EVALUATION OF THE *IN VIVO* AND *IN VITRO* ANTIDIARRHEAL EFFECTS OF METHANOL LEAF EXTRACTS OF *Boscia salicifolia* IN MICE AND ISOLATED RABBIT JEJUNUM

[1]MAILAFIYA M*, [2]ELON R. I, [4]JOHNNY J AND [3]ATINGA V.

[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Gombe State University, Gombe State, Nigeria
[2]Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Gombe State University, Gombe State, Nigeria
[3]Department of Pharmacognosy and Drug development Faculty of Pharmaceutical Sciences, Gombe State University, Gombe State, Nigeria
[4]Department of Science Laboratory Technology, Federal Polytechnic Kaltungo, Gombe State
*Corresponding Author: mariamailafiya@gmail.com

ABSTRACT

Diarrhoea is one of the leading causes of preventable death in developing countries and mainly affects children and infants. The leaves of *Boscia salicifolia* has been used as an herbal remedy in the treatment of diarrhea in many parts of Northern Nigeria and West Africa. Thus, this study was aimed at investigating this enthnomedical claims of the plant extract on the gastrointestinal tracts. The fresh leaves were collected from Kalshingi town of Akko Local Government area Gombe State in August. They were dried for a week under the sun and the grounded into powder. Six hundred (600 g) grams of the powdered leaves were subjected to successive cold extraction with 80 % methanol. The extract was used for acute toxicity, phytochemical screening, antidiarrheal and spasmolytic evaluation of acetylcholine, histamine and serotonin induced contractions on rabbit jejunum. The preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenes, steroid and glycosides. The effect methanol leaf extract of *B. salicifolia* on castor oil-induced diarrhoea in mice showed a significant reduction ($p<0.05$) in the diarrheal drops with increase percentage inhibition of diarrhea 45.95, 40.54, and 29.72% at 380, 760, and 1140 mg/kg doses of the extract, respectively. The standard drug (loperamide 5 mg/kg) inhibited diarrhoeal droppings by 51 %. The extract significantly ($p<0.001$) decreases the force of rabbit ileum contraction induced by acetylcholine, histamine and serotonin at 7×10^{-10} g/ml, 1×10^{-7} g/ml and 1×10^{-7} g/ml respectively. The methanol leaf extract of *Boscia salicifolia* was found to be relatively safe and possess significant anti-diarrhoeal activity, and this justifies the use of this plant in the treatment of diarrhoea in the traditional settings.

Keywords: *Boscia salicifolia*, diarrhea, *in vivo, in vitro*, spasmolytic, castor oil

INTRODUCTION

Diarrhea is described as a change in the individual's bowel habits that results in significantly more frequent and/or loose feces (Armon *et al*., 2001). Increased gastrointestinal motility and secretion, decreased fluid absorption, and electrolyte

and water loss characterizes diarrhea (Sulaiman *et. al*., 2017). Diarrhea, especially in children, is one of the primary causes of mortality and morbidity in underdeveloped nations (Ugboko *et al*., 2020).

Antimotility and antisecretory medicines are the cornerstones of diarrhea treatment. Opioids and their derivatives are still frequently utilized also, opioid antidiarrheals such as diphenoxylate, difenoxin, and loperamide are commonly employed (Bruntun *et al*., 2008). However, the use of herbal remedies to treat diarrheal disorders has risen in recent years, and it is believed that around a third of all diarrheal diseases are now treated with herbal remedies (Balemba *et el.,* 2010).

A vast majority of the people of developing countries rely on herbal drugs for the management of diarrhoea. Considering this fact, the World Health Organization has instituted a diarrhoeal disease control programme, which includes studies of traditional medicinal practices, together with the elevation of health education and prevention approaches (Ugboko *et al.,* 2020).
Boscia salicifolia (*B. salicifolia*) is a small to medium deciduous tree with dark grey bark that is rough and corky. The leaves are pirally arranged, seldom tightly clustered, lanceolate up to 15 cm in length, and dull green (Lemmens, 2013). From Senegal through Niger, Northern Nigeria, and East Africa, this tree with drooping Folix-like leaf grows to a height of 13 cm in the dry Savanna zone and is frequently associated with termites and mounds (Yakubu *et al*., 2014). Ghana, Nigeria, Cameroon, Uganda, Kenya, Tanzania, Malawi, Zambia, Botswana, and Zimbabwe are among the countries where the plants can be found (Yakubu *et al.,* 2014).
Root decoctions are used as aphrodisiacs and to cure diarrhea, inflammations, oedema, and psychological illnesses. Pounded leaves are used to treat wounds, abscesses, furuncles, swollen glands, haemorrhoids, and itchy skin across its distribution range. Leaf ash is taken as antidote for poisoning and to treat tuberculosis. Powdered leaves in water are added to food of livestock to treat diarrhea (Danjuma and Darda'u, 2013).
Therefore, the aim of this study is to investigate the effects of methanol leaf extract of *B. salicifolia* on castor oil-induced diarrhea in mice and its spasmolytic effects on Acetylcholine (ACh), Histamine, and Serotonin (5-HT) induced rabbit jejunum contractions.

MATERIALS AND METHOD

Sample collection

Leaves of *B. salicifolia* (*capparaceae*) were freshly collected from Kalshingi town of Akko Local Government area, Gombe State in August 2019. The leaves were identified with the voucher number 166 by a taxonomist in the Department of Biological Sciences, Faculty of Science, Gombe State University of Gombe, Nigeria.

Preparation of extract

The dried leaves of *B. salicifolia* were size reduced using mortar and pestle in which 600 g were weighed into a container and macerated for 7 days with 80% methanol. The solvent was evaporated at room temperature to yield a thick extract which was suspended in distilled water and then

successively fractionated with n- hexane, chloroform, ethyl acetate and n-butanol to yield n-hexane, chloroform, ethyl acetate and n-butanol fraction respectively.

Animal

Rabbits weighing (1.6-2.5 kg) and mice of either sex were used weighing (21-31 g) in the animal house of the Faculty of Pharmaceutical Sciences, Gombe State University, Nigeria. The animals were housed in transparent plastic cages padded with wood shavings, under standard conditions of temperature, relative humidity, and light/dark cycles. The animals were fed on standard feeds and water. All mice were acclimatized to the working (lab) environment one week prior to the experiment.

Preliminary Phytochemical screening of methanol leaf extract of *Boscia salicifolia*

The leaf extract and fractions of *B. salicifolia* were prepared in suitable forms for the screening of alkaloids, saponins, tannins, glycosides, flavonoids, and terpenes using standard laboratory procedures described by Trease and Evans (1989) and Sofowora (1993).

Acute toxicity studies of methanol leaf extract of *Boscia salicifolia*

The method previously described by Lorkes (1983) was adopted. In the first phase, the animals were divided into three groups of three mice each and were given 10, 100 and 1000 mg/kg body weight of the extract respectively. In the second phase, four mice were used and one each for 600, 1600, 2900 and 5000 mg/kg of the crude extract were administered to the animals orally.

Effect of Methanol leaf extract of *Boscia Salicifolia* on Castor oil-induced diarrhoea

This experiment was based on the procedure employed by Awouters *et al,* (1978), with minor changes. The study employed twenty-five Swiss albino mice that had been fasted for eighteen hours and divided into five groups of five mice each. Group I got 2 mL 0.9 % normal saline, whereas groups II, III, and IV received 380, 760, and 1140 mg/kg body weight of the methanol extract, respectively. The conventional anti-diarrheal medicine loperamide was given orally at a dose of 5 mg/kg body weight to Group V. All mice were given a one-hour oral pre-treatment before receiving the dose of 1.0 ml castor oil per oral. The animals were caged individually and examined for the occurrence of diarrhoea hourly for 5 hours following the castor oil challenge (Shaphiullah et al., 2003). Diarrhoea was defined as the presence of watery stool, staining the absorbent paper beneath the cage. The absence was recorded as diarrhoea protection and the percentage protection was calculated (Diurno et al., 1996). (Akah and Offiah, 1996)

Isolation and preparation of Rabbit jejunum

A modified technique by Amos *et al*., (1998) was used. The rabbits were euthanized, and the jejunum was quickly transferred into a petri dish containing Tyrode solution. Then sections of the jejunum were cut and separated from adhering tissues. Suitable lengths (2 cm)

were fixed with a tissue clamp and suspended in 25 mL organ bath (Ugo Basile S.R.L., Varese, Italy) containing Tyrode's solution and connected to a 7003-F force transducer model, the transducer was connected to an iworx Data capsule (17304 Ugo basili Data-capsule) attached with a USB cable to a laptop computer for data acquisition. A preload of 1 g was attached to the tissue. The experiment started by launching the labscribe software, and calibrated the units to grams force of contraction after the tissue was allowed to equilibrate for thirty (30) minutes, the preparation was washed three times during the thirty minutes period.

Procedure

With minor modifications, the method of Uchendu *et al* (1999) was used. On the isolated rabbit jejunum, the mechanism of *B. Salicifolia's* relaxant effect on acetylcholine-, histamine-, and serotonin-induced contractions was investigated. In the absence or presence of the extract The phytochemical screening of *B. salicifolia* extract and its fraction showed that it contained alkaloids, saponins, tannins, flavonoid, glycoside and terpenoids (Table 1).

(0.03-0.3 mg/ml), acetylcholine (Ach; 1 x 10-7 g/ml; 0.1 ml) was added to the bath.

Before washing the tissues, the recording was paused, and the tissues were allowed to recuperate. Each application of test chemicals was separated by 5 minutes. Instead of Acetylcholine, the experiment was performed with 0.2 ml of Histamine (1×10^{-5} g/ml) and 0.2 ml of Serotonin (1×10^{-5} g/ml). Acetylcholine (n=4), histamine (n=4), and serotonin (n=4) were used to measure the influence on gram force of contraction and maximal responses.

Statistical Analysis

Data were statistically analysed and expressed as the mean ± standard error of mean (SEM). Statistical significance was determined using one way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test at 95 % confidence interval using the SPSS statistical software package (v.20.0; SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

Table 1: Phytochemical constituents of Methanol leaf extract of *Boscia salicifolia*

TEST	Crude extract	n-Hexane	Chloroform	Ethyl acetate	N-butanol
Alkaloids	+	-	-	-	-
Flavanoids	+	-	-	+	+
Steroids	+	-	-	-	-
Saponins	+	-	-	-	+
Glycosides	+	-	-	-	+
Tannins	+	-	-	-	+
Terpenoids	+	+	+	-	-

Key: - = absent; + = present

Studies have revealed that tannins have an antispasmodic and muscle relaxant effect, flavonoid inhibits prostaglandin E_2-induced intestinal secretion, saponins inhibit histamine release and terpenoids inhibit the release of prostaglandins (Mekonnen *et al*., 2018). Therefore, the antidiarrheal activity of the plant extract may be attributed to these phytochemical constituents.

Spasmolytic effect was also shown by the methanol leaf extract of *B. salicifolia* acccording to Mekonnen *et al*., (2018) who reported the presence of alkaloids, flavonoids, saponins and tannins. Toxicity/safety assessment of the methanol leaf extract of *B. salicifolia* revealed that the LD_{50} (3800 mg/kg) was slightly toxic (Lorkes, 1983) (Table 2.0). The result indicated only one death at dose of 5000 mg/kg

Table 2: Acute toxicity studies of methanol leaf extract of *Boscia Salicifolia* administered orally to Swiss albino mice

Experiments	Doses (mg/kg bw)	No dead mice after 24 hrs
Phase I	10	0/3
	100	0/3
	1000	0/3
Phase II	1600	0/1
	2900	0/1
	5000	1/1

Acute toxicity was carried out in two Phases, each dose group of Phase-I made up of 3 mice those in phase-II have 1 mouse per group (LD_{50}= 3800mg/kg)

The methanol leaf extract of *B. salicifolia* significantly ($p< 0.05$) reduced diarrheal drops in castor oil treated mice in the five hours observation period compared with the

negative control, Timothy *et al.,* (2017) and Michel *et al.,* (2019) also obtained similar results with *Faiderhbia albida* and *Bixa orellana* respectively.

The doses (1140, 760 and 380 mg/kg) of the extract inhibited the incidence of diarrhea by 29.7, 40.5 and 45.9% respectively while loperamide inhibited diarrhea by 51.3% as shown in (Table 3.0).

Table 3: Effect of antidiarrheal activity of methanol leaf extract of *Boscia salicifolia* on castor oil-induced diarrhea in mice.

Treeatments	**Dose (*Mg/kg*)**	**Number of watery stools**				**% Inhibition**
		Hard	**Soft**	**Semi-watery**	**Watery**	
Normal Saline	2 ml	8.25±1.25	2.75±2.06	4.50±0.67	9.25±1.89	-
Loperamide	5	2.00±1.63*	2.25±0.95	4.75±2.06	4.75±0.96*	51.35135
Extract	380	4.50±2.52	2.00±1.82	3.75±1.70	4.25±2.22*	45.94595
Extract	760	4.00±2.45*	3.25±1.25	2.75±1.70	3.75±1.26*	40.54054
Extract	1140	3.50±1.72*	2.75±0.96	2.75±0.96	2.75±0.96*	29.72973

Results were expressed as mean ± standard error mean, (n=4) *=significant relative to normal saline used as control ($p < 0.05$). Normal saline was given at a dose of 2 ml/kg. Normal saline and Loperamide were used as control with n=4 for extract group.

The extract showing similar activity with loperamide when tested at 5 mg/kg. Loperamide, a widely used drug in the management of diarrhea was found to antagonize diarrhoea induced by castor oil similar to studies by Ahmadua *et al*., (2007) and Suleiman *et al*., (2017). Ricinolate is a major constituent of castor oil that is metabolized to ricinoleic acid which is responsible for the diarrhea property of castor oil (Mekeon *et al*., 1999). Ricinoleic acid stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also involves stimulation of release of endogenous prostaglandin E_2 increasing water secretion and diarrhea by over 80 % (Musa *et al*., 2008).

The methanol leaf extract of *B. salicifolia* significantly ($p< 0.05$) decreased the ACh, Histamine and 5-HT induced force of contraction on isolated rabbit ileum also similar with Suleiman *et al*., (2017). The extract exerted a reduction in spontaneous contraction of the rabbit jejunum induced by acetylcholine, histamine, and serotonin. The contractile effect of both acetylcholine (7×10^{-5} g/ml), histamine (1×10^{-7} g/ml) and serotonin (1×10^{-7} g/ml) were antagonized in a concentration dependent manner by the extract (Tables 4, 5 and 6)

Table 4: Effects of *B. salicifolia* on Acetylcholine-induced Contraction on rabbit ileum

Groups	Final bath Conc. (per ml)	Mean Force response (g)	Mean percentage Response (%)
Acetylcholine	0.7 ng	6.71±0.199	100
Boscia salicifolia + Acetylcholine	0.03 mg, 7 x 10^{-5} g	0.66±0.15*	9.84
Boscia salicifolia + Acetylcholine	0.07 mg, 7 x 10^{-5} g	0.58±0.03*	8.64
Boscia salicifolia + Acetylcholine	0.1 mg, 7 x 10^{-5} g	0.30±0.03*	4.47
Boscia salicifolia + Acetylcholine	0.3 mg, 7 x 10^{-5} g	0.24±0.01*	3.58

Values indicated by asterisks (*) are significantly different ($p<0.05$) compared to the acetylcholine (control) group using Dunnett's Post Hoc test.

Table 5: Effects of *B. salicifolia* on Serotonin-induced Contraction on rabbit ileum

Group	Final bath Conc. (per ml)	Mean Force response (g)	Mean Percentage response (%)
Serotonin	1 x 10^{-7} g	1.985±0.992	100
Boscia salicifolia + serotonin	0.03 mg 1 x 10^{-7} g	0.225±0.07*	11.34
Boscia salicifolia + serotonin	0.07 mg 1 x 10^{-7} g	0.07±0.01*	3.53
Boscia salicifolia + serotonin	0.1 mg 1 x 10^{-7} g	0.03±0.01*	1.51
Boscia salicifolia + serotonin	0.3 mg 1 x 10^{-7} g	0.04±0.01*	2.02

Values indicated by asterisks (*) are significantly different ($p<0.05$) compared to the acetylcholine (control) group using Dunnett's Post Hoc test.

Table 6: Effects of *B. salicifolia* on Histamine-induced Contraction on rabbit ileum

Group	Final bath Conc. (per ml)	Mean Force response (grams)	Mean Percentage Response (%)
Histamine	1 x 10^{-7} g	0.792±0.124	100
Boscia salicifolia + Histamine	0.03 mg. 1 x 10^{-7} g	0.501±0.102*	63.26
Boscia salicifolia + Histamine	0.07 mg, 1 x 10^{-7} g	0.211±0.04*	26.64
Boscia salicifolia + Histamine	0.1 mg, 1 x 10^{-7} g	0.158±0.03*	19.95
Boscia salicifolia + Histamine	0.3 mg, 1 x 10^{-7} g	0.128±0.04*	16.16

Values indicated by asterisks (*) are significantly different ($p<0.05$) compared to the acetylcholine (control) group using Dunnett's Post Hoc test

It is well known that when these agonists are added to a bathing medium containing rabbit jejunum, they illicit contractile responses, that the action of acetylcholine is mediated through M_2 and M_3 muscarinic receptors while 5-HT binds to specific receptors like 5-HT_3 and 5-HT_4 receptors to evoke gut motility and that the predominant contractile effect of histamine on gastrointestinal motility is via H_1 receptors (Blessing *et al.,* 2020).

The observed dose-dependent relaxant effects of the extract against contractile responses due to histamine, serotonin, and acetylcholine suggests that its antispasmodic effect may involve antagonizing both histaminergic, serotonergic and cholinergic receptors (Lee *et al.,* 1997; Brown and Taylor, 2006; Pasricha, 2006). This further corroborates the ability to protect the mice against diarrhea induced by castor oil. This study therefore suggests that *Boscia salicifolia* extract possesses anti-diarrheal and antispasmodic properties.

CONCLUSION

It could therefore be concluded that the Methanol leaf extract of *Boscia salicifolia* was found to be relatively safe in mice and confirmed to possess anti-diarrheal and gastrointestinal relaxant activity as revealed by reduction in castor oil-induced diarrheal drops and antagonizing histaminergic, serotonergic and cholinergic receptors. The presence of some phytochemical constituents was confirmed which includes; alkaloids, saponins, tannins, flavonoids, steroids, terpenoids and glycosides. Hence, this study supports the use of the plant in the treatment of diarrhea in the traditional settings.

REFERENCES

Armon K, Stephenson T, MacFaul R, Eccleston P, Werneke U. (2001). An evidence and consensus based guideline for acute diarrhoea management. *Archives of Disease in Childhood*; 85:132-42.

Awouters, F. C. J. E. Niemegeers, F. M. Lenaerts, and P. A. J. Janssen, 1978. "Delay of castor oil diarrhoea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis," *Journal of Pharmacy and Pharmacology*, vol. 30, no. 1, pp. 41–45.

Ahmadua, A. A., Zezi, A. U., and Yaro, A. H. (2007). Anti-diarrheal activity of the leaf extracts of Daniellia oliveri Hutch and Dalz (Fabaceae) and Ficus sycomorus Miq (Moraceae). *African Journal of Traditional, Complementary and Alternative Medicines*, *4*(4), 524-528.

Akah P.A and Offiah V.N (1996). Gastrointestinal effects of Allamanda cathartica leaf extracts. *International Journal of pharmacognosy*. 30; 213-217.

Amos, S., Okwuasaba, F.K., Gamaniel, K., Akah, P. and Wambebe, C. (1998). Inhibitor effects of the aqeous extract of *Pavetta crassipes* leaves on gastrointestinal and uterine smooth muscle preparations isolated from rabbits, guinea pigs and rats. *Journal of Ethnopharmacology,* 61**:** 209-213.

Balemba, O. B. Bhattarai Y, C. Stenkamp-Strahm, M. S. B. Lesakit, and G.M.Mawe, (2010.) "The traditional antidiarrheal remedy, Garcinia buchananii stem bark extract, inhibits propulsive motility and fast synaptic potentials in the guinea pig distal colon," *Neurogastroenterology & Motility*, vol. 22, no. 12, pp.1332–1339.

Brunton L.L, Parker K.L, Blumenthal D., and I. Buxton, (2008) "Treatment of disorders of bowel motility and water flux; antiemetics; agents used in biliary and pancreatic disease," in *Goodman and Gilman's Manual of Pharmacology and Therapeutics*, pp. 633–652, McGraw-Hill, New York, NY, USA,

Blessing O. Omolaso, Francis S. Oluwale, Olugbenga A. Odukanmi, Julius K. Adesanwo, Ahmed A. Ishola, Kayode E. Adewole. (2020). Evaluation of the gastrointestinal antimotility effect of Anacardium occidentale stem bark extract: a mechanistic study of antidiarrheal activity. *Journal of Pharmaceutical analysis,* 48:2095-1779

Brown, J.H., Taylor, P., (2006). Cholinergic agonist. In: Brunton L.L, Parker K., Lazo, J, S., *Pharmacological basis of Therapeutics.* 11th ed. McGraw-Hill, New York, pp, 183-200.

Danjuma M. N and Darda'u H (2013). An ethno-survey of medicinal rees of kabobi village, NorthenKatsina, Nigeria. *Academic Research International*. Vol.4.No.3 ISSN: 2223-9944

Diurno M. U, Izzo A. A, Mazzoni B, Bologgnese A, Capaso F (1996). Antidiarrhoeal activity of new Thiazolidinones related to loperamide. *J.pharm.Pharmacol*. 45:1054-1059.

Khan T, Ahmad M. Spasmolytic and spasmogenic activities of crude extract and subsequent fractions of Paeonia emodi. Pharmazie. 2007 Jun; 62(6):476-7. PMID: 17663201.

Lee, C.W., Sarna, S.K. Singaram, C., Casper, M.A., (1997). Ca^{2+} channel blockadw by verapamil inhibits GMCs and diarrhea during small intestinal inflammation. American Journal of Physiology 273, 785-794.

Lemmens, R.H.M.J. (2013). Boscia salicifolia Oliv. In: Schmelzer, G.H. & Gurib-Fakim, A. (Editors). PROTA (Plant Resources of Tropical Africa / Ressources végétales de

l'Afrique tropicale), Wageningen, Netherlands; p1-6.

Lorke D (1983). A new approach to acute toxicity testing. Arch Toxicol. 54: 275-287

Mekonnen, B., Asrie, A. B., & Wubneh, Z. B. (2018). Antidiarrheal activity of 80% methanolic leaf extract of Justicia schimperiana. *Evidence-Based Complementary and Alternative Medicine*, *2018*.

Mekeon, T.A., Lin, A. and Stafford, A.E. (1999). Biosynthesis of ricinoleate in castor oil. Advance Experimenatal and Medical Biology, Pp. 46437- 46447.

Michel archangel, Fokma Tagne, Hypolyte Akaou, Paul Aime Noubissi, Angele Foyet Fondigo. (2019). ''Effect of the hydroethanol extract of *Bixa Orellana* Linn (Bixacea) leaves on castor oil induced diarrhea in Swiss Albino Mice'' *Gastroenterology Research and Practice*, vol. 2019. Article ID 6963548, 8 pages

Musa, A. M., Sule, M. I., Haruna, A. K., Ilyas, M., Iliya, I., Yaro, A. H., and Magaji, M. G. (2008). Preliminary gastrointestinal studies of methanol extract of Indigofera pulchra willd in rodents. *Nigerian journal of pharmaceutical sciences*, *7*(1), 86.

Pasricha, P.J., (2006). Treatment of disorders of bowel motility and water flux. In: Brunton L.L, Parker K., Lazo, J, S., *Pharmacological basis of Therapeutics.* 11th ed. McGraw-Hill, New York, pp, 983-1008.

Suleiman, M. M., Oyelowo, B. B., Abubakar, A., Mamman, M., & Bello, K. T. (2017). A controlled study to investigate anti-diarrhoeal effect of the stem-bark fractions of *Terminalia avicennioides* in laboratory animal models. *International journal of veterinary science and medicine*, *5*(1), 14–22. https://doi.org/10.1016/j.ijvsm.2017.04.002

Shaphiullah, M., Bachar, S. C., Kundu, J. K., Begum, F., Uddin, M. A., Roy, S. C., & Khan, M. T. H. (2003). Antidiarrheal activity of the methanol extract of Ludwigia hyssopifolia Linn. *Pak J Pharm Sci*, *16*(1), 7-11.

Sofowara E.A (1993): Recent Trends in Research into African medicinal plants. *J. Ethnopharmacol,* 38: 209-214.

Suleiman Mohammed M, Oyelowo Balkisu B, Abubakar Ahmed, Mamman Mohammed, Bello Kamar-deen T. (2017). A controlled study to investigate anti-diarrhoeal effect of the stem-barkfractions of Terminalia avicennioides in laboratory animal models, *International Journal of Veterinary Science and Medicine* 5: 14–22.

Trease G.E and Evans M.D (1989). A Textbook of pharmacognosy, 13th

ed. Baillier, Tindal and Causee London, 144-689.

Timothy S.Y , Wazis C. H, Midala T.A.S , Joseph O.S , Sabastine A. Z , Nachanaa T , Oiza F.D. (2017). ‘’Evaluation of Anti-Diarrhoeal Activity of Different Bark Extracts of *Faidherbia albida* (Delile) A (Chav) in Albino Rats’’ *Bima journal of Science and Technnology* Vol. 1(2), 122-130

Ugboko, H. U., Nwinyi, O. C., Oranusi, S. U., and Oyewale, J. O. (2020). Childhood diarrhoeal diseases in developing countries. *Heliyon*, *6*(4), e03690.

Uchendu, C.N., 1999. Role of Ca^{2+} on uterine force stimulated by a glycoside from the root of *Dalbergia saxatilis*. Indian J. Physiol. Pharmacol., 43: 171-178.

Yakubu M. B, Usman I. A and Ado M. T (2014). Isolation and characterization of bioactive constituent of *Bosciasalicifolia Olive (Capparaceae).Nigerian Journal of chemical research.* Vol.19. pp 31-32