Magna Scientia Advanced Biology and Pharmacy

eISSN: 2582-8363

Cross Ref DOI: 10.30574/msabp

Journal homepage: https://magnascientiapub.com/journals/msabp/

(RESEARCH ARTICLE)

Check for updates

Evaluation of antidiarrheal properties of leaf extracts of *Acioa barteri* (Chrysobalanaceae) in Wister albino mice

Ogechi O. Anyanwu *, Chukwubuikem C. Okolo, Innocent N. Okpoli and Festus B.C Okoye

Department of Pharmaceutical and Medicinal Chemistry, Nnamdi Azikiwe University, Awka, Nigeria.

Magna Scientia Advanced Biology and Pharmacy, 2023, 09(02), 090–097

Publication history: Received on 28 June 2023; revised on 27 August 2023; accepted on 29 August 2023

Article DOI: https://doi.org/10.30574/msabp.2023.9.2.0052

Abstract

Diarrhea remains one of the most important health issues worldwide, with high morbidity and mortality rates, accounting for more than two million deaths annually. Oral rehydration therapy (ORT) is central to the management of diarrhea, and is sufficient to prevent complications due to dehydration in most patients while the disease runs its course. However, ORT has no effect on the duration of the disease or frequency of bowel motions, and any agent that could meet these needs would therefore be a useful addition to ORT. The aim of the study is to evaluate the antidiarrheal properties of the leaf extract of *Acioa barteri* (Chrysobalanaceae) in Winster albino mice. The pulverized leaves were extracted by cold maceration using methanol with for three (3) days.Castor oil (1ml/kg) was used to induce diarrhea in mice through the oral route of administration followed by two different doses of plant extract (250mg/kg and 500mg/kg) administered orally to the test groups while Loperamide (2mg/kg body weight) was used as the positive control. Gastrointestinal motility test (Charcoal meal test) and Castor oil induced enteropooliong were also used to evaluate the antidiarrheal activity of *A. barteri*. All samples were administered once, and the duration of the study was six hours. The result shows that in castor oil induced diarrhea, the extract, 250mg/kg and 500mg/kg showed significant inhibition of defecation by 43.52 % and 71.48 % respectively and 2mg/kg of loperamide showed an inhibition of 70.80 %. The gastrointestinal motility test of the extract, 250mg/kg and 500mg/kg showed significant inhibition of defecation by 40.66% and 80.48% respectively while 2mg/kg of loperamide showed an inhibition of 78.92 %. From the result of the castor oil induced enteropooling, the extract, 250mg/kg and 500mg/kg showed significant inhibition of defecation by 51.27 % and 82.41 % respectively and 2mg/kg of loperamide showed an inhibition of 79.89%. The result clearly indicates antidiarrheal properties of Acioa barteri leaf extract, which could be a therapeutic option against diarrheal disease and useful therapeautic addition to the oral rehydration therapy.

Keywords: Antidiarrheal; *Acioa barteri;* Gastrointestinal; Castor oil; Loperamide

1. Introduction

Diarrheal (which children are more susceptible to) is the second leading causes of death of children under five years old especially in developing countries (Saralaya *et al*., 2010; Lucas & Gilles, 2009; Gupta &Mahajan, 2005). Diarrhea is gastrointestinal disorder, characterized by an increase in stool frequency and change consistency (Amole *et al*., 2010), and increased frequency of bowel movements, wet stool and abdominal pains (Ezekwesili *et al*., 2004; Wang *et al.*, 2015). Recently, international organizations encourage studies pertaining to the treatment and prevention of diarrheal diseases using traditional medical practices (WHO, 2004: Tannaz *et al*., 2010; Park, 2000). Pathologically, Diarrhea can be interpreted as an incidence of daily stool exceeding 200 g comprised of 60 to 95% of water (Maes *et al*., 2003). Diarrhea occurs as the result of a nonspecific intestinal response to various stimuli, including infections, drugs, and inflammatory bowel disease. Enteropathogens like Escherichia coli, Shigella flexneri, Salmonella typhi, Staphylococcus aureus, and Candida albicans are the major causative agents that can provoke diarrhea (Konaté *et al*., 2015; Thapar & Sanderson, 2004). Diarrhea occurs when absorptive and secretory changes increase the volume of water that enters the

* Corresponding author: Ogechi O Anyanwu

colon to a level beyond its absorptive capacity (Dupont and Vernisse, 2009). According to researchers (Alam & Ashraf, 2003; Field, 2003; Morley, 2000), diarrhea arises always as a result of one or more of four basic mechanisms: osmotic diarrhea, secretory diarrhea, exudative diarrhea, and diarrhea associated with motility disorders. Developing countries are particularly vulnerable to the consequences of diarrhea (Dalby-Payne, 2004). Diarrhea can be triggered by many different factors, such as infections, food intolerance, intestinal disorders, among others (Young and Schmidt, 2004; Pimentel et al., 2010; Al Jarousha et al., 2011; Barnoy et al., 2011), and also occurs as a symptom of many other diseases including diabetes mellitus, inflammatory bowel disease, etc. (Farthing, 2000; Benson et al., 2004; Semenya and Maroyi, 2012). Currently, the treatment for diarrhea is non-specific and is usually aimed at reducing the discomfort and inconvenience of frequent bowel movements (Brunton, 2008; Suleiman et al., 2008). Bioactive constituents are responsible for the antiinflammatory actions of *Jatropha curcas* (Anyanwu *et al*., 2018). Natural products from plants have served human beings as a natural source for treatments and therapies from ancient times (Okolo *et al*., 2022).

Acioa barteri (Chrysobalanceae) has higher medicinal value, can be extensively employed to extract the natural compounds which are beneficial to human being. The genus *Acioa* is used as a chewing stick (Okwu and Ekeke, 2003) and the methanolic extracts effect on the hepatocellular damage and lipid profile have been reported (Aloh *et al*., 2015). The leaves extract of *Acioa barteri* is rich in phytochemicals and revealed the presence of tannins, saponins, terpenoids, flavonoids, alkaloid, steroids and cardiac glycosides (Anyanwu *et al*., 2020). *Acioa barteri* seemed to be less toxic justifying from its wide use in folklore medicine. Indeed, the leaves extract (250mg/kg and 500mg/kg) showed no toxicity in experimental mice. After 24 hours and 48 hours of administration of the extract (250mg/kg and 500mg/kg) in group 1 and 2, there was no significant difference from the mass (weight) of the animals compared to control respectively, but group 3 showed significant difference after administration of extract at dose of 1000mg/kg compared to control. However at a higher dose, 1000mg/kg, the methanol leaves extract initiated hypersensitivity, cytotoxicity and increased aggressiveness and eventually caused mortality of experimental albino mice. This toxicity involves a drastic reduction ($p<0.05$) in the activities of the alkaline phosphate (ALP), aspertate transaminase (AST) and Alanine transminase (ALT) in the liver (Anyanwu *et al*., 2020).

2. Material and methods

2.1. Plant Collection and Identification

Fresh leaves of *Acioa barteri* plants were collected from Amudi village, Ezinihitte Mbaise in Imo State, Nigeria. The leaves were identified and authenticated by Dr. Garuba Omosun of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia state.

2.2. Extraction of Plant Material

A method described by Anyanwu *et al*., 2020 was used for the plant extraction. The cleaned, healthy plant leaves were washed, dried at room temperature and pulverized. About 1500g of the pulverized leaves was subjected for extraction of active constituents by cold maceration using methanol as the solvent. The extraction was allowed to stand for 72 hours (3 days) with slight and timely shaking. The resultant mixture was first filtered through muslin cloth and subsequently filtered with Whatman (No. 1) filter paper. The filtrate was then concentrated using a rotary evaporator maintained at temperature 40℃ -50℃ to obtain a constant weight of the extract.

2.3. Experimental animals

Albino Swiss mice (15–25 g) and Wistar rats (120-150 g) of both sexes were obtained from the colony breed of the animal house of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka Anambra State. Animals were handled in compliance with the National Institute of Health guidelines for the care and use of laboratory animals (Pub. No. 85-23, revised 1985).

2.4. Animal grouping and dosing

The experimental animals were randomly divided into four groups (Negative control, positive control and two test groups) comprising of five animals in each group. Negative controls received the vehicle (10 ml/kg, distilled water) and positive controls receive Loperamide (2 mg/kg). The test groups (group 3 and 4) received different doses (low and high extract in mg/kg).

2.5. Determination of antidiarrheal activity

2.5.1. Castor oil induced diarrhea

The method described by Ezekwesili *et al*., 2010 was employed for this study with little modifications. Four groups of swiss albino mice were used (n = 5). Castor oil (1ml/kg) was used to induce diarrhea in mice through the oral route of administration.

After one hour of castor oil treatment, two different doses of plant extract (250 and 500 mg/kg body weight orally) were administered orally to the test groups. Groups 1 received normal saline orally (1ml/kg) and serve as control, while group 2 serving as standard, received Loperamide (2mg/kg body weight). Group 3 and 4 were treated orally with 250 and 500 mg/kg body weight of the extract. The animals were housed in individual metal cages lined with white non-absorbent paper. Faecal output was assessed by collecting the faecal material for 6 hours after drug administration, dried for 2 hours then weighed. The percentage feacal output (% FOP) was calculated using the formula below:

$$\% \text{ FOP } = \frac{\text{Ft}}{\text{Fc}} \text{x } 100$$

Where Ft = mean faecal weight of each group, Fc = faecal weight of control group

$$\% \text{ Inhibition of defecation } = \frac{\text{Mo} - \text{M}}{\text{Mo}} \text{X } 100$$

Where, Mo: Mean defecation of control, M: Mean defecation of test sample/standard drug.

2.5.2. Gastrointestinal motility test (Charcoal meal test)

The effect of *A. barteri* on normal gastrointestinal motility was assessed using the methods described by Ezekwesili *et al*., (2010). Four groups of albino mice were used for the experiment (n = 5 each). The animals were fasted for 24 hours with access to water before the experimental interventions. Group 1 received normal saline (1ml/kg body weight) which served as control; group 2 received 2mg/kg body weight of standard drug (loperamide) while group 3 and 4 were treated orally with 250 and 500 mg/kg body weight of the extract . After 5 minutes, 1ml of charcoal meal (5 % charcoal suspension in 2% aqueous tragacanth) was administered orally to each animal. The mice were sacrifice by cervical dislocation followed by opening of the abdomen and the entire length of the intestine was removed and placed lengthwise on a white paper and intestinal distance moved by the charcoal meal from pylorus to caecum was measured after one hour.

The peristaltic index and percentage of inhibition was calculated by using formula according to Than *et al*., 1989.

$$\text{Peristalsis index } = \frac{\text{Distance travelled by charcoal meal}}{\text{Length of small intestine}} \text{X } 100$$

$$\% \text{ inhibition } = \frac{\text{Dc} - \text{Dt}}{\text{Dc}} \text{X } 100$$

Where, Dc: Mean distance travelled by the control, Dt: Mean distance travelled by the test group.

2.5.3. Castor oil induced enteropooling

The effect of extract on intraluminal fluid accumulation was determined using the method described by Robert *et al*., 1976. Swiss albino mice were grouped and treated with castor oil as follows: Group 1 received normal saline (1ml/kg body weight) which served as control; group 2 received 2mg/kg body weight of standard drug (loperamide) while group 3 and 4 were treated orally with 250 and 500 mg/kg body weight of the extract.

One hour after treatment, the mice were sacrificed by cervical dislocation. The abdomen of each mouse was opened and the whole length of the intestine, from the pylorus to the caecum, was ligated, dissected and carefully removed. The small intestine was weighed, and the intestinal content was collected by milking into a graduated tube to measure the volume. The empty intestine was reweighed and the difference between the two weights was calculated. Finally, the percentage of reduction of intestinal secretion and weight of intestinal contents was determined by using the following formulas.

$$\% \text{ of inhibition by using MVSIC } = \frac{\text{MVICC} - \text{MVICT}}{\text{MVICC}} \text{X } 100$$

Where MVSIC is the mean volume of the small intestinal content, MVICC is the mean volume of the intestinal content of the control group, & MVICT is the mean volume of the intestinal content of the test groups.

$$\% \text{ of inhibition by using MWSIC } = \frac{\text{MWICC} - \text{MWICT}}{\text{MWICC}} \text{X } 100$$

Where MWSIC is the mean weight of the small intestinal content, MWICC is the mean weight of the intestinal content of the control, and MWICT is the mean weight of the intestinal content of the test group.

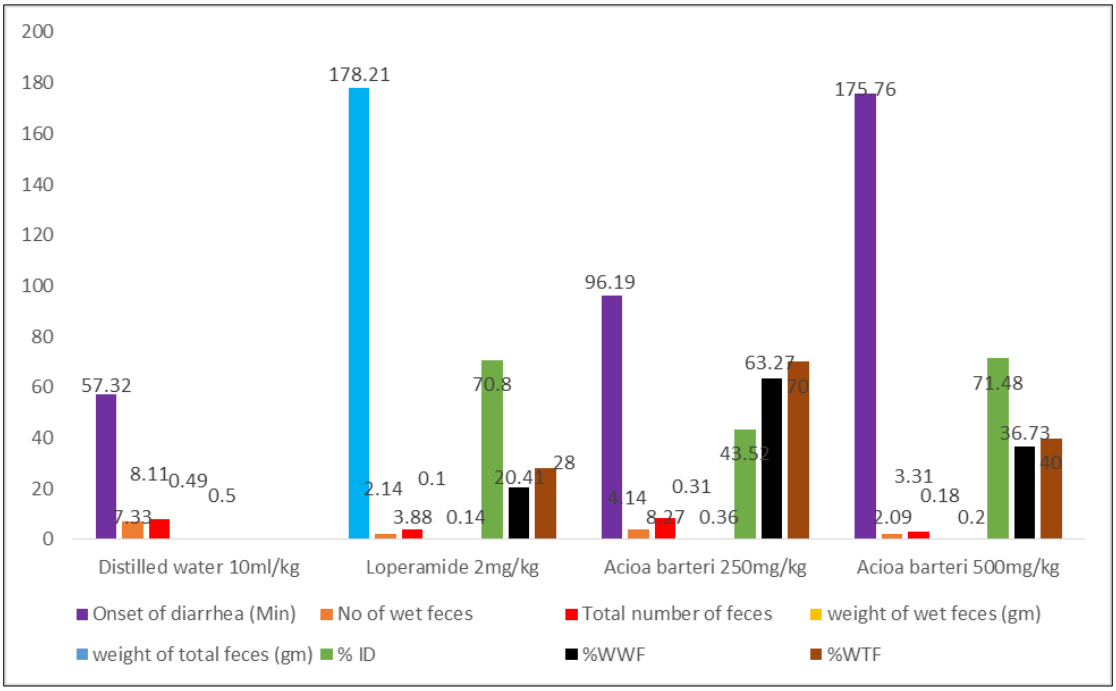
2.6. Statistical Analysis

Results were analyzed using One Way ANOVA (Tuckey post hoc test) and expressed as mean ± SEM (n = 5). A p value less than 0.05 were considered statistically significant.

3. Results

3.1. Castor oil induced diarrhea

Diarrhea was seen in all control animals after 1 hour of castor oil administration. Significant ($p<0.05$) inhibition of diarrheal effects was produced by the ingestion of different dose of methanol leaf extract of *Acioa barteri* across the treatment groups. The 500mg/kg of *Acioa barteri* gave the highest activity inhibitions of 71.48%. This inhibition displayed was higher than that of 2mg/kg of the standard antidiarrheal drug loperamide (70.80%). A moderate but significant inhibition was as well achieved by 250mg/kg (43.52%). The result is shown in Figure 1 below.

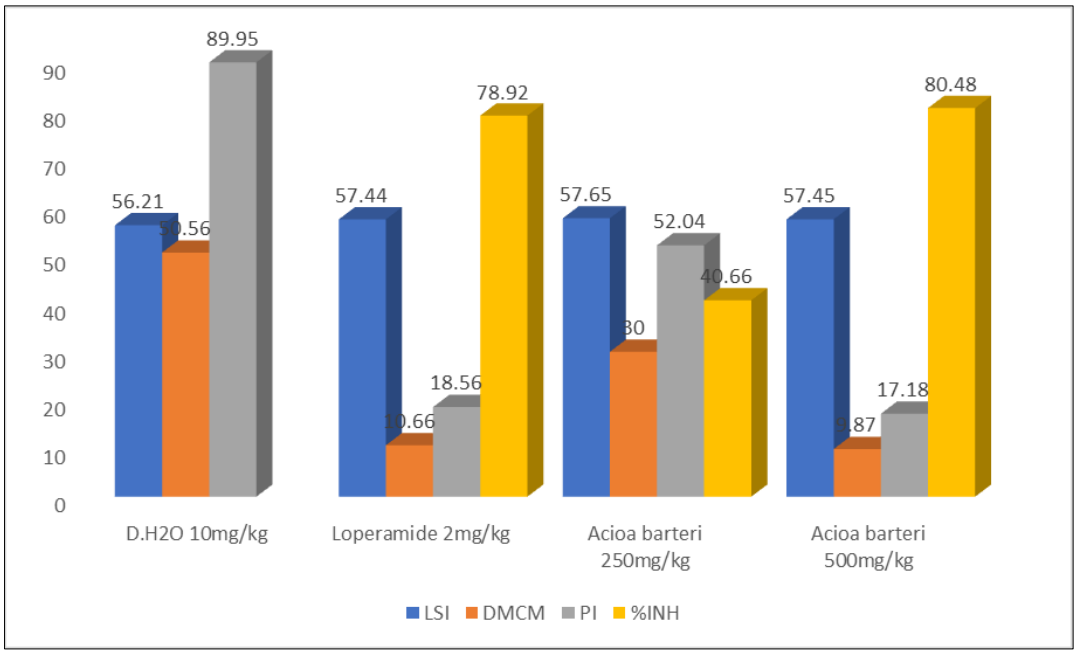


% ID= % inhibition of diarrhea %WWF= % weight of wet feces WTF= % weight of total feces

Figure 1 Antidiarrheal effects of methanol leaves extract of *Acioa barteri* on castor oil induced diarrheal model in mice

3.2. Gastrointestinal motility test (charcoal meal test)

The extract significantly inhibited the intestinal transit of charcoal meal at all tested doses. The data revealed that the percentage reduction of gastrointestinal transit of charcoal was 40.66% ($p < 0.05$) and 80.48% ($p <0.001$) at doses of 250 mg/kg and 500 mg/kg respectively. The entire result is dose dependent as shown in figure 2 below.

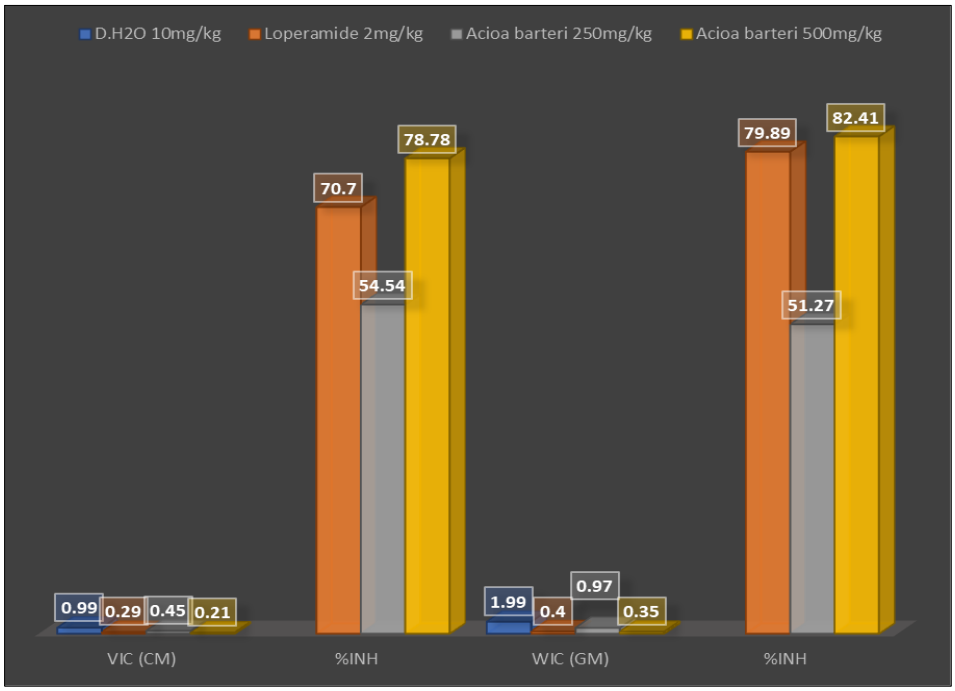


LSI= Length of small intestine, DMCM= Distance moved by charcoal meal, PI= Peristatic index and %INH= % inhibition

Figure 2 Effect of methanol leaves extract of *Acioa barteri* on castor oil induced intestinal transit in mice

3.3. Castor oil induced enteropooling

In this test, the extract showed a significant reduction in both the average volume and weight of intestinal contents (AVIC and AWIC) at all tested doses compared to control. The percentage inhibition of volume of intestinal contents was found to be 54.54% ($p < 0.05$), 78.78% ($p < 0.001$), at doses of 250 mg/ kg and 500 mg/kg respectively. The result is dose dependent as shown in figure 3.



VIC= Volume of intestinal contents (ml), WIC= Weight of intestinal contents (gm) and %INH= % inhibition

Figure 3 Effects of methanol leave extract of *Acioa barteri* on gastrointestinal fluid accumulation in mice

4. Discussion

There was a dose-dependent decrease in the number of wet faeces passed in castor oil-induced rats by 43.52% and 71.48% at 250mg/kg and 500 mg/kg, respectively. However, at equivalent concentration of the extract and loperamide, 2mg/kg body weight, loperamide appeared to produce a greater inhibition than 250mg/kg of the extract, but lesser inhibition than 500mg/kg of the extract. The results obtained showed that the extract of *Acioa barteri* has a great potential to reduce the frequency of stool at doses comparable to that of the conventional drug used as control. The presence of castor oil in the guts stimulates the secretion of prostaglandins through the activation of COX receptors, which in turn induces gastrointestinal motility. Hypermotility is one of the pathophysiological conditions that characterize diarrhea and hence positive result to it is a direct indication of the pathological condition. The charcoal meal test was carried out to determine the effect of *Acioa barteri* extract on gut motility. Loperamide was used as a positive control because it has been known as a conventional antidiarrhoea agent and hence, an inhibitor of castor oil-induced diarrhea. As shown in figure 2, it was observed that the extract of *Acioa barteri* reduced the intestinal motility of charcoal meal by 40.66% and 80.48% for the 250mg/kg and 500 mg/kg doses of the extract respectively as compared to an inhibition of 78.93% by 2 mg/kg dose of loperamide. A reduction in intestinal transit time shows that the extract was able to reduce the motility of the gut and thus reduce diarrhea. Normal saline, which was the negative control, showed no inhibition, as it will normally not have effects on diarrhea. The result also showed that at 2 mg/kg body weight of loperamide and 250mg/kg of the extract, the level of inhibition produced by the extract was lesser than that produced by loperamide, and at 2mg/kg of loperamide and 500mg/kg of the extract, the level of inhibition produced by the extract was greater than that produced by loperamide. From the result on castor oil induced enteropooling (gastrointestinal fluid accumulation), it is seen in figure 3 that the percentage inhibition of the volume of intestinal content by 2mg/kg of loperamide, 250mg/kg and 500mg of the extract were 70.70%, 54.54% and 78.78% respectively. Also, the percentage inhibition of the weight of intestinal content of 2mg/kg of loperamide, 250mg/kg and 500mg/kg of the extract were 79.89%, 51.87%, 82.41% respectively. A reduction in the volume and weight of intestinal content shows that the extract has the propensity to reduce or inhibit diarrhea.

5. Conclusion

The *in vivo* antidiarrheal activities of methanol extract of *A. barteri* leaf were successfully determined and measured with respect to normal saline control, using castor oil induced diarrhea, gastrointestinal motility test and castor oil induced enteropooling. The results of the study suggest that methanol extract of the leaves of *Acioa barteri* possesses antidiarrheal properties evidenced by the percentage inhibition of wet faces, intestinal motility of charcoal meal, volume and weight of intestinal content correspond with an increased dose of the crude extract. According to the results obtained, the dose of the extract when compared to the dose of the standard drug produces equivalent effect.

Compliance with ethical standards

Disclosure of conflict of interest

The author declares no conflict of interest.

References

[1] Adeyemi, O. O., Akindele, A. J. (2008). Antidiarrhoeal activity of the ethyl acetate extract of *Baphia nitida* (papilionaceae). Journal of Ethnopharmacology, 116:407

[2] Al Jarousha, A. M., ElJarou, M. A., and ElQouqa, I. A. (2011). Bacterial enteropathogens and risk factors associated with childhood diarrhea. Indian Journal of Pediatrics; 78, 165–170. doi: 10.1007/s12098-010-0249-0

[3] Alam, N. H., Ashraf, H. (2003). Treatment of infectious diarrhea in children. Pediatric Drugs, 5 (3): 151-65

[4] Aloh, G. S., Obeagu, E. I., & Kanu, S. N. (2015). Effects of methanol extract of Acioa barteri on Hepatocellular damage and lipid profile of albino rat.

[5] Amole, O. O., Salahdeen, H. M., Onyeahialam, A. E. (2010). Evaluation of the antidiarrheal effect of Lannea welwitschii Hiern (Anacardiaceae) bark extract. African Journal of Pharmacy and Pharmacology, 4(4): 165-169.

[6] Anyanwu, O. O., Ngwoke, K. G., & Okoye, F. B. C. (2018). Bioactive constituents responsible for the antiinflammatory actions of Jatropha curcas. Journal Medicinal Botany, 2, 07-15.

[7] Anyanwu O.O, Barikor G.T, Okoye F.B.C (2020) Preliminary Phytochemical and Acute Toxicity Studies of Methanol Leaf Extract of *Acioa barteri.* Pharm Research, (4) 1: 2574 - 777

[8] Barnoy, S., Baqar, S., Kaminski, R. W., Collins, T., Nemelka, K., Hale, T. L., et al. (2011). Shigella sonnei vaccine candidates WRSs2 and WRSs3 are as immunogenic as WRSS1, a clinically tested vaccine candidate, in a primate model of infection. Vaccine 29, 6371–6378. doi: 10.1016/j.vaccine.2011.04.115

[9] Benson, A. B., Ajani, J. A., Catalano, R. B., Engelking, C., Kornblau, S. M., Martenson, J. A. Jr., et al. (2004). Recommended guidelines for the treatment of cancer treatment-induced diarrhea. Journal of Clinical Oncology: 22, 2918–2926. doi: 10.1200/JCO.2004.04.132

[10] Brunton, L. L. (2008). Agents for Control of Gastric Acidity and Treatment of Peptic Ulcers Goodman Gilman's The Pharmacological Basis of Therapeutics, 11th Edn. New York, NY: McGaw-Hill, 623–652.

[11] Dalby-Payne, J., Elliott, E. (2004). Gastroenteritis in Children. Clinical Evidence, 12: 443-54.

[12] Dupont, C., & Vernisse, B. (2009). Anti-diarrheal effects of Diosmectite in the treatment of acute diarrhea in children. Pediatric Drugs, 11(2), 89-99.

[13] Ezekwesili, C. N., Obiara, K. A., and Ugwu, O. P. (2004). Evaluation of antidiarrheal properties of crude aqueous extract of Ocimum gratissimum L. (Labiatae) in rats. Biochemistry 16, 122–131.

[14] Ezekwesili, J. O., Nkemdilim, U. U., & Okeke, C. U. (2010). Mechanism of antidiarrhoeal effect of ethanolic extract of Psidium guajava leaves. *Biokemistri,* 22(2):87.

[15] Farthing, M. J. (2000). Diarrhoea: a significant worldwide problem. International Journal of Antimicrobial Agents; 14, 65–69. doi: 10.1016/S0924-8579(99)00149-1

[16] Field, M. (2003). Intestinal ion transport and the pathophysiology of diarrhea. Journal of Clinical Investigation, 111 (9): 931-43

[17] Gupta, M.C. &Mahajan, B.K. (2005). Textbook of Preventive and Social Medicine (3rd ed.) India. Jaype e Brothers medical Publisher (P) Ltd.

[18] Konaté, K., Yomalan, K., Sytar, O., Brestic, M. (2015) Antidiarrheal and antimicrobial profiles extracts of the leaves from Trichilia emetica Vahl. (Meliaceae). Asian Pacific Journal of Tropical Biomedicine, 5(3):242–8. https://doi.org/10.1016/ S2221-1691(15)30012-5

[19] Lucas, A. O. & Gilles, H.M. (2009). A new short textbook of preventive medicine for the tropics. Ibadan: Bounty Press Limited.

[20] Maes, B. D., Dalle, I., Geboes, K., Oellerich, M., Armstrong, V. W., Evenepoel, P., ... & Vanrenterghem, Y. F. C. (2003). Erosive enterocolitis in mycophenolate mofetil-treated renal-transplant recipients with persistent afebrile diarrhea. Transplantation, 75(5), 665-672.

[21] Morley, D. (2000). Paediatric Priorities in the developing world. London. Butter worth.

[22] Okolo, C. C., Mbachu, N. A., Anyanwu, O. O., Ngwoke, K. G., & Okoye, F. B. (2021). Screening for anti-inflammatory activity of extract and fractions of Morinda lucida stem bark and detection of bioactive secondary metabolite. *GSC Biological and Pharmaceutical Sciences*, *16*(1), 031-039.

[23] Okwu, D. E., & Ekeke, O. (2003). Phytochemical screening and mineral composition of chewing sticks in South Eastern Nigeria. Global Journal of Pure and Applied Sciences, 9(2), 235-238.

[24] Park K. (200). Park's text book of preventive and social medicine. Jabalpur: M/s Banarsidas Bharat Publishers, p. 172-175.

[25] Pimentel, M., Hwang, L., Melmed, G. Y., Low, K., Vasiliauskas, E., Ippoliti, A., et al. (2010). New clinical method for distinguishing DIBS from other gastrointestinal conditions causing diarrhea: the LA/IBS diagnostic strategy. Digestive Disease and Sciences: 55, 145–149. doi: 10.1007/s10620-008- 0694-z

[26] Robert, A., Nezamis, J. E., Lancaster, C., Hanchar, A. J., Klepper, M. S. (1976). Enteropooling assay: a test for diarrhea produced by prostaglandins. Prostaglandins, 11(5):809–28.

[27] Saralaya, M. G., Patel, P., Roy, M. P. S. P., & Patel, A. N. (2010). Research article antidiarrheal activity of methanolic extract of moringa oleifera lam roots in experimental animal models. International Journal of Pharmaceutical Research, 2(2), 25-29.

[28] Semenya, S. S., and Maroyi, A. (2012). Medicinal plants used by the Bapedi traditional healers to treat diarrhea in the Limpopo province, South Africa. Journal of Ethnopharmacology, 144(2) 395–401. doi: 10.1016/j.jep.2012.09.027

[29] Suleiman, M. M., Dzenda, T., and Sani, C. A. (2008). Antidiarrhoeal activity of the methanol stem-bark extract of Annona senegalensis pers. (Annonaceae). Journal of Ethnopharmacology, 116, 125–130. doi: 10.1016/j.jep.2007. 11.007.

[30] Tannaz, B., Poonam, D., Brijesh, S., Pundarikakshudu, T., Arvind, N., Noshir, A. (2010). Newer insights into the mechanism of action of Psidium guajava L. leaves in infectious diarrhoea. BMC Complement Alternative Medicine, 10: 33.

[31] Thapar, N., Sanderson, I. R. (2004). Diarrhoea in children: an interface between developing and developed countries. Lancet, 363 (9409): 641-53.

[32] Wang, S., Zhao, Y., Zhang, J., Huang, X., Wang, Y., Xu, X., et al. (2015). Antidiarrheal effect of Alpinia oxyphylla Miq. (Zingiberaceae) in experimental mice and its possible mechanism of action. Journal of Ethnopharmacology, 168, 182–190. doi: 10.1016/j.jep.2015.03.066

[33] World Health Organization. World health report[R]. Geneva: WHO; 2004, p. 120-125.

[34] Young, V. B., and Schmidt, T. M. (2004). Antibiotic-associated diarrhea accompanied by large-

[35] scale alterations in the composition of the fecal microbiota. Journal of Clinical Microbiology: 42, 1203–1206. doi: 10.1128/JCM.42.3.1203- 1206.2004

[36] WHO (2004). The treatment of Diarrhoea. A manual of physicians and other Health workers' Geneva: World Health Organization.