BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 2, October 2025 | Pages: 591-599 | DOI: 10.14421/biomedich.2025.142.591-599 ISSN 2540-9328 (online)

Evaluation of Selected Pharmacological Properties of a Polyherbal Extract (Aju *Mbaise*) in Experimental Rats

Robert Ikechukwu Uroko[1,*], Nnah Solomon Ijioma[2], Henry Nnaemeka Ogbonna[1], Nancy Oluomach Uchenna[1]
[1]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
[2]Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Corresponding author*
ir.uroko@mouau.edu.ng

Manuscript received: 22 March, 2025. Revision accepted: 18 August, 2025. Published: 01 October, 2025.

Abstract

Aju Mbaise, an herbal combination widely utilized in southeastern Nigeria for managing postpartum complications and alleviating menstrual pain, was evaluated for analgesic, anti-inflammatory, and anti-diarrheal properties in rats. Specific objectives included evaluating anti-diarrheal effects, examining anti-inflammatory effects and assessing analgesic properties of the herbal combination. The study involved subjecting the Aju Mbaise polyherbal extract (APE) to phytochemical analysis and acute toxicity testing. Anti-diarrheal effects were evaluated by administering charcoal as a meal and using castor oil-induced models. Anti-inflammatory effects were assessed through the carrageenan-induced paw oedema model, and analgesic properties were examined using the acetic acid-induced pain model. Phytochemical analysis identified alkaloids, tannins, phenols, steroids, cardiac glycosides, terpenoids, flavonoids, and saponins in the extract. The acute toxicity value exceeded 5000 mg/kg body weight, indicating safety. In animal studies, APE exhibited significant inhibitory effects on intestinal motility, reduced wet stool frequency, and influenced the castor oil-induced diarrhoea and enhanced anti-inflammatory activities. Additionally, it demonstrated a reduction in acetic acid-induced pain in rats. The APE with its diverse phytochemical composition, possesses anti-diarrheal, anti-inflammatory, and analgesic properties. However, further research is needed to establish ideal dosages and potential adverse effects.

Keywords: *Aju Mbaise* polyherbal extract; Analgesic, Castor oil; Diarrhoea; Inflammation; Motility.

INTRODUCTION

Medicinal plants as complementary treatment medications are now accepted globally (Ekor, 2014). The global population currently depends on medicinal plants to treat various illnesses and in fact, it is envisaged that the acceptability of medicinal plants as a means of treatment will experience a further upsurge due to the availability, accessibility, cheapness, and reported effectiveness of these medicinal plants in alleviating a wide range of health complications (Sofowora et a*l.,* 2013; Ekor, 2014; Salmerón-Manzano et a*l.,* 2020). The meltdown in global economies, coupled with the poor socio-economic status of most individuals, especially across developing countries of Africa and Asia, also appears to have contributed to the growing interest in herbal medicine (James et a*l.,* 2018; Glover, 2021). This is because most individuals living in these regions of the world find it difficult to cope with the demand for sophisticated treatments (Goodman et al*.,* 2022). *Aju Mbaise* polyherbal extract (APE) is only one of the herbal preparations currently used against diseases (Ijioma et al*.,* 2019a, b).

Aju Mbaise is a polyherbal comprising various medicinal plant parts, including stems, bark, leaves, and roots. *Aju Mbaise* polyherbal formulation comprises different plants identified as *Barteria fistulosa, Napoleona vogelii, Euphorbia convolvuloides, Spondias mombin, Uvaria chamae, and Ceiba pentandra* (Ijioma et al*.* 2019b; Ijioma et al*.,* 2020). The researchers also reported notable alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, phenols, and cardiac glycosides within the extract (Ijioma et al*.,* 2020). Although reports on the medicinal uses of the medicinal plant appear scanty, its constituent plants have been used to manage diarrhoea and other forms of gastrointestinal diseases (Uroko et al*.,* 2022). *Aju Mbaise* extract improves lipid profile, electrolyte balance, haematological profile, male fertility indices and prevents oxidative stress and hepatic injury in rats (Uroko et al*.,* 2022).

The passage of frequent watery or unformed stools characterizes diarrhoea, which is prevalently implicated in the death of children aged 1 – 5 worldwide and a significant contributor to the current global disease burden, especially in developing countries with limited access to good water supply, and poor breastfeeding

practices (Birru et al., 2016). Major causes of diarrhoea are gastrointestinal infection of bacterial, viral, and parasitic origin, contaminated food or drinking water, poor hygiene, and motility disorders of the gastrointestinal tract (Teke et al., 2010). Current management strategies, including oral rehydration and the use of drugs, have not achieved the desired outcomes due to a lack of funds on the part of many affected individuals and also a lack of compliance on the part of those who can afford the drugs (Jin *et al.,* 2008; Ezezika et al., 2021). Medicinal plants have proven viable alternative treatment sources over the years due to their availability, accessibility, and effectiveness (Ogbonna et al., 2020). Several medicinal plants have shown significant anti-diarrhoeal activities (Ayalew et al., 2022). Due to their vast phytochemical contents, medicinal plants can play a beneficial role as, antimicrobial, anti-inflammatory and anti-cytotoxic agents while reducing inflammation and protecting from cellular damage (Nwokafor et al., 2020; Udekwu et al., 2020). The fact that pains and inflammations have been associated with diarrhoea (Bentley et al., 2004; Panikkath et al., 2014; Birru et al., 2016), this study evaluates a strategy for managing all three symptoms with a single agent as an area of interest. Therefore, the study assessed in rats the anti-diarrheal, anti-inflammatory, and analgesic effects of the polyherbal extract of *Aju Mbaise.*

MATERIALSS AND METHODS

Chemicals and Drugs

Drugs used for this study include Loperamide, Castor oil, and Aspirin (Emzor Drugs, Nigeria), while chemicals include Ethanol and activated charcoal (BDH Company, United Kingdom). Reagents include electrolyte test kits for sodium, potassium, chloride, and bicarbonate (Randox Laboratories, United Kingdom).

Plant Materials and Preparation of Extract

Aju Mbaise polyherbal was obtained from Ahiara Mbaise Local Government Area of Imo State, Nigeria, and was allowed to dry and this was done using a laboratory bench for 21 days. The dried sample was pulverized into a coarse powder in a locally made manual blender. Two hundred and fifty (250) grams of the pulverized material was immersed in 1500 millimetres of ethanol within 48 hours and intermittent stirring every 2 hours before being filtered to obtain a filtrate containing the extract in solution. Subsequently, the filtrate was subjected to drying in a hot air oven at 40°C, resulting in a greenish paste extract that weighed 16.90 g, equating to a percentage yield of 6.76%. The extract was prepared and subsequently stored at very low temperatures in a refrigerator until required, and it is now identified as APE.

Phytochemical Analysis of *Aju Mbaise* Polyherbal Extract

The assay, identification and quantification of phytochemicals in APE were carried out according to the protocols outlined in Evans (2009). The extract's presence and amounts of alkaloids, phenols, cardiac glycosides, saponins, steroids, flavonoids, terpenoids and tannins were assayed.

Experimental Animals

A total of one hundred and twenty-one (121) adult male Wistar albino rats with average weight (150.24 ± 1.33 g) were used for the different designs adopted in the study. Twenty-one (21) of the rats were used for acute toxicity assay of the extract, seventy-five (75) for the three models of anti-diarrhoea tests (25 for each model) and twenty-five (25) for anti-inflammatory study and analgesic studies. The rats were procured from the Institution's Animal House of the Department of Zoology and Environmental Biology. The rats were housed in stainless steel metabolic cages at room temperature (27 ± 3℃) and 35 ± 5% humidity in well-ventilated laboratory. The rats had *ad libitum* access to clean water and Chikun Feed Finisher Mash (17.0 % crude protein, 3000.00 Kcal/kg metabolizable energy, Chikun Feeds Depot, Ibadan, Nigeria) for 12-hour day light/dark cycles. The rats were maintained and managed in compliance with international standards for the care and use of laboratory animals (NRC, 2011). Before each experiment, the rats were starved for 24 hours.

Acute Toxicity (LD_{50}) Evaluation of *Aju Mbaise* Polyherbal Extract

Lorke's method was used to evaluate the acute toxicity of APE (Lorke, 1983). The test involved two stages, with the first stage using 9 Wistar rats that were separated into 3 groups of A, B, and C which consisted of 3 rats each. The groups received 10, 100, and 1000 mg/kg of APE, respectively. The animals were observed for toxicity signs and death within 24 hours, and no deaths were recorded across the groups. Consequently, the study progressed to the second stage, which involved another set of 9 rats divided into 3 groups (A, B, and C) and administered single oral doses of APE at 1600, 2900, and 5000 mg/kg, respectively. The animals were again checked for signs of toxicity and death within 24 hours, with no deaths recorded across the groups. As a confirmatory test, the highest dose (5000 mg/kg) was repeated on different sets of 3 rats. Acute toxicity values calculated using Lorke's formula stated as: $LD_{50} = \sqrt{A\ x\ B}$, where A = Maximum dose that did not result in any deaths and B = Minimum dose that caused mortality in all animals within a group.

Anti-Diarrhoeal Activity of *Aju Mbaise* Polyherbal Extract

Model 1: Effect of the polyherbal extract on intestinal transit

The study adopted the method of Ijioma et al. (2019b), assigning 25 rats into five groups of five rats each, after a 24-hour starvation period. Group 1 served as control, while group 2 received 0.5 mg/kg body weight of the standard drug (Loperamide) via the oral route. Groups 3, 4, and 5 received oral administrations of 250, 500, and 1000 mg/kg of APE, respectively. After 30 minutes, each rat received 10 ml/kg body weight of 10% activated charcoal before being sacrificed under anesthesia with chloroform and dissected open to expose the visceral content. The small intestine's length was measured, and the distance covered by the charcoal meal was also measured and converted to percentage of the entire length. The percentage inhibition of intestinal motility was calculated as indicated in the equation below:

$$\% \text{ inhibition} = \frac{\text{DT in control} - \text{DT in test x100}}{\text{DT in control}}$$

where DT = Distance travelled by charcoal meal.

Model 2: Effect of the polyherbal extract on castor oil-induced diarrhoea in rats

For model 2, a separate set of 25 adult rats fasted for 24 hours and divided into 5 groups of 5 rats each according to the method used by Ugwuja et al. (2022). The rats received the same treatment as in model 1 and, 30 minutes later, were given castor oil (1 ml) orally before being housed in individual cages coated with absorbent paper for three hours to monitor episodes of diarrhoea. Wet and dry stools were counted, and their weights were recorded, as well as when the diarrhoea first started (the latent phase). After each period, the absorbent papers were changed, and the average stools passed by the treatment and control groups were compared. The percentage average number of diarrheal faeces passed in the control group was calculated.

The inhibition percentage of wet faeces and stool frequency induced by the extract was determined by calculating the variance between the number of faeces in the control group (NC) and the amount of faeces in the treated group (NT), divided by NC and multiplied by 100. % Inhibition of bowel movement = $[(N_C - N_T)/N_C] \times 100$, where: N_C = Number of wet faeces/stools of control group and N_T = Number of wet faeces/stools of treated group (Ijioma et al., 2020).

Model 3: Effect of the polyherbal extract on castor oil-induced gastrointestinal fluid accumulation and serum electrolytes concentration in rats

Using the method described by Ugbogu et al. (2023), another set of 30 rats was given the same treatments as model 2 using the same protocols. In this instance, six groups of five adult albino rats each were used. After an hour of administering castor oil, the rats were euthanized, and their small intestines were removed from the pyloric region to the caecum, after ligating both ends. Each intestine's weight with its contents within was determined before the contents were expelled out, and the empty intestine was then weighed again. The weight of the intestinal content was determined by subtracting the weight of the empty intestine from the weight of the full intestine. Percentage of diarrhoeal activity was evaluated using the relationship:

$$\% \text{ Activity} = \frac{\text{WIC in control} - \text{WIC in test x100}}{\text{WIC in control}}$$

where WIC = weight of intestinal content.

The concentrations of serum electrolytes were also determined by examining blood samples collected from each rat. Randox commercial test kit for electrolytes including sodium, potassium, chloride and bicarbonate were used in accordance with the methods of Terri & Sesin (1958), described by the manufacturer, Randox Laboratories, United Kingdom, for each test as outlined below:

Potassium ion (K^+): The principle is that sodium tetraphenylboron in a specifically prepared mixture can produce a colloidal suspension. The turbidity of this suspension is directly proportional to the concentration of potassium in the sample. For potassium, three test tubes labelled blank, sample and standard were each made to contain 1.0 ml of potassium reagent, before 10 µl of the distilled water, sample and standard were added to their respective tubes. The content of each tube was mixed and allowed to stand for 3 minutes at room temperature before absorbance values of each were read at 500 nm in a spectrophotometer after zeroing with the blank. The K^+ the concentration was calculated using the formula:

$$\frac{Absorbance\ of\ test}{Absorbance\ of\ standard}\ x\ Concentration\ of\ standard,$$

where concentration of standard was 5.0 mEq/L.

Sodium ion (Na^+): This test is predicated on the principle of precipitating sodium, as a triple salt, sodium magnesium uranyl acetate, with the excess uranium being reacted with ferrocyanide to produce a chromophore whose absorbance is inversely proportional with the concentration of sodium in the sample being tested. In sodium electrolyte analysis, two stages of test–filtrate preparation and colour development. In the filtration phase, test tubes labelled blank, standard and sample were made to contain 1.0 ml of the filtrate reagent, followed by adding 50 µl of sample to all tubes and 1 ml of distilled water to the blank. All tubes were shaken vigorously and mixed continuously for 3 minutes. The mixtures were then centrifuged for 10 minutes and supernatant fluids were used for the test in the colour development phase which involved the addition of 1 ml

of the acid reagent to each tube before reading absorbance values after zeroing the spectrophotometer at 550 nm. Sodium electrolyte concentration was calculated using the formula: $\frac{\text{Abs.of Blank} - \text{Abs.of sample}}{\text{Abs.of Blank} - \text{Abs.of STD}}\ x\ \text{Conc. of STD (mEq/L)}$

Chloride ion (Cl^-): The principle here is that chloride ions form a soluble non-ionized compound with mercuric ions and will displace thiocyanate ions from non-ionized mercuric thiocyanate. The released thiocyanate ions then react with ferric ions to form a colour complex whose intensity is proportional to the chloride concentration in the sample. In the test, three test tubes labelled blank, sample and standard were each made to contain 1.0ml of chloride reagent, before 10 µl of the distilled water, sample and standard were added to the respective tubes. The content of each tube was mixed and allowed to stand for 3 minutes at room temperature before absorbance values of each were read at 500 nm in a spectrophotometer after zeroing with the blank. The Cl^- concentration was calculated using the formula:

$$\frac{Absorbance\ of\ test}{Absorbance\ of\ standard}\ x\ Concentration\ of\ standard$$

Evaluation of the Anti-Inflammatory Effects of the Polyherbal Extract

Twenty-five mature rats were assigned to 5 groups with each group having 5 rats. The standard group (Group 2) received an anti-inflammatory drug (100 mg/kg Aspirin), while the control group (Group 1) received 0.2 ml of ordinary saline. Using the oral method, groups 3, 4, and 5 received 250, 500, and 1000 mg/kg of APE, respectively. The animals' paw circumferences (PC) were measured and recorded before these treatments. After 30 minutes following treatment, the rats were subjected to acute inflammation (paw oedema) using a sub-plantar injection of 0.1 ml of 1% λ-carrageenan (in 0.9% saline solution) into the right hind paw of each rat. After that, the PC of the treated rats and control were measured and recorded at different time intervals (30 minutes, 1 hour, and 2 hours) following induction. The percentage inhibition of oedema was estimated using the following relationship, and the degree of oedema was determined according to the method employed by Ijioma et al. (2019) as the difference between the initial and final PC values:

$$\text{Percentage inhibition of oedema} = \frac{\text{PC in control} - \text{PC in test x 100}}{\text{PC in control}}.$$

Evaluation of Analgesic Effects of the Polyherbal Extract

Same twenty-five (25) matured rats assigned to 5 groups used for the anti-inflammatory study were used and were given same treatments. Thirty minutes after treatment, acute pain was inducted by intraperitoneal administration of 10 ml/kg body weight of 0.6% acetic acid solution. Thereafter, the number of writhes made by each rat in 30 minutes was ascertained for both the control and test groups. Percentage inhibition of pain (analgesic activity) was calculated using the relationship employed by Ugbogu et al. (2023):

$$\%\ \text{inhibition of pain} = \frac{\text{Number of writhes in control} - \text{Number of writhes in test x 100}}{\text{Number of writhes in control}}$$

Data Analysis: SPSS (Statistical Products and Service Solutions) Version 20.0, IBM SPSS Incorporated, Chicago, IL was utilized to analyse the data. Experimental data collected were subjected to a one-way analysis of variance (ANOVA) to obtain significant means. The various significant means were separated using the Duncan Multiple Range Test post-hoc analysis. Values with $p<0.05$ were regarded as being statistically significant.

RESULTS AND DISCUSSION

RESULTS

Phytochemical Composition of *Aju Mbaise* Polyherbal Extract

The extract's phytochemicals, which were quantified, include saponins, tannins, phenolics, flavonoids, steroids, terpenoids, glycosides, and alkaloids, and they are presented in Table 1

Table 1. Phytochemical composition of *Aju Mbaise* polyherbal extract.

Parameters	Qualitative test	Qualitative test result (mg/100g)
Saponins	++	8.33 ± 0.12
Tannins	++	5.71 ± 0.16
Phenolics	+++	13.18 ± 0.30
Flavonoids	++	7.19 ± 0.22
Steroids	++	5.65 ± 0.14
Terpenoids	+	2.52 ± 0.11
Cardiac glycosides	+	3.63 ± 0.09
Alkaloids	+++	14.91 ± 0.32

Key: *+++ = Present in high quantity, ++ = Present in moderate quantity, + = Present in low quantity and - = Absent*

Acute Toxicity (LD_{50}) of *Aju Mbaise* Polyherbal Extract

No mortality occurred across all test groups and in all stages of acute toxicity evaluation of the extract, including the highest dose of 5000 mg/kg body weight. After treatment, the animals appeared physically stable, and all survived till the end of 24 hours and a further seven days of the LD_{50} test, except those administered

5000 mg/kg, which at the initial moments after administration were calm with decreased physical activity. However, these animals all survived to the end of the test period as 0% mortality was recorded. The LD_{50} value for the extract was >5000 mg/kg (Table 2).

Table 2. Acute toxicity (LD_{50}) of *Aju Mbaise* polyherbal extract.

Group	Dose (mg/kg)	Number of death	Observation
Phase 1			
1	10	0/3	Animals displayed normal physical activity and appeared to be in good health
2	100	0/3	Animals displayed normal physical activity and appeared to be in good health
3	1000	0/3	Animals displayed normal physical activity and appeared to be in good health
Phase 2			
1	1600	0/3	Animals exhibited normal activity and were physically stable
2	2900	0/3	Animals exhibited normal activity and were physically stable
3	5000	3/3	No mortality. Animals were calm for up to 2 hours before regaining activity.

LD_{50} >5000 mg/kg

Anti-Diarrhoeal Activity of *Aju Mbaise* Polyherbal Extract

Model 1: Effect of APE on intestinal transit

The treated rats exhibited significantly inhibited intestinal motility ($p<0.05$) when compared to the control group. The inhibition occurred in a dose-dependent manner, causing a decrease in charcoal meal transit in rats. The treatment was comparable to Loperamide activity but demonstrated greater activity than Loperamide at the highest dose (100 mg/kg).). Table 3 shows the inhibitory effect of APE on intestinal motility in rats.

Table 3. *Aju Mbaise* polyherbal extract's inhibitory effects on intestinal motility of rats.

Treatments	Length of intestine (cm)	Distance travelled by charcoal meal (cm)	Percentage charcoal meal movement (%)	Percentage inhibition of motility (%)
Control	92.60 ± 2.07^{a}	75.60 ± 2.23^{c}	81.00 ± 6.92^{c}	0.00 ± 0.00^{a}
Loperamide 0.5 mg/kg	90.00 ± 1.58^{a}	56.80 ± 3.56^{b}	63.20 ± 3.70^{b}	25.40 ± 1.82^{b}
Extract 250 mg/kg	91.20 ± 2.95^{a}	57.80 ± 2.28^{b}	63.40 ± 2.70^{b}	24.20 ± 2.28^{b}
Extract 500 mg/kg	91.60 ± 4.83^{a}	54.20 ± 2.17^{b}	$59.20 \pm 3.03^{a,b}$	27.40 ± 3.29^{b}
Extract 1000 mg/kg	91.00 ± 6.21^{a}	45.00 ± 3.77^{a}	52.60 ± 3.50^{a}	33.00 ± 4.47^{c}

The results are shown as mean ± standard deviation. ^{a,b,c} = Means on the same column with different letter superscripts differ significantly ($p<0.05$)

Model 2: Effect of APE on castor oil-induced diarrhoea in rats

In castor oil-induced diarrhoea rats, the extract exhibited a significant inhibitory effect on the induction of diarrhoea at doses of 250, 500, and 1000 mg/kg, comparable to Loperamide. Specifically, the extract reduced the incidence of diarrhoea by 57.29 ± 2.93%, 66.66 ± 2.95%, and 84.77 ± 2.73%, respectively, when compared to the control ($p<0.05$). The extract-treated groups had a significantly decreased number and weight of wet stools with longer latent periods compared to the control group (Table 4).

Table 4. Inhibitory effect of *Aju Mbaise* polyherbal extract on castor oil-induced diarrhoea in rats.

Treatments	Latent period (minutes)	Number of wet stool	Weight of wet stool (g)	Number of Dry stool	Weight of dry stool (g)	% inhibition of diarrhoea
Control	30.60 ± 5.86^{a}	7.20 ± 0.84^{c}	4.82 ± 0.18^{d}	1.40 ± 0.55^{a}	$1.41 \pm 0.28^{a,b}$	0.00 ± 0.00^{a}
Loperamide 0.5 mg/kg	56.80 ± 1.64^{c}	0.80 ± 0.05^{a}	0.71 ± 0.09^{a}	2.60 ± 0.48^{b}	2.62 ± 0.33^{c}	89.10 ± 6.22^{d}
Aju Mbaise 250 mg/kg	48.00 ± 1.23^{b}	2.60 ± 0.55^{b}	1.87 ± 0.23^{c}	1.20 ± 0.08^{a}	1.10 ± 0.07^{a}	57.29 ± 2.93^{b}
Aju Mbaise 500 mg/kg	70.00 ± 2.45^{d}	1.60 ± 0.48^{a}	1.26 ± 0.37^{b}	2.80 ± 0.47^{b}	2.57 ± 0.42^{c}	66.66 ± 2.95^{c}
Aju Mbaise 1000 mg/kg	73.80 ± 0.84^{d}	0.80 ± 0.04^{a}	0.56 ± 0.06^{a}	2.20 ± 0.44^{b}	1.97 ± 0.21^{b}	84.77 ± 2.73^{d}

The results are shown as mean ± standard deviation. ^{a,b,c} = Means on the same column with different letter superscripts differ significantly ($p<0.05$)

Model 3: Effect of APE on castor oil-induced gastrointestinal fluid accumulation and serum electrolytes concentration in rats:

In addition, the extract significantly reduced the intestinal weight in the enteropooling version of the castor oil-induced diarrhoea rats compared to Loperamide-treated rats (Table 5). Serum electrolyte composition analysis revealed a significant increase in sodium and potassium ions in the extract-treated groups, while the levels of chloride and bicarbonate remained

unchanged compared to the diarrhoea control group (Table 6). The control group had significantly lower serum levels of sodium and chloride ions. However, in groups treated with the extract, serum levels of these electrolytes increased significantly. In comparison to the control, the extract-treated groups had significantly higher K^+ levels. The HCO_3- levels between the extract-treated group and the control group were not statistically different.

Table 5. Inhibitory effect of *Aju Mbaise* polyherbal extract on the weight of intestinal contents in castor oil-induced diarrhoea model in rats.

Treatments	Weight of filled intestine (cm)	Weight of empty intestine (g)	Weight of intestine content (g)	% Activity
Control	15.59 ± 0.51^{d}	4.58 ± 0.23^{a}	11.01 ± 0.41^{d}	0.00 ± 0.00^{a}
Loperamide 0.5 mg/kg	12.34 ± 0.20^{b}	$4.73 \pm 0.36^{a,b}$	7.60 ± 0.31^{b}	58.60 ± 0.71^{e}
Aju Mbaise 250 mg/kg	13.93 ± 0.20^{c}	4.93 ± 0.09^{b}	9.01 ± 0.22^{c}	17.20 ± 0.53^{b}
Aju Mbaise 500 mg/kg	12.17 ± 0.24^{b}	4.94 ± 0.04^{b}	7.23 ± 0.28^{b}	32.85 ± 2.57^{c}
Aju Mbaise 1000 mg/kg	11.48 ± 0.38^{a}	5.01 ± 0.07^{b}	6.48 ± 0.29^{a}	43.65 ± 1.42^{d}

The results are shown as mean ± standard deviation. ^{a,b,c} *= Means on the same column with different letter superscripts differ significantly (p<0.05)*

Table 6. Effect of *Aju Mbaise* polyherbal extract on serum electrolyte levels in castor oil-induced diarrhoea in rats.

Treatments	Na^+ mEq/L	K^+ mEq/L	Cl^- mEq/L	HCO_3^- mEq/L
Control	107.93 ± 1.24^{a}	4.11 ± 0.09^{a}	79.37 ± 0.58^{a}	19.36 ± 0.14^{a}
Loperamide 0.5 mg/kg	118.72 ± 3.08^{c}	4.48 ± 0.27^{b}	86.50 ± 0.72^{b}	$19.50 \pm 0.09^{a,b}$
Aju Mbaise 250 mg/kg	111.78 ± 2.35^{b}	$4.49 \pm 0.19^{b,c}$	86.76 ± 2.28^{b}	19.60 ± 0.13^{b}
Aju Mbaise 500 mg/kg	118.16 ± 2.568^{c}	$4.62 \pm 0.04^{b,c}$	$87.80 \pm 1.06^{b,c}$	$19.51 \pm 0.27^{a,b}$
Aju Mbaise 1000 mg/kg	119.90 ± 2.29^{c}	$4.72 \pm 0.12^{b,c}$	89.52 ± 1.88^{c}	$19.51 \pm 0.10^{a,b}$

The results are shown as mean ± standard deviation. ^{a,b,c} *= Means on the same column with different letter superscripts differ significantly (p<0.05)*

Anti-Inflammatory Effect of *Aju Mbaise* Polyherbal Extract in Carrageenan Induced Paw Oedema in Rats

The extract, similar to Aspirin, significantly reduced the degree of paw oedema in rats at all administered doses compared to the control ($p<0.05$). The extract's inhibitory effect on carrageenan-induced paw oedema was 47.83 ± 1.90%, 77.35 ± 3.97%, and 85.05 ± 1.09% for 250, 500, and 1000 mg/kg doses, respectively, compared to the effect of Aspirin at 56.93 ± 1.59%. Comparing the extract-treated groups to the control groups, inflammation-related indicators showed a significant difference ($p<0.05$). The levels of Interleukin 1b and prostaglandin E2 were lower in the extract-treated groups compared to the control (Tables 7 and 8).

Table 7. Anti-inflammatory effect of *Aju Mbaise* polyherbal extract in rats.

Treatments	Initial paw circumference (cm)	30 minutes paw post circumference (cm)	1 hour paw post circumference (cm)	2 hours paw post circumference (cm)
Control	$2.44 \pm 0.17^{a,1}$	$3.82 \pm 0.21^{b,2}$	$4.22 \pm 0.20^{d,3}$	$4.20 \pm 0.47^{d,3}$
Aspirin 100 mg/kg	$2.70 \pm 0.10^{b,1}$	$3.26 \pm 0.25^{a,2}$	$3.66 \pm 0.13^{b,2,3}$	$3.42 \pm 0.48^{b,3}$
Aju Mbaise 250 mg/kg	$2.74 \pm 0.21^{b,1}$	$3.20 \pm 0.12^{a,2}$	$3.94 \pm 0.27^{c,3}$	$3.66 \pm 0.15^{c,3}$
Aju Mbaise 500 mg/kg	$2.72 \pm 0.08^{b,1}$	$3.04 \pm 0.13^{a,3}$	$3.20 \pm 0.11^{a,2}$	$3.00 \pm 0.01^{a,2}$
Aju Mbaise 1000 mg/kg	$2.58 \pm 0.18^{a,b,1}$	$3.26 \pm 0.06^{a,4}$	$3.06 \pm 0.17^{a,3}$	$2.84 \pm 0.23^{a,2}$

The results are shown as mean ± standard deviation, ^{a,b,c} *= Means on the same column with different letter superscripts differ significantly (p<0.05),* ^{1,2,3,4} *= Means on the same row with different number superscripts differ significantly (p<0.05)*

Table 8. Anti-inflammatory effect of *Aju Mbaise* polyherbal extract in rats.

Treatments	Change in paw circumference after 2 hours	% inhibition of inflammation	IL-1b ng/ml	PGE-2 ng/ml
Control	1.72 ± 0.30^{c}	0.00 ± 0.00^{a}	1.32 ± 0.03^{d}	73.26 ± 1.42^{a}
Aspirin 100 mg/kg	0.72 ± 0.16^{b}	56.93 ± 1.59^{c}	0.89 ± 0.03^{b}	64.94 ± 1.31^{b}
Aju Mbaise 250 mg/kg	0.94 ± 0.05^{b}	47.83 ± 1.90^{b}	1.01 ± 0.02^{c}	71.66 ± 2.70^{a}
Aju Mbaise 500 mg/kg	0.32 ± 0.06^{a}	77.35 ± 3.97^{d}	0.81 ± 0.02^{a}	68.84 ± 1.20^{c}
Aju Mbaise 1000 mg/kg	0.24 ± 0.05^{a}	85.05 ± 1.09^{d}	0.84 ± 0.06^{a}	66.77 ± 2.34^{c}

The results are shown as mean ± standard deviation. ^{a,b,c} *= Means on the same column with different letter superscripts differ significantly (p<0.05)*

Analgesic Effect of *Aju Mbaise* Polyherbal Extract in Acetic Acid-Induced Pain in Rats

The extract also showed significant analgesic activity similar to that of Aspirin when compared with the control ($p<0.05$), as the extract-treated rats made fewer writhes than the control with 36.76 ± 3.80%, 66.01 ± 2.79% and 77.54 ± 3.01% analgesic activities for 250, 500 and 1000 mg/kg treatment dose levels respectively (Table 9).

Table 9. Anti-analgesic effect of *Aju Mbaise* polyherbal extract in rats.

Treatments	% Writhes after 30 minutes	% Analgesic activity
Control	59.80 ± 2.44^{d}	0.00 ± 0.00^{a}
Aspirin 100 mg/kg	14.00 ± 2.35^{a}	69.37 ± 1.13^{c}
Aju Mbaise 250 mg/kg	33.80 ± 1.64^{c}	36.76 ± 3.80^{b}
Aju Mbaise 500 mg/kg	18.80 ± 2.17^{b}	66.01 ± 2.79^{c}
Aju Mbaise 1000 mg/kg	11.60 ± 1.95^{a}	77.54 ± 3.01^{d}

The results are shown as mean ± standard deviation. ^{a,b,c} *= Means on the same column with different letter superscripts differ significantly ($p<0.05$)*

DISCUSSION

The findings of this study indicate that the APE has an LD_{50} value exceeding 5000 mg/kg body weight, suggesting that it is safe for oral consumption and may not cause systemic toxicity in rats. These findings can potentially be extrapolated to humans and other animals. The non-toxic effect of APE had earlier been reported by Ijioma et al. (2019a), following acute and sub-acute *in vivo* toxicity studies, and has only been confirmed by the findings of this study. International standards for acute toxicity studies indicate that a major outcome in testing toxic substances is mortality and that where no mortality occurs, the agent being investigated may be considered a safe medication for use (OECD, 2001). The findings of this study are consistent with the report by Erhirhie et al. (2018), who classified any substance with an LD_{50} value above 5000 mg/kg as non-toxic. In plant-based substances, toxicity usually results from consuming intolerable amounts of phytochemical components in plants (Bode & Dong, 2015). However, this study's findings have shown that the amount of the various phytochemical agents in APE was well tolerated in rats, hence translating into positive pharmacological effects that could be harnessed for managing human health challenges. In this study, APE has shown significant antidiarrhoeal, anti-inflammatory, and analgesic effects in rats.

The three-model approach adopted in the investigation of the anti-diarrhoeal potential of APE all yielded positive outcomes as results obtained suggest that APE may be a valuable agent for treating diarrhoea. In the charcoal meal transit model, the inhibition of both transit and intestinal motility observed suggests that the APE contains bioactive compounds which act by inhibiting acetylcholine activity in the gastrointestinal tract. The crucial role played by acetylcholine in the initiation and maintenance of intestinal motility has been extensively studied (Iino & Nojyo, 2006; Nezami & Srinivasan, 2010; Mercan et al*.,* 2020). Acetylcholine induces contractions of the gastrointestinal smooth muscle through cholinergic signalling, which is mediated by muscarinic acetylcholine receptors present on the surface of smooth muscle cells in the intestine (Iino & Nojyo, 2006). Inhibition of acetylcholine activity is a well-established mechanism for the anti-diarrheal effects of drugs (Colovic *et al.,* 2013). Additionally, castor oil-induced diarrhoea in rats was significantly lowered by the polyherbal extract. Typically released from castor oil by intestinal lipase, ricinoleic acid is a hydroxylated fatty acid that irritates and inflames the intestinal mucosa. This causes prostaglandins and nitric oxide to be released, which in turn increases gastrointestinal secretion, motility, and epithelial permeability and causes diarrhoea (Sharma et al*.,* 2010). The extract may have inhibited the successful promotion of this cascade of activities by inhibiting either the release of ricinoleic acid from castor oil or interfering with intestinal lipase activity. The anticholinergic/spasmolytic activity of the extract in the charcoal meal transit study and anti-enteropooling and ability to remove fluid from the intestinal lumen in the castor oil models all support the claim that the extract may be a good anti-diarrhoeal agent. Agents with such effects have been employed in managing diarrhoea (Sharma et al*.,* 2010). These activities of the extract may not be unconnected with its combined effects of composite phytochemical agents. Tannins, alkaloids and saponins have been implicated in inhibiting intestinal motility and fluid accumulation in the gastrointestinal lumen (Palombo, 2006; Ayinde & Owolabi, 2009). The presence of these phytochemical agents in APE was reported in a previous communication (Ijioma et al*.* 2019a). Another mechanism for achieving antidiarrhoeal effect by agents like Loperamide is the antisecretory pathway. Loperamide alters intestinal absorption and inhibits its secretion by binding to the δ receptor via a cascade of biochemical activities reported by Tagne et al. (2019), resulting in the maintenance of low and high intracellular concentrations of Na^{+} and K^{+} respectively and the inhibition of the diarrhoea causing potentials of ricinoleic acid in castor oil (Ijioma et al*.* 2020). APE may have also achieved its antidiarrhoeal effect via this mechanism, having produced similar activity as Loperamide.

Like Aspirin, APE significantly inhibited carrageenan-induced paw oedema in rats. Carrageenan induces rat paw oedema by first increasing histamine and serotonin activities in the tissues, increasing local synthesis of prostaglandin, and later by bradykinin, leukotrienes, and leukocyte infiltrations and also biosynthesis of prostaglandin by inducible cyclooxygenase (Guay et al*.* 2004; Adebayo et al*.* 2015). The extract's anti-inflammatory effect may have interfered with or reasonably inhibited these biochemical processes leading to less inflammation in the extract-treated rats. The findings of the analgesic study indicate

that the APE has a significant and dose-dependent analgesic effect in rats using the acetic acid pain-induced model. This model induces pain and inflammation in the abdominal cavity through the activation of nociceptors, and the writhing observed in the treated animals is a result of intense endogenous pain (Gregory et al. 2013; Regmi and Shah, 2020). The observed decrease in the levels of interleukin-1b and prostaglandin E2 in APE-treated rats compared to the control group provides additional evidence supporting the argument in favour of the anti-inflammatory and analgesic effects of APE (Mirtella et al. 1995; Muraki et al. 2004). These inflammatory markers usually increase in inflammatory conditions and the opposite may indicate the presence of an anti-inflammatory agent, which in this study is APE.

CONCLUSIONS

APE inhibited diarrhoea by reducing intestinal motility/peristalsis, wet stool frequency, fluid accumulation in the gastrointestinal lumen and loss of electrolytes. These effects suggest that the extract may be a good antidiarrhoeal agent but may be further dissected to characterize other useful phytochemicals eliciting these beneficial effects. Further studies may also provide more support for our current findings.

Acknowledgements: Authors are grateful to Kingsley Chijioke Ugwuanyi an academic staff of Department of Zoology and Environmental Sciences, Blessing Ekwu and Odinaka Ugwuege, undergraduate students of Department of Biochemistry, MOUAU for their assistance during the experimental protocols and data collection.

Authors' Contributions: Uroko R.I., Ogbonna H.N. and Uchenna, N.O., designed the study and carried out the laboratory analyses. Uroko R.I., carried out the statistical analysis of the data obtained from the study. Whereas, Ijioma SN., and Uroko RI, carried out the literature survey and drafted the manuscript for publication. All the coauthors read the manuscript and made necessary input before the final version of the manuscript was approved for publication.

Competing Interests: The authors declare that there are no competing interests.

Funding: The authors declare no external funding for the study.

REFERENCES

Adebayo, S. A., Dzoyem, J. P., Shai, L., J. & Eloff, J. N. (2015). The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African. *BMC Complementary & Alternative Medicine*, 15, 159. https://doi.org/10.1186/s12906-015-0669-5

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 & Therapies*, 22, 8. https://doi.org/10.1186/s12906-021-03498-6

AyindE, B. A., & Owolabi, O. J. (2009). Effects of the aqueous extract of *Ficus capensis* Thunb. (Moraceae) leaf on gastrointestinal motility. *Journal of Pharmacognosy & Phytotherapy*, 1(3), 31-35.

Bentley, C., Laubach, H., Spalter, J., Ginter, E., & Jensen, L. (2004). Relationship of cryptosporidiosis to abdominal pain and diarrhea in Mayan Indians. *Revista do Instituto de Medicina Tropical de São Paulo*, 46(4), 235-237.

Birru, E. M., Asrie, A. B., Adinew, G. M., & Tsegaw, A. (2016). Antidiarrheal activity of crude methanolic root extract of *Idigofera spicata* Forssk. (Fabaceae). *BMC Complementary & Alternative Medicine*, 16, 272. https://doi.org/10.1186/s12906-016-1252-4

Bode, A. M., & Dong, Z. (2015). Toxic phytochemicals and their potential risks for human cancer. *Cancer Prevention Research*, 8(1), 1-8.

Colovic, M. B., krstic, D. Z., Lazarevic-pasti, T. D., Bondzic, A. M., & Vasic, V. M. (2013). Acetylcholinesterase inhibitors: Pharmacology and toxicology. *Current Neuropharmacology*, 11(3), 315-335.

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 4, 177. https://doi.org/10.3389/fphar.2013.00177

Erhirhie, E. O., Ihekwereme, C. P., & Ilodigwe, E. E. (2018). Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. *Interdisciplinary Toxicology*, 11(1), 5-12.

Evans, W. C. (2009). *Trease and Evans Pharmacognosy*. 16th Edition, Elsevier Health Sciences, Edinburgh, United Kingdom.

Ezezika, O., Ragunathan, A., El-bakri, Y., & Barrett, K. (2021). Barriers and facilitators to implementation of oral rehydration therapy in low-and middle-income countries: A systematic review. *PLoS One*, 16(4), e0249638. https://doi.org/10.1371/journal.pone.0249638

Glover, D. M. (2021). Traditional medicines in a global economy: Resource sustainability and resilience in the traditional Tibetan medical practice of ingredient substitution. *Human Ecology*, 49(1), 33-42.

Goodman, O. O., Adejoh, S. O., Adeniran, A., Emechebe, A. C., & Kuyinu, Y. A. (2022). We love orthodox medicine but still use our '*Elewe omo*': Utilization of traditional healers among women in an urban community in Nigeria. *Journal of Family Medicine & Primary Care*, 11(1), 215-223.

Gregory, N. S., Harris, A. L., Robinson, C. R., Dougherty, P. M., Fuchs, P. N., & Sluka, K. A. (2013). An overview of animal models of pain: Disease models and outcome measures. *The Journal of Pain*, 14(11), 1255-1269.

Guay, J., Bateman, K., Gordon, R., Mancini, J., & Riendeau, D. (2004). Carrageenan-induced paw edema in rat elicits a predominant prostaglandin E2 (PGE2) response in the central nervous system associated with the induction of microsomal PGE2 synthase-1. *The Journal of Biological Chemistry*, 279(23), 24866-24872.

Iino, S., & Nojyo, Y. (2006). Muscarinic M2 acetylcholine receptor distribution in the guinea-pig gastrointestinal tract. *Neuroscience*, 138(2), 549-559.

Ijioma, S. N., Osim, E. E., Nwankwo, A. A., Kanu, K. C. and Orieke, D. (2020). Southeast Nigerian polyherbal (*Aju Mbise*): A potential uterotonic and tocolytic agents. *Scientific African*, 8, e00393. https://doi.org/10.1016/j.sciaf.2020.e00393

Ijioma, S. N., Osim, E. E., Nwankwo, A. A., Nwosu, C. O., & Ekeleme, C. M. (2019a). Antioxidant potentials and effects on the hematology and osmotic fragility scores of a polyherbal formulation used in southeast Nigeria. *Journal of Basic and Clinical Physiology & Pharmacology*, 30(4), 20170099. https://doi.org/10.1515/jbcpp-2017-0099

Ijioma, S., Osim, E., Nwankwo, A., Kanu, K., Orieke, D., & Ezike, J. (2019b). Antimotility effect of a southeast Nigerian polyherbal combination (Ajumbise): An in-vitro and in-vivo evaluation. *Animal Research International*, 16(3), 3494-3502.

James, P. B., Wardle, J., Steel, A. & Adams, J. (2018). Traditional, complementary and alternative medicine use in Sub-Saharan Africa: A systematic review. *BMJ Global Health*, 3(5), e000895. https://doi.org/10.1136/ bmjgh-2018-000895

Jin, J., Sklar, G. E., Min Sen OH, V., & Chuen L.I., S. (2008). Factors affecting therapeutic compliance: A review from the patient's perspective. *Therapeutics and Clinical Risk Management*, 4(1), 269-286.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, 54, 275-287.

Mercan, N., Toros, P., Söyler, G., Hanoglu, A., & Kükner, A. (2020). Effects of *Corchorus olitorius* and protacatechuic acid on diabetic rat testis tissue. *International Journal of Morphology*, 38(5),1330-1335.

Mirtella, A., Tringali, G., Guerriero, G., Ghiara, P., Parente, L., Preziosi, P., & Navarra, P. (1995). Evidence that the interleukin-1 beta-induced prostaglandin E2 release from rat hypothalamus is mediated by type I and type II interleukin-1 receptors. *Journal of Neuroimmunology*, 61(2), 171-177.

MurakI, T., Fujimori, K., Ishizaka, M., Ohe, Y., Urade, Y., Okajima, F., & Ishikawa, K. (2004). Effects of interleukin-1beta and prostaglandin E2 on prostaglandin D synthase production in cultivated rat leptomeningeal cells. *Journal of Cerebral Blood Flow & Metabolism*, 24(4), 409-418.

Nezami, B. G., & Srinivasan, S. (2010). Enteric nervous system in the small intestine: pathophysiology and clinical implications. *Current Gastroenterology Reports*, 12, 358-365.

NRC (2011). *Guide for the Care and Use of Laboratory Animals.* Eighth Edition, Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, Division on Earth and Life Studies, The National Research Council, National Academies Press, Washington, DC., USA. https://grants.nih.gov/grants/ olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf

Nwokafor, C. V., Udensi, C. G., Ogbonna, H. N., Udekwu, C. E., Nwankpa, U. D., Amanze, E. K., Chibuzor, W. N,. & Okeke, K. C. (2020). Antimicrobial activities of moringa, neem and ginger plant extracts against bacteria associated with the spoilage of fruit juice. *South Asian Journal of Research in Microbiology*, 7(4), 21-30.

OECD (2001). *OECD Annual Report 2001.* Organisation for Economic Co-operation and Development, Paris, France. https://doi.org/10.1787/annrep-2001-en

Ogbonna, H. N., Nwankpa, U. D., Aloh, G. S., & Ibeh, R. C. (2020). Effect of methanol extract of unripe *Carica papaya* pulp on lipid profile and liver function of alloxan-induced diabetic rats. *International Journal of Biochemistry Research & Review*, 29(4), 1-11.

Palombo, E. A. (2006). Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. *Phytotherapy Research*, 20(9), 717-724.

Panikkath, R., Costilla, V., Hoang, P., Wood, J., Gruden, J. F., Dietrich, B., Gotway, M. B., & Appleton, C. (2014). Chest pain and diarrhea: a case of *Campylobacter jejuni*-associated myocarditis. *The Journal of Emergency Medicine*, 46(2), 180-183.

Regmi, B., & Shah, M. K. (2020). Possible implications of animal models for the assessment of visceral pain. *Animal Models & Experimental Medicine*, 3(3), 215-228.

Salmerón-manzano, E., Garrido-cardenas, J. A., & Manzano-agugliaro, F. (2020). Worldwide research trends on medicinal plants. *International Journal of Environmental Research and Public Health*, 17(10), 3376. https://doi.org/10.3390/ijerph17103376

Sharma, P., Vidyasagar, G., Singh, S., Ghule, S., & Kumar, B. (2010). Antidiarrhoeal activity of leaf extract of *Celosia argentea* in experimentally induced diarrhoea in rats. *Journal of Advanced Pharmaceutical Technology & Research*, 1(1), 41-48.

Sofowora, A., Ogunbodede, E., & OnayadE, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional, Complementary & Alternative Medicines*, 10(5), 210-229.

Tagne, F. M. A., Akaou, H., Noubissi. P. A., Foyet F. A., Rékabi, Y., & Wambe, H. (2019). Effect of the hydroethanolic extract of *Bixa orellana* Linn (Bixaceae) leaves on castor oil-induced diarrhea in Swiss albino mice. *Journal of Gastroenterology Research & Practice,* 2019: e6963548. https://doi.org/10.1155/2019/6963548

Teke, G. N., Kuiate, J. R., Kuete, V., Teponno, R. B., Tapondjou, L. A., & Vilarem, G. (2010). Antidiarrheal activity of extracts and compound from *Trilepisium madagascariense* stem bark. *Indian Journal of Pharmacology*, 42(3), 157-163.Terri, A.E. and Sesin, P.G. (1958). *American Journal of Clinical Pathology*, 29:86.

Udekwu, C. E., Ebhohon, S., Ibeh, R. C., Ogbonna, H. N., & Nwankpa, U. D. (2020). Effect of Aqueous Stem Extract of *Loranthus micranthus* Linn on anti-microbial sensitivity, cytotoxicity, and in-vitro anti-inflammatory indices on human red blood cells. *Asian Journal of Research in Biochemistry*, 6(3), 32-40.

Ugbogu, E. A., Okoro, H., Emmanuel, O., Ugbogu, O. C., Ekweogu, C. N., Uche, M., Dike, E. D., & Ijioma, S. N. (2024). Phytochemical characterization, anti-diarrhoeal, analgesic, anti-inflammatory activities and toxicity profile of *Ananas comosus* (L.) Merr (pineapple) leaf in albino rats. *Journal of Ethnopharmacology*, 319(Pt 2), 117224. https://doi.org/10.1016/j.jep.2023.117224

Ugwuja, F.N., Ezebuiro, F.C., Omodamiro, O.D., Ijioma, S.N., & Mukah, F.E. (2022). Antimicrobial activity and anti-diarrheal potentials of *Psidium guajava* linn leaf extract in experimental rat models. *Animal Research International*, 19(2), 4530-4542.

Uroko, R. I., Aaron, C. F., Uche, M. E., Aguwamba, C., Ogwo, E. U., Nweje-anyalowu, P. C., & Ijioma, S. N. (2022). Effect of *Aju Mbaise* on sperm morphology, semen quality, sex hormonal levels, gonadosomatic index and testicular histology of Avodart-induced rats. *Plant Biotechnology Persa*, 4(2), 22-37.

THIS PAGE INTENTIONALLY LEFT BLANK