 0.05$ was considered statistically significant.

RESULTS

Result of phytochemical analysis of *A. laxiflora* leaves extract

The phytochemical analysis revealed that the crude extract is rich in alkaloids, flavonoids, terpenoids and cardiarc glycosides (Table 1).

Table 1: Results of phytochemical analysis of the leave extract of *A. laxiflora.*

S/N	Phytochemical constituents	Relative abundance
1	Alkaloids	+
2	Saponins	-
3	Tannins	-
4	Flavonoids	+
5	Steriods	-
6	Terpenoids	+
7	Cardiarc glycosides	+
8	Proteins	+
9	Carbohydrates	+
10	Reducing sugars	_

Key
- = Not present

+ = Present

Result of Acute toxicity test

In the acute toxicity and lethality test, results (Table 2) indicated no physical and behavioural change such as diarrhea, sleepiness, loss of appetite, coma or death. The LD_{50} was thus calculated to be ≥ 5000 mg/kg.

Table 2: Results of acute toxicity (LD_{50}) test.

Phase	Dose (mg/kg)	Mortality
Phase one	10	0/3
	100	0/3
	1000	0/3
Phase two	1600	0/1
	2900	0/1
	5000	0/1

Anti-diarrhea test result

Oral pretreatment of mice with different doses of the extract and fractions showed a significant ($p < 0.001$) delay on the onset of diarrhoea, with the higher dose of the extract exhibiting the better effect. In addition, the extract significantly reduced the frequency of defecation and the number of wet stools when compared with control ($p < 0.01$). Percentage of faecal output was also reduced by different doses of the extract and fractons, in which the higher dose of n-hexane fraction (250 mg/kg) producing a better effect compared to any of the groups as shown in table 3 below.

Table 3: The Antidiarrhoeal effects of the leave extract and fractions of *A. laxiflora* on castor oil-induced diarrhoeal model in mice.

Group	Time of onset of diarrhea(min)	Total No. of wet stool	Total weight of wet stool(g)	% inhibition of defecation
10 ml/kg distilled water	0.34±0.14	17.15±2.54	8.62±2.44	-
2mg/kg loparamide	1.26±0.21**	4.11±0.96**	0.96±0.04**	78.03
50mg/kg crude extract	0.38±0.09^{ns}	13.10±1.17^{ns}	6.24±1.57^{ns}	23.61
100mg/kg crude extract	1.14±017**	7.20±1.36*	1.35±0.32**	58.01
250mg/kg crude extract	0.58±0.13^{ns}	9.36±1.84*	4.87±1.06*	45.42
100 mg/kg N-hexane fraction	1.09±0.11**	6.35±1.16**	1.24±0.07**	62.97
250 mg/kg n-hexane	1.14±0.01**	5.00±2.11**	0.86±0.16**	70.84
100 mg/kg ethylacetate fraction	0.54±0.08^{ns}	8.91±1.47*	3.06±0.09*	48.04
250 mg/kg ethylacetate fraction	0.49±0.18^{ns}	9.00±1.66*	3.55±0.58^{ns}	47.52
100 mg/kg Butanol fraction	0.43±0.06^{ns}	11.54±2.04^{ns}	5.11±0.13^{ns}	32.71
250mg/kg butanol fraction	0.33±0.01^{ns}	12.04±0.91^{ns}	6.01±1.07^{ns}	31.89

All values are expressed as mean ± standard error of the mean (SEM), (n = 5) *$p < 0.05$, ** $p < 0.01$, ns= non significsnt as compared with Control group. Data was analyzed by one way ANOVA followed by Dunnett Multiple Comparisons Test.

Table 4: Effect of the leave extract and fractions of *A. laxiflora* on Gastrointestinal motility in Mice.

Group	Distance travelled by charcoal meal(cm)	Total length of the intestine (cm)	%distance travelled by the charcoal meal(cm)	%inhibition
10 ml/kg distilled water	38.14±4.16	45.36±5.34	84.08±4.74	
2mg/kg loperamide	17.14±4.26	41.26±7.76	41.54±6.01*	51
50mg/kg crude extract	28.27±5.34	43.24±6.18	65.38±5.76^{ns}	22
100mg/kg crude extract	14.48±2.16	39.54±4.87	36.62±3.52*	56
250mg/kg crude extract	25.66±7.12	44.19±4.26	58.07±5.69^{ns}	31
100 mg/kg n-Hexane fraction	15.17±4.26	38.44±5.26	39.46±4.66*	53
250 mg/kg n-Hexane fraction	14.52±2.91	40.10±3.11	36.20±4.01*	57
100 mg/kg Ethylacetate fraction	21.68±4.14	40.05±5.17	54.13± 4.66*	36
250 mg/kg Ethylacetate fraction	26.99±5.01	41.99±3.68	64.28±3.41^{ns}	24
100 mg/kg Butanol fraction	25.87±4.55	42.14±3.67	61.39±4.11^{ns}	27
250 mg/kg butanol fraction	24.00±0.20	35.05±9.03	68.47	19

All values are expressed as mean ± standard error of the mean (SEM), (n = 5) *p < 0.05, ** p < 0.01, ns= non significant as compared with Control group. Data was analyzed by one way ANOVA followed by Dunnett Multiple Comparisons Test.

Table 5: The effects of leave extract and fractions of *A. laxiflora* on castor oil induced enteropooling in mice.

Group	Weight of intestine with fluid (g)	Weight of empty intestine (g)	Volume of intestinal fluid (ml)
10 ml/kg distilled water	2.14±0.25	0.44±0.06	1.70±0.28
2mg/kg loperamide	1.96±0.43 ns	0.34±0.08 ns	1.62±0.33 ns
50 mg/kg crude extract	2.07±0.24 ns	0.44±0.05 ns	1.63±0.47 ns
100 mg/kg crude extract	2.05±0.51 ns	0.48±0.03 ns	1.57±0.28 ns
250mg/kg crude extract	1.87±0.45 ns	0.58±0.08 ns	1.29±0.32 ns
100 mg/kg n-Hexane fraction	1.98±0.52 ns	0.33±0.04 ns	1.65±0.28 ns
100 mg/kg Ethylacetate fraction	2.11±0.52 ns	0.43±0.05 ns	1.68±0.41 ns
100 mg/kg Butanol fraction	2.02±0.18 ns	0.43±0.04 ns	1.59±0.26 ns

All values are expressed as mean ± standard error of the mean (SEM), (n = 5) *p < 0.05, ** p < 0.01, ns= non significsnt as compared with Control group. Data was analyzed by one way ANOVA followed by Dunnett Multiple Comparisons Test.

DISCUSSION

Therapeutic uses of medicinal plants as alternative medicine lead to this present study to ascertain antidiarrhea property of *Alchornea laxiflora*. Acute toxicity study on the methanol leaf extract of the plant which is greater than 5000mg/kg showed high safty margin to the use of the plant This supports the believe that green plants are safer.

Phytochemical screening on the plant showed moderately presence of alkaloids, flavonoids, terpenoids, cardiac glycosides, carbohydrates and presence of protein; this correlates with an earlier study done by Borokini *et al.*[13] These active phytochemicals are known for their medicinal activity as well as physiological actions; as such they confer the therapeutic potentials of all medicinal plants. Previous studies showed that terpenoids and steroids like phytosterols have a capacity to inhibit the production of prostaglandin E2 which has a crucial role in the stimulation of intestinal secretions.[4] According to similar studies done on different plants suggested that the presence of tannins and flavonoids increase colonic water and electrolyte reabsorption. Tannins are known for making intestinal mucosa more resistant by reducing secretion, normalizing deranged water transport and reduction of intestinal transit.[14] Other secondary metabolites like terpenoids and saponins also have the ability to inhibit the release of autacoids like prostaglandins and histamines. Phytochemicals such as phenolic compounds and alkaloids also inhibit intestinal motility.[15] In addition, flavonoids are also found to display a wide range of biological activities including inhibition of enzymes such as prostaglandin synthase, cyclooxygenase and lipoxygenase that might mainly contribute to its anti-diarrhoeal activity.[16]

Castor oil is the most commonly used diarrhea inducer in mice models. Ricinoleic acid, the active metabolites of castor oil is responsible for the diarrhea-inducing properties of castor oil.[4] In the castor oil-induced diarrheal model, the crude leaves extract and fractions of *A. laxiflora* significantly prolonged the diarrheal onset and the frequency of stooling at 100mg/kg crude extract, 100 and 250 mg/kg n-hexane fraction with percentage inhibition of 58.01, 62.97 and 70.84 % respectively when compared to the standard drug (Loperamide 78.03%). The significant antidiarrheal activity of this plant could be due to the probable localization of secondary metabolites to inhibit castor oil-induced fluid secretion. Surprisingly, at the maximum tested doses of the n-hexane fraction (250mg/kg) showed almost comparable effect with the standard drug.

With regard to castor oil–induced gastrointestinal motility, the plant extract and fractions significantly (P < .05) inhibited the propulsive movement of charcoal marker at 100mg/kg crude extract and the test doses of n-hexane whereas the ethylacetate ans butanol fractions of the plant extract was unable to produce significant inhibitory effect on the distance traveled by charcoal meal as compared with the negative control group. This finding suggested that the plant extract and n-hexane fraction has antimotility effect which opposed the effect of castor oil on the gastrointestinal motility of the mice in a similar manner to loperamide.[17] The reduction of GI motility is one of the mechanisms by which antidiarrhoeal agents can act. For example, the standard drug (loperamide) used in this study acts by activation of μ receptors that inhibit the release of acetylcholine to enhance phasic colonic segmentation and inhibit peristalsis, thus increasing intestinal transit time [18]. The report was compared with similar reports done by Degu *et al* and Sisay *et al*.[19,20]

The third model in this study was castor oil-induced enteropooling model, which is aimed to assess the potential inhibition of secretory components in the gastrointestinal tract after castor oil administration for the induction of diarrhoea. Outcomes in this model proved that the crude extract and fractions of *A. laxiflora* showed no significant reduction in both average weight and volume of intestinal contents at all test doses as compared with the negative control.

CONCLUSION

The results of this study revealed that extract and n-hexane fraction of *A. laxiflora* endowed with significant antidiarrheal activity. It inhibited the frequency of defecation and reduced greatly the wetness of faecal excretion. Moreover, it also produces an inhibitory effect on castor oil induced gastrointestinal motility. These antidiarrheal activities of the extract and fraction may be attributed to the presence of phytochemicals including alkaloids, flavonoids and terpinoids that act individually or collectively. These findings provide a scientific support for a traditional use of the stem of *A. laxiflora* as diarrhoea remedy.

ACKNOWLEDGEMENT

We thank Management of Nnamdi Azikiwe University, Awka, Anambra state for providing necessary facilities to carry out this work.

Conflict of Interests
Declared none.

REFERENCE

1. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, et al. Child health epidemiology referencegroup of WHO and UNICEF. Global, regional and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet, 2012; 379: 2151–61.
2. WHO. Diarrheal disease [Internet]. [cited 2015 January 28]. Available at: http://www.who.int /mediacentre/factsheets/fs330/en/, 2013.
3. Hanwa UA, Kaita AH, Sule MI,Ahmadu AA and Magaji MG. Antidiarrheal activity of the leaf extract of *Stereopermum kunthianum.* Biological and Environmental Sciences Journal for the Tropics, 2007; 4(2): 82-86.
4. Amsalu D, Belayneh K, Diriba A and Gobezie TT. Evaluation of the Antidiarrheal Activity of Hydromethanol Crude extracts of *Ruta chalepensis* and *Vernonia amygdalina* in Mice. Evidence-Based Complementary and Alternative Medicine, 2020; 1: 1-6.
5. Nascimento GG, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Braz J Microbiol, 2000; 31: 247-256.
6. Sisay M, Engidawork E and Shibeshi W. Evaluation of the antidiarrheal activity of the leaf extracts of Myrtus communis Linn (Myrtaceae) in mice model. BMC Complementary and Alternative Medicine. 2017; 17: 103.
7. Uhunmwangho ES, Rasaq NO and Ojemekele, O. Protective Effect of Alchornea laxiflora Methanolic Leaf Extract on CCL4-induced Hepatotoxicity and Reproductive Toxicity in Male Wistar Rats, 2016; 14(1): 1–7.
8. Evans WC. Trease & Evans pharmacognosy. 15th Edn. W. R. Saunders, London, 2002; 214-393.
9. Sofowora A. Medicinal plants and traditional medicine in Africa. 2nd Edn. Spectrum Books Limited, Ibadan, Nigeria, 1993; 1: 153. 21.
10. Lork, D. A new approach to acute toxicity testing. Arch Toxicol, 1983; 54: 275-287.
11. Akah, P. A; Offiah, V. N. Gastrointestinal effects of leaf extracts, Internationl jurnal of Pharmacognosy, 1996; 30: 213 217.
12. Abdullahi AL, Agho MO, Amos S, Gamaniel KS, Watanabe C. Antidiarrhoeal activity of the aqueous extract of *Terminalia avicennoides* roots. Phytother Res, 2001; 15: 431-434.
13. Borokini TI, Omotayo FO. Phytochemical and ethnobotanical study of some selected medicinal plants from Nigeria. J Med Plants Res., 2012; 6: 1106 - 1118.
14. Degu A, Engidawork E, Shibeshi W. Evaluation of the anti-diarrheal activity of the leaf extract of Croton macrostachyus Hocsht. ex Del. (Euphorbiaceae) in mice model. BMC Complement Altern Med, 2016; 16: 1–11.
15. Mishra CK, Sasmal D. In-vivo evaluation of anti-diarrhoeal activity of Ethanolic fruit and root extracts of Carissa carandas Linn. (Apocynaceae). Int J Drug Dev Res., 2015; 7: 216–21.
16. Hossain E, Ko SY, Yang CJ. Dietary supplementation of green tea byproducts on growth performance, meat quality, blood parameters and immunity in finishing pigs. J Med Plants Res., 2012; 6: 2458–67.
17. Jemal A. Evaluation of In Vivo Antidiarrheal Activities of Hydroalcoholic Leaf Extract of Dodonaea viscosa L. (Sapindaceae) in Swiss Albino Mice. Journal of Evidence-Based Integrative Medicine, 2019; 24: 1-10
18. Faure C. Role of Antidiarrhoeal drugs as adjunctive therapies for acute Diarrhoea in children. Int J Pediatr, 2013; 2013: 1–14.
19. Sisay M, Engidawork E, Shibeshi W. Evaluation of the antidiarrheal activity of the leaf extracts of Myrtus communis Linn (Myrtaceae) in mice model. BMC Complement Altern Med, 2017; 17: 1–11.
20. Degu A, Engidawork E, Shibeshi W. Evaluation of the anti-diarrheal activity of the leaf extract of Croton macrostachyus Hocsht. ex Del. (Euphorbiaceae) in mice model. BMC Complement Altern Med, 2016; 16: 1–11.