Acute Toxicity and in-Vivo Antidiarrhoeal Activity of Leaf Extracts of Acacia Nilotica from Nasarawa west, Nasarawa State, Nigeria

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ABSTRACT

The *in vitro* antibacterial activity of leaf extracts of *Acacia nilotica* has been demonstrated by several studies, including ours. Scientific evidence for in vivo use and safety need to be investigated. This study evaluates the acute toxicity and *in vivo* antidiarrhoeal activity of crude methanolic, aqueous, ethyl acetate and n-hexane leaf extracts of *Acacia nilotica* from Nasarawa West, Nasarawa State, Nigeria. The acute toxicity and lethality (LD_{50}) were determined in mice using standard protocol. Mice infected with diarrhoea-causing bacteria *Escherichia coli*, *Salmonella paratyphi* and *Shigella dysenteriae* to induce diarrhea were treated with various doses of the extract, and observed for their response. All the crude extracts were acutely safe at the doses tested as there was zero death of the mice throughout the two weeks of the experiment. The oral LD_{50} is therefore, greater than 2000 mg/kg body weight. The crude extracts at 400 mg/kg and 200 mg/kg demonstrated antidiarrheal effect on bacteria-induced diarrhea in mice by their ability to significantly delay in the onset of diarrhea compared to the control group. The total number of stools (faeces) was significantly reduced and the amount of wet stool, which is influenced by fluid secretion, was significantly reduced in all the extract-treated groups compared to the control group. The results have shown that the leaf extracts of *Acacia nilotica* tested are both safe and have great potential in the treatment of diarrhoeal infection.

Keywords: Antibacterial, *Acacia nilotica*, Treatment, Diarrhea.

INTRODUCTION

Diarrhea, defined as a condition with a symptom of having 3 or more loose or liquid bowel movements per day or more frequently than normal for the individual, is one of the most common diseases for all age groups [1]. In 2019, an estimated 5.2 million children under 5 years died mostly from preventable and treatable causes; children aged 1 to 11 months accounted for 1.5 million of these deaths while children aged 1 to 4 years accounted for 1.3 million deaths; newborns (under 28 days) accounted for the remaining 2.4 million deaths [2]. Diarrhea can arise from a multiple of causes including bacterial, parasitic or viral infections, indigestion, or can be a symptom of diseases like cholera [3]. Common bacterial causes of diarrhea in man include *Aeromonas*, *Cryptosporidium*, *Campylobacter*, *Salmonella*, *Shigella*, *Vibrio* as well *Escherichia coli* [4,5]. Waterborne diseases including infectious or noninfectious diarrhea is one of the leading causes of morbidity and mortality in developing countries in which Nigeria and Africa are not exempted [6].

Antibiotics are the major remedy of infections like diarrhea, however significant increase in antibiotics resistance has been observed in common human pathogens worldwide [7]. The use of herbal drugs in the treatment of diarrhea is a common practice in many countries of Africa. These plants, which abound in the environment, enjoy wide acceptability by the population and serve as alternatives to orthodox medicines.

Importance of the traditional indigenous medicines greatly emphasized by the World Health Organization (WHO), as these medicines are being used by many rural people in the developing countries for the first safety in health care till now [2]. A diarrhoeal disease control programme, including indigenous medical therapy along

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with evaluation of health education and prevention approaches, has recently been launched by WHO [8]. There is sufficient support of national and international organizations for the studies on treatment of diarrhoeal diseases where medicinal plants are becoming hopeful source of antidiarrhoeal drugs Therefore, indigenous medicinal plants are playing significant alternative role to antibiotics [9].

Plants have shown *in vitro* antimicrobial activities against diarrhea-causing bacteria [10,11]; and the *in vivo* value against bacteria-induced diarrhea in animal studies had been evaluated to confirm its potential medicinal use. Plants have chemical constituents, and are intrinsically toxic [12]. Hence, the need to evaluate their safety in animal studies, to support their possible use as herbal remedies against diseases. Toxicity is defined as the relative ability of a substance to cause adverse effects in living organisms [13]. Toxicity studies may be classified as acute, subacute/subchronic and chronic effects depending upon the quantity and duration of administration of the agents [14]. Acute toxicological studies investigate the toxic effects (harm or death) produced by a single large-dose exposure to a toxicant lasting no longer than 24 h. The starting point for toxicological classification of chemicals uses the LD_{50} value, which is the dose administered in acute toxicity testing that causes death in 50% of experimental animals [15]. This study investigates the acute toxicity and *in vivo* antidiarrheal potential of leaf extracts of *Acacia nilotica* collected from Nasarawa West Senatorial District (Nasarawa West), Nasarawa State, Nigeria.

MATERIALS AND METHODS

A. Test Plant Extract

Crude methanolic, aqueous, ethyl acetate and n-Hexane leaf extracts of *Acacia nilotica* collected from Nasarawa West Senatorial District (Nasarawa West), Nasarawa State, Nigeria were used for the study using protocol described by [11]. The extracts were stored at 4°C until required for use.

B. Acute Toxicity Testing

1. Bacterial Test Strains

The bacterial Test Strains used in the study, *Escherichia coli* (ATCC 25922), *Salmonella paratyphi* and *Shigella dysenteriae,* were obtained from the Department of Microbiology and Biotechnology at the National Institute for Pharmaceutical Research and Development (NIPRD) Abuja. All isolates were preserved in Tryptone Soy Broth (TSB: Oxoid Ltd., England) containing 15% glycerol, and stored at -80°C. When needed for use, the isolates were revived by streaking on Tryptone Soy Agar (TSA: Oxoid Ltd., England) plates and incubating at 37°C for 24 hours. The isolates were confirmed by their morphological, cultural and biochemical characteristics.

2. Preparation of Inoculum

Fresh cultures were prepared from frozen stocks every two weeks on TSA overnight at 37°C. A single colony was inoculated into a 10 ml TSB media and incubated at 37°C for 24 h. The suspension was standardized according to the Clinical and Laboratory Standards Institute (CLSI) with sterile normal saline (0.9% NaCl in water) to turbidity equivalent to 0.5 McFarland scale approximately1-2×10^8cfu/ml [16] and diluted further to 1 x 10^6cfu/ml.

C. Acute Toxicity Testing

The acute toxicity of the extracts was determined using healthy female albino mice (18 -24 g). The mice were housed under standard environmental condition of temperature (26±2^{o}C), relative humidity, and 12-hour light/dark cycle; and were fed with standard rodent diet and allowed free access to water *ad libitum.* All experiments were performed in accordance with Standard Operating Procedures for Studies involving whole animal as verified by the NSUK Animal Care and Use Research Ethics Committee (Appendix 1).

The method of Oral acute toxicity test was conducted in accordance to the Organization for Economic Cooperation and Development (OECD) Test Guidelines 425 limit test method at a dose of 2000 mg/kg [17].

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Twenty Female mice were collected and fasted overnight. The mice were weighed and randomized into 4 groups of 5 mice each. Each group received a single dose of test extracts at 2000 mg/kg body weight while the second (control) received water at volumes of 10 ml/kg body weight. Animals were observed during the first 8 hours with special attention given to the first critical four hours, and periodically for the next 24 hours and then daily for 14 days. Parameters observed in each mouse included general signs and symptoms of toxicity such as abdominal writhing, hyperactivity, sedation, convulsion, tremors, diarrhoea, grooming, paralysis and death after drug administration [18].

D. Evaluation of the in vivo antidiarriaheal activity of the crude extracts using an animal model

The mice were randomly grouped into 4 groups, each containing 5 mice. These groups were designated as negative controls (group I), positive control and the remaining three as treatment groups. Negative control mice were treated with 2% tween 80 (10 ml/kg). At the same time, positive controls were treated with loperamide (Brawn Laboratories Ltd, India) (3 mg/kg) for bacterial-induced diarrhea. The three treatment groups were treated with doses of 200 mg/kg (group IV), 400 mg/kg (group III), and 800 mg/kg (group II) of crude extracts of *Acacia nilotica*. These doses were determined based on previous acute toxicity studies results that showed LD50 of the leaf extract was greater than 2000 mg/kg [19].

Diarrhea was induced in the mice using bacterial test strains and the antidiarrheal activity of the crude extracts was tested according to the method used by [19]. The mice were fasted for 18 h for food but had free access to water. The fasted mice were randomly grouped and treated as mentioned in the grouping and dosing sections above. After 1 h of dosing, 0.5 ml of bacterial test strain was administered orally. Then each mouse was placed in a separate cage lined with transparent paper. The transparent paper was changed every 1 hr for 4 hrs of observation. During the observation period, time of onset of diarrhea (time of first diarrhea following bacterial test strain administration), number and weight of total and wet feces were measured. All, 100% of the feces of the negative control group were assumed to be wet feces (diarrhea). The percentage of diarrhea inhibition was calculated according to the following formula [19].

$$\% \text{ ID} = \frac{\text{Mean number of (WFNC} - \text{WFT)}}{\text{Mean number of WFNC}} \text{ x } 100$$

Where, ID = inhibition of diarrhea, WFNC = wet feces in the negative control group, WFT = wet feces in the test group.

Data Analysis

All experiments were carried out in triplicate. Data obtained were analyzed by one way analysis of variance and means were compared by Duncan Multiple Range Test (SPSS 20.0 version). Differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Following administration of the leaf extract on the experimental animals (mice) no signs of toxicity were observed from all extracts after administration (Table 1). No delayed toxicity was observed in the mice during the 14 days' period. There were no eyes, skin and fur changes. No death was recorded throughout the observation period. The absence of death in the mice for all extracts throughout the two weeks of the experiment suggests that the test extracts are acutely safe. The oral LD_{50} is therefore, greater than 2000 mg/kg.

Table 1: Toxicity and mortality during acute toxicity study of aqueous extract of *Acacia nilotica in Mice*

Groups	**Dose (Mg/kg B.w)**	**No of mice with signs of toxicity/ Normal behaviour (ST/NB)**	**No of mortality/ Survival (D/S)**
1 (Control)	0	0/3*	0/3
2	200	0/3	0/3

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VII July 2024

3	400	0/3	0/3
4	2000	0/3	0/3

A. w = Body weight, ST = sign of toxicity, NB = Normal behaviour, D = Death, S = Survival; Asterisk (*) under ST/NB: numerator (0) is the number of death animal recorded and denominator (3) is the number of used animals.

The extract did not cause death or change in physical appearance and morphological characteristics in the treated animals throughout the 14-day observation period after single oral administration of 2000 mg/kg doses of aqueous extract of *A. nilotica* in the acute toxicity study (Table 1). This result is similar to the one conducted elsewhere [20]

Survival of mice after oral administration of 2000 mg/kg body weight of the extract (Table 1), up to fourteen days (observation period) implies that the estimated oral median lethal dose (LD50) of the extract at 5000 mg/kg body weight is non-toxic [20]. According to the OECD 423 guideline, absence of mortality after oral administration of 2000 mg/kg b.w of the extract corresponds to a LD50 value of 5000 mg/kg [17]. This suggests that acute oral administration of the extract is safe, and may also explain the reason why the leaf portion of the plant is widely used in traditional treatment of diseases [17], [19].

The results of the antidiarrheal effect of the crude extracts on bacteria-induced diarrhea in mice, shown in Tables 2 to Table 5, suggest that the extracts possesses antidiarrheal effect [21]. The onset of diarrhea was significantly delayed in all the extract-treated groups compared to the control group. The aqueous crude extract at 400 mg/kg body weight and 200 mg/kg body weight, as well as the ethyl acetate extract at 400 mg/kg, showed a significant delay in the onset of diarrhea compared to the control group. The total number of stool (faeces) was significantly reduced in all the extract-treated groups when compared to the control group. The crude extract at 400 mg/kg and 200 mg/kg, as well as the ethyl acetate extract at 400 mg/kg, showed a significant reduction in the total number of stools compared to the control group. The amount of wet stool, which is influenced by fluid secretion, was significantly reduced in all the extract-treated groups compared to the control group. The crude extract at 400 mg/kg and 200 mg/kg, as well as the ethyl acetate extract at 400 mg/kg.

This suggest that as the dose of the crude extracts was increased, the numbers of colonies were reducing until there was a clear plate, but there was no significant difference when concentrations used was compared, this could be attributed to the fact that antimicrobial activities of substance has active ingredient reaching an organism [20]. When compared with positive control there were no significant difference between the treatment groups and positive control, this may suggest that the plant extract exhibits antibacterial activity and could be employed in the treatment of infectious diseases. In the same vein when treatment groups were compared with negative control, there was significant difference between the treatment groups. This suggests that plant extracts may have antibacterial activity [21]. The antibacterial screening of crude extract of *Acacia nilotica* carried out *in vivo* revealed antibacterial activity against *Escherichia coli, Salmonella paratyphi and Shigella dysenteriae* at concentration of 200mg/kg and 400mg/kg. The aqueous extract exhibited higher activity.

Table 2: Evaluation of *in vivo* antidiarrheal activity of the crude methanolic leaf extract of *Acacia nilotica*

Effect	**Types of Treatments**				
	Control (Negative)	800 mg/kg AQ	400 mg/kg AQ	200 mg/kg AQ	Loperamide (Positive control)
Onset of Diarrhea (Min)	35.40±2.69	172.20±7.61*	166.60±18.62*	61.20±3.93	180.60±3.61
Total Number of faeces (TNF)	18.20±0.37	8.00±1.00*	8.20±0.66*	12.20±0.86*	7.60±0.81

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VII July 2024

Wet faeces (NWF)	13.80±0.49	3.40±0.51*	3.80±1.39*	8.00±1.26*	3.00±0.84
Formed faeces WTF(g)	4.40±0.51	4.40±0.68	4.20±1.05	4.20±0.58	1.20±0.58
Change in Weight (WWF(g)	2.84±0.15	2.20±0.49	1.70±0.15	1.49±0.34	0.60±0.22
%ID	-	39.23	28.45	21.27	75.10

Values are represented as Mean ± S.E.M. n=5; One-way ANOVA was used for differences in mean followed by Dunnett's test for multiple comparison; * $p<0.5$ significantly different from control. Where, ID = inhibition of diarrhea, WFNC = wet feces in the negative control group, WFT = wet feces in the test group, ITFO = Inhibition of total fecal output, TFC = total feces of control, and TFT = total feces of treated.

Table 3: Evaluation of *in vivo* antidiarrheal activity of the crude aqueous leaf extract of *Acacia nilotica*

Effect	**Treatments**				
	Control	**800 mg/kg AQ**	**400 mg/kg AQ**	**200 mg/kg AQ**	**Loperamide**
Onset of Diarrhea (Min)	35.40±2.69	195.80±30.37*	167.20±46.36*	60.40±8.85*	180.60±3.61*
Total Number of faeces (TNF)	18.20±0.37	1.80±0.73	3.60±1.57	6.60±0.51	7.60±0.81
Wet faeces (NWF)	13.80±0.49	1.20±0.58	1.60±0.68	2.80±0.66	3.00±0.84
Formed faeces WTF(g)	4.40±0.51	0.60±0.40	2.00±0.89	3.80±0.73	1.20±0.58
Change in Weight (WWF(g)	2.84±0.15	0.56±0.24	0.06±0.39	0.61±0.22	0.60±0.22
%ID	-	83.98	71.55	38.95	75.14

Values are represented as Mean ± S.E.M. n=5; One-way ANOVA was used for differences in mean followed by Dunnett's test for multiple comparison;

* $p<0.5$ significantly different from control.

Table 4: Evaluation of *in vivo* antidiarrheal activity of the crude ethyl acetate leaf extract of *Acacia nilotica*

Effect	**Treatments**				
	Control	**800 mg/kg EA**	**400 mg/kg EA**	**200 mg/kg EA**	**Loperamide**
Onset of Diarrhea (Min)	35.40±2.69	135.60±6.90	187.40±23.14	124.00±5.22	180.60±3.61
Total Number of faeces (TNF)	18.20±0.37	3.60±0.68	4.40±1.29	3.60±0.51	7.60±0.81
Wet faeces (NWF)	13.80±0.49	1.40±0.24	1.20±0.58	2.20±0.37	3.00±0.84
Formed faeces WTF(g)	4.40±0.51	2.20±0.58	3.20±0.73	2.80±0.40	1.20±0.58

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VII July 2024

Change in Weight (WWF(g)	2.84±0.15	0.70±0.46	0.93±0.38	1.70±0.28	0.60±0.22
%ID	-	59.94	42.82	37.85	75.14

Values are represented as Mean ± S.E.M. n=5; One-way ANOVA was used for differences in mean followed by Dunnett's test for multiple comparison;

* p<0.5 significantly different from control.

Table 5: Evaluation of *in vivo* antidiarrheal activity of the crude n-hexane leaf extract of *Acacia nilotica*

Effect	**Treatments**				
	Control	**800 mg/kg EA**	**400 mg/kg EA**	**200 mg/kg EA**	**Loperamide**
Onset of Diarrhea (Min)	35.40±2.69	198.64±19.54	211.20±12.89	139.20±8.51	180.60±3.61
Total Number of faeces (TNF)	18.20±0.37	9.00±2.07	7.00±1.58	6.40±0.93	7.60±0.81
Wet faeces (NWF)	13.80±0.49	1.60±0.75	3.00±1.64	3.00±0.89	3.00±0.84
Formed faeces WTF(g)	4.40±0.51	3.10±2.01	3.20±1.53	4.20±0.68	1.20±0.58
Change in Weight (WWF(g)	2.84±0.15	0.42±0.09	0.65±0.13	0.65±0.31	0.60±0.22
%ID	-	51.38	46.96	32.87	75.14

Values are represented as Mean ± S.E.M. n=5; One-way ANOVA was used for differences in mean followed by Dunnett's test for multiple comparison; * p<0.5 significantly different from control.

Discussion Continues

The aqueous, ethyl acetate, methanol and n-Hexane leaf extracts of *Acacia nilotica* from Nasarawa West in Nasarawa State Nigeria have already been shown to possess antibacterial activity against common bacterial agents of diarrhea [11]. This study that evaluated their acute toxicity and *in vivo* antidiarrheal activity in animal model has demonstrated their acute toxicity and potential for use against diarrhea caused by these bacterial agents. The index of acute toxicity which is the LD_{50} was not calculated as no death was recorded, however it was estimated to be greater than 5g/kg. According to Lorke, LD_{50} values greater than 2,000 mg/kg are of no practical toxicological interest. Other objectives involve determination of the most important clinical signs attributable to exposure to high dose of the test substance, time of onset, remission and recovery from those signs, sequence and timing of events leading to death of test subjects in instance where lethality is not recorded [17] since there is zero signs of toxicity observed from all extracts after administration. There is no delayed toxicity observed in the mice during the 14 days' period. There were no eyes, skin and fur changes. No death was recorded throughout the observation period. The absence of death in the mice for all extracts throughout the two weeks of the experiment suggests that the test extracts are acutely safe. The oral LD_{50} is therefore, greater than 2000 mg/kg. This result corresponds to several other research results [19][20].

CONCLUSION

The use of plants in the treatment of infectious has been extensively applied by the local populace in the world. This study signifies the potential of *Acacia nilotica* leaf extracts as a source of therapeutic agent. Therefore, the great potential of *Acacia nilotica* in the treatment of microbial infection such as diarrhea was elucidated, while the bactericidal activity of the extract showed that the plant is of medicinal importance. In addition, the activity exhibited by the extracts against tested bacteria species, that are associated with various

infectious diseases, may provide scientific justification for the ethnomedicinal uses of the plant.

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We give God the glory for the life and wisdom granted us thus far.

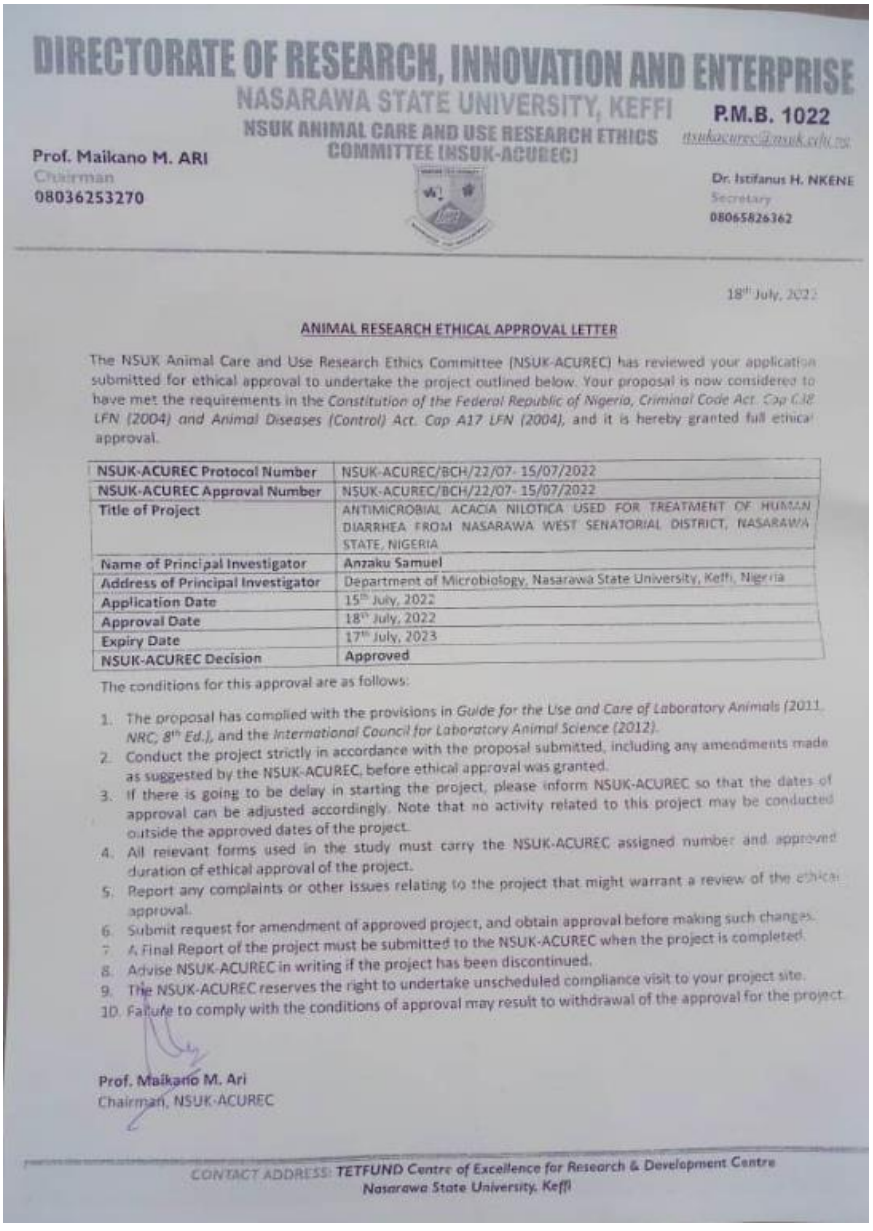
REFERENCES

1. Pandey A, Patel AK, Ariharan S, Kumar V, Sharma RK, Kanhed S, Nigam VK, Keshri A, Agarwal A, Balani K (2018). Enhanced tribological and bacterial resistance of carbon nanotube with Ceria- and Silver-Incorporated Hydroxyapatite Biocoating. Nanomaterials 8(6): 363.
2. World Health Organization (WHO). 2020. Legal status of traditional medicine and complementary/alternative medicine:a worldwide review. Available from: http://libdoc.who.int/hq/2001/WHO_EDM_TRM_2001.2.pdf
3. Stuempfig ND, Seroy J. Viral gastroenteritis. Stat Pearls. Treasure Island, FL: Stat Pearls Publishing, 2022.
4. Sharma P, Kumari B, Dahiya S, Kulsum U, Kumar S Manral N, Pandey S, Kaur P, Sood S, Das BK et al. Azithromycin resistance mechanisms in typhoidal salmonellae in India: A 25-year analysis. Indian Journal of Medical Research, 2019; 149: 404–411.
5. Wang YF, Ni ZY, Dong M, Cong B, Shi WQ, Gu YC, Kiyota H. (2019). Secondary Metabolites of Plants from the Genus Saussurea; Chemistry and Activity. Chemistry and Biodiversity, 7, 2623-2659.
6. Null C, Stewart CP, Pickering AJ, Dentz HN, Arnold BF, Arnold CD, Benjamin-Chung J, Clasen T, Dewey KG, Fernald LCH, Hubbard AE, Kariger P, Lin A, Luby SP, Mertens A, Njenga SM, Nyambane G, Ram PK, Colford JM.2018. Effects of water quality, sanitation, handwashing, and nutritional interventions on diarrhoea and child growth in rural Kenya: a cluster-randomised controlled trial. Lancet Health. Mar;6(3): e316-e329.
7. Desta GT, Adela Alemu M, Tsegaw A, Belete TM, & Adugna BY. (2021). Antidiarrheal Effect of 80% Methanol Extract and Fractions of Clerodendrum myricoides (Hochst.) Vatke (Lamiaceae) Leaf in Swiss Albino Mice. Evidence-based complementary and alternative medicine: eCAM, 2021, 9369173. https://doi.org/10.1155/2021/9369173.
8. Azwanida MK, Pietersen DW, Kotzé A, Dalton DL, Jansen R. Knowledge and uses of African pangolins as a source of traditional medicine in Ghana. PLoS ONE., 2020; 10: e0117199. doi: 10.1371/journal.pone.0117199.
9. Ma B, Wang X, Lei C, Tang Y, He J, Gao Y, … Wang H. Identification of Three Novel PmGRI1 Genomic Resistance Islands and One Multidrug Resistant Hybrid Structure of Tn7-like Transposon and PmGRI1 in Proteus mirabilis. Antibiotics, 2021; 10(10): 1268. doi:10.3390/antibiotics10101268.
10. Eve A, Aliero AA, Nalubiri D, Adeyemo RO, Akinola SA, Pius T, Nabaasa S, Nabukeera S, Alkali B, Ntulume I (2020). In vitro antimicrobial activity of crude extracts of Artocarpus heterophyllus seeds against selected diarrhea-causing superbug bacteria. Scientific World Journal 1: 9813970.
11. Anzaku S, Ngwai YB, Ishaleku D, Igbawua IN. Antibacterial Activity Profile of crude leaf extracts of Acacia nilotica used for the treatment of human diarrhea in Nasarawa West, Nasarawa State, Nigeria. International Journal of Modern Pharmaceutical Research, 2024, 8(7): 13-18.
12. Subramanian K, Sankaramourthy D, Gunasekaran M (2018). Toxicity Studies Related to Medicinal Plants. Natural Products and Drug Discovery- An Integrated Approach. Page 491-505.
13. UNL Environmental Health and Safety. Toxicology and Exposure Guidelines [Internet]. Lincoln: University of Nebrasha; 2002 [cited 2017 Sep 22]. p. 28. Available from: https://ehs.unl.edu/documents/tox_exposure_guidelines.pdf
14. Denny KH, Stewart CW. Acute, sub-acute, sub-chronic and chronic general toxicity testing for preclinical drug development. In: A Comprehensive Guide to Toxicology in Preclinical Drug Development. Elsevier Inc.: London; 2013. pp. 87-105.
15. WHO. The WHO Recommended Classification of Pesticides by Hazard, and Guidelines to Classification. Geneva: WHO Press; 2010. pp. 1-81.
16. Cheng AG, Kim HK, Burts ML, Krausz T, Schneewind O, and Missiakas DM 2009. Genetic requirements for Staphylococcus aureus abscess formation and persistence in host tissues. FASEB J.

23:3393–3404
17. OECD (2001). Organization for Economic Co-operation and Development Guidelines for the testing of chemicals/section 4: Health effects test No. 423. Acute oral toxicity - Acute toxic class method.
18. Apu, A., Muhit, M., Tareq, S., Pathan, A., Jamaluddin, A., & Ahmed, M. (2010). Antimicrobial Activity and Brine Shrimp Lethality Bioassay of the Leaves Extract of Dillenia indica Linn. Journal of young pharmacists: JYP, 2(1), 50–53. https://doi.org/10.4103/0975-1483.62213
19. Ayalew, M., Bekele, A., Mengistie, M. G., & Atnafie, S. A. (2022). Evaluation of the antidiarrheal activity of 80% methanol extract and solvent fractions of the leaf of Bersama abyssinica fresen (Melianthaceae) in mice. BMC complementary medicine and therapies, 22(1), 8. https://doi.org/10.1186/s12906-021-03498-6
20. Alli, L. A., Adesokan, A. A., Salawu, O. A., & Akanji, M. A. (2015). Toxicological studies of aqueous extract of Acacia nilotica root. Interdisciplinary toxicology, 8(1), 48–54. https://doi.org/10.1515/intox-2015-0005
21. Tadesse WT, Hailu AE, Gurmu AE, Mechesso AF. Experimental assessment of antidiarrheal and antisecretory activity of 80% methanolic leaf extract of Zehneria scabra in mice. BMC Complement Altern Med. 2014;14(1):460.

APPENDIX I

Ethical Clearance



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18th July, 2022

ANIMAL RESEARCH ETHICAL APPROVAL LETTER

The NSUK Animal Care and Use Research Ethics Committee (NSUK-ACUREC) has reviewed your application submitted for ethical approval to undertake the project outlined below. Your proposal is now considered to have met the requirements in the *Constitution of the Federal Republic of Nigeria, Criminal Code Act. Cap C38 LFN (2004) and Animal Diseases (Control) Act. Cap A17 LFN (2004)*, and it is hereby granted full ethical approval.

NSUK-ACUREC Protocol Number	NSUK-ACUREC/BCH/22/07- 15/07/2022
NSUK-ACUREC Approval Number	NSUK-ACUREC/BCH/22/07- 15/07/2022
Title of Project	ANTIMICROBIAL ACACIA NILOTICA USED FOR TREATMENT OF HUMAN DIARRHEA FROM NASARAWA WEST SENATORIAL DISTRICT, NASARAWA STATE, NIGERIA
Name of Principal Investigator	**Anzaku Samuel**
Address of Principal Investigator	Department of Microbiology, Nasarawa State University, Keffi, Nigeria
Application Date	15th July, 2022
Approval Date	18th July, 2022
Expiry Date	17th July, 2023
NSUK-ACUREC Decision	**Approved**

The conditions for this approval are as follows:

1. The proposal has complied with the provisions in *Guide for the Use and Care of Laboratory Animals (2011, NRC, 8th Ed.)*, and the *International Council for Laboratory Animal Science (2012)*.
2. Conduct the project strictly in accordance with the proposal submitted, including any amendments made as suggested by the NSUK-ACUREC, before ethical approval was granted.
3. If there is going to be delay in starting the project, please inform NSUK-ACUREC so that the dates of approval can be adjusted accordingly. Note that no activity related to this project may be conducted outside the approved dates of the project.
4. All relevant forms used in the study must carry the NSUK-ACUREC assigned number and approved duration of ethical approval of the project.
5. Report any complaints or other issues relating to the project that might warrant a review of the ethical approval.
6. Submit request for amendment of approved project, and obtain approval before making such changes.
7. A Final Report of the project must be submitted to the NSUK-ACUREC when the project is completed.
8. Advise NSUK-ACUREC in writing if the project has been discontinued.
9. The NSUK-ACUREC reserves the right to undertake unscheduled compliance visit to your project site.
10. Failure to comply with the conditions of approval may result to withdrawal of the approval for the project.

Prof. Maikano M. Ari
Chairman, NSUK-ACUREC

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