Journal of Sustainable Veterinary & Allied Sciences
P-ISSN-2695-2661 e-ISSN: 2811-1346
JoSVAS 2024 Dec. Vol 6 Issue 4: 217-228

Original Research

Phytochemical composition, antioxidant, brine shrimp lethality and antidiarrheal activities of methanol extract of *Erythrina senegalensis* leaf.

***[1]Ukwueze, I. J., [1]Elezua O. P., [2]Ukwueze, C. O., [1]Nweze, E. C., [1]Onoja, S. O., [1]Madubuike, K. G., [1]Ifenkwe, D. C.**

[1]Department of Veterinary Physiology and Pharmacology, [2]Department of Veterinary Surgery and Radiology, Michael Okpara University Agriculture Umudike, Umuahia, Abia State Nigeria.

**Correspondence:* ukwueze.ifunanya@mouau.edu.ng *+2347036038280*

ABSTRACT

This study investigated the phytochemical composition, antioxidant, and antidiarrheal activities of the methanol extract of *Erythrina senegalensis* (MEES) leaf. The fresh leaf *Erythrina senegalensis* was processed and extracted using cold maceration method with 80% hydro-methanol. The total phenols and flavonoids content were determined using standard protocols. The ferric reducing antioxidant power and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay of MEES were evaluated at 25-400 µg/mL concentration. The antidiarrheal activity of MEES was determined using castor-oil induced diarrhea, enteropooling and intestinal transit model in mice at the dose of 100, 200 and 400 mg/kg. The total flavonoid concentration and total phenol concentration of MEES are 252.33 ± 0.67 mg RE/g MEES and 36.05 ± 0.35 mg GAE/g MEES, respectively. The MEES produced concentration-dependent increase in free radical scavenging activity, which was significantly ($p < 0.05$) lower than that of ascorbic acid. At four hours post treatment, the MEES (100, 200 and 400 mg/kg) and loperamide (3 mg/kg) produced 27, 32, 40 and 99% inhibition of diarrhoea respectively, when compared with the distilled water treated group. The MEES (200 and 400 mg/kg) and loperamide (3 mg/kg) produced 13, 25 and 52% inhibition of peristaltic index respectively, relative to the distilled water treated group. The MEES (100, 200 and 400 mg/kg) and loperamide (3 mg/kg) produced 19, 12, 15 and 28% inhibition of intraluminal fluid content accumulation respectively, when compared to the distilled water treated group. The MEES demonstrated mild antidiarrheal property which validates its use as antidiarrheal agent in folkloric medicine.

. **Keywords:** Antidiarrheal, antioxidant, enteropooling, *Erythrina senegalensis*, phytochemical,

INTRODUCTION

Diarrhea stands as a critical global public health issue, posing significant threats of illness and death across various age groups and geographical regions. Children under the age of 5 years in developing countries are especially vulnerable, facing severe consequences (Meng *et al*., 2011). Diagnosis relies on specific criteria such as stool frequency, consistency, and volume, with the World Health Organization defining diarrhea as increased bowel movements with excessive water content (WHO, 20O5; Chu *et al*., 2020). Imbalances in intestinal processes, disrupting water and electrolyte equilibrium, often precipitate diarrhea (Bartel & Gau, 2012; Emudainohwo *et al*., 2015).

Diarrhea ranks as a leading cause of mortality among children under 5 years, surpassing combined deaths from AIDS, malaria, and measles (WHO, 2005; Liu *et al*., 2012). It holds the unfortunate position as the second leading cause of death, following pneumonia, in this age group in developing nations (Lanata *et al*., 2013). Classification of diarrhea hinges on factors like disease course, presence of infectious agents, and stool characteristics, manifesting with symptoms such as fever, weakness, and abdominal discomfort.

Efforts to manage diarrhea focus on minimizing both morbidity and mortality. Conventional antidiarrheal drugs are commonly employed, yet accessibility and efficacy can be challenging (WHO, 2005; Chu *et al*., 2020). Consequently, many individuals, including both rural and urban dwellers, are turning to medicinal plants as an alternative treatment. This trend reflects a shift towards

http://doi.org/10.54328/covm.josvas.2024.195

seeking affordable and effective solutions for various health conditions, highlighting the need for diverse and accessible healthcare options.

Erythrina senegalensis DC, commonly known as coral flower or parrot tree, is a thorny shrub with vibrant red flowers belonging to the Fabaceae family (Atsamo *et al*., 2011). It holds strong cultural significance in West Africa and, known as "echichi" in Igbo, "minjirya" and "showoh" in Hausa and Tiv, respectively and "Ologun sheshe" in South Western Nigeria (Saidu *et al*., 2000; Udem *et al*., 2010). Traditional healers utilize its stem, leaves and root bark for various ailments, such as malaria, liver disease, gastrointestinal disorders, diarrhea, amenorrhea, secondary infertility, nose bleeding etc (Doughari, 2010; Atsamo *et al*., 2011). Some phytochemical constituents such as alkaloids, flavonoids, tannins, saponins, and terpenoids have been identified in the various parts of the plant (Rwang *et al*., 2016). Despite extensive scientific research on its toxicity (Obidah *et al*., 2014), antihypertensive, antidiabetic (Bilanda *et al*., 2020), and hepatoprotective and antioxidant (Donfack *et al*., 2008) properties, there is a notable gap regarding its antidiarrheal effects, prompting further investigation. This study investigated the phytochemical composition, antioxidant and antidiarrheal activities of the methanol extract of *Erythrina senegalensis* leaf.

MATERIALS AND METHODS

PLANT COLLECTION AND IDENTIFICATION

Fresh leaves of *Erythrina senegalensis* was collected from Ameke Afara Ibeku in Umuahia North Local Government Area, Abia State. The plant was identified and authenticated by Mr. S. C. Ibe in the Department of Forestry, Michael Okpara University of Agriculture, Umudike and a voucher specimen (MOUAU/CVM/VPP/2020/03) kept in the departmental herbarium unit.

PREPARATION OF EXTRACT

The fresh leaves of *Erythrina senegalensis* was washed under clean running water immediately after collection and air dried to a constant weight, and then pulverized into coarse powder using a contact milling machine. The quantity of the plant material was weighed with an electronic balance, and then macerated (cold maceration) in 80% analytical grade methanol at a ratio of 277g of plant material:1.383 Liters of methanol. The content of the bottle was vigorously agitated intermittently every 3 hours for 72 hours. The mixture was filtered through a Whatman filter paper into an already weighed beaker at room temperature. The filtrate was concentrated to dryness under pressure using a Rotary evaporator (Cole-Parmer type N-1110, China), and the percentage yield (w/w) of the plant extract was calculated using the formular below.

$$\% \text{ Yield} = \frac{W1}{W2} \times \frac{100}{1}$$

Where: W_1 = Weight of dried MEES; W_2 = Weight of plant material

The methanol extract of *Erythrina senegalensis* (MEES) was stored in a refrigerated at 4°C until commencement of the experimental study.

EXPERIMENTAL ANIMALS AND THEIR MANAGEMENT

Apparently healthy adult mice of either sexes, weighing between 28-30 grams were procured from the Laboratory Animal House, of the Department of Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, for the study. The experimental animal was housed in a wire cage and feed with pelleted grower mesh (vital feeds) and portable water *ad libitum*. The ethical approval for the use of laboratory animal was obtained from the College of Veterinary Medicine Research Ethics committee, Michael Okpara University of Agriculture, Umudike (MOUAU/CVM/REC/202409).

TOTAL PHENOL CONTENT

Total phenol content (TPC) in MEES was determined using the FC method as described by Do *et al*. (2014), with minor modifications. The freeze-dried MEES was dissolved in distilled water to a concentration of 50 μg/mL. The calibration curve was established using gallic acid (0–60 μg/mL). The diluted MEES or gallic acid (1.6 mL) was added to 0.2 mL FC reagent (5-fold diluted with distilled water) and mixed thoroughly for 3 minutes. Sodium carbonate (0.2 mL, 10% w/v) was added to the mixture and the mixture was allowed to stand for 30 minutes at room temperature. The absorbance of the mixture was measured at 760 nm using a UV–VIS spectrophotometer V-550 model (Jasco, Tokyo, Japan). TPC of MEES was expressed as milligram gallic acid equivalent per gram MEES (mg GAE/g MEES).

TOTAL FLAVONOID CONTENT

The total flavonoid content (TFC) of the MEES was investigated using the aluminum chloride colorimetry method described by Li *et al*. (2015) with slight modifications. In brief, the MEES sample was diluted with methanol to produce 100 μg/mL solution. The calibration curve was prepared by diluting rutin in methanol (0–100 μg/mL). The diluted MEES or rutin (2.5 mL) was mixed with 0.15 ml of 5% $NaNO_2$. After 5 minutes, 0.15 mL of 10% (w/v) aluminium chloride solution was added, and the mixture was allowed to stand for another 5 minutes, after which 1 ml of 1 molar NaOH was added. The mixture was kept at room temperature for 15 minutes. Then absorbance of the mixture was measured at 415 nm using a UV–VIS spectrophotometer. TFC was expressed as milligram rutin equivalent per gram MEES (mg RE/g MEES).

IN-VITRO ANTIOXIDANT ACTIVITIES OF MEES

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) photometric assay

The free radical scavenging activity of the MEES was investigated by the DPPH assay (Mensor *et al.*, 2001) using spectrophotometer. The MEES at concentrations (25, 50, 100, 200 and 400) µg/mL each was mixed with 1 mL of 0.5 mM DPPH (in methanol) in a cuvette. The absorbance at 517 nm was taken after 30 minutes of incubation in the dark at room temperature. The experiment was done in triplicate.

The percentage antioxidant activities were calculated as follows.

% antioxidant activity (AA) = 100-[{(ABS sample—ABS blank) ×100}/ABS control]

One mililitre of methanol plus 2.0 mL of the MEES was used as the blank while 1.0 mL of the 0.5 mM DPPH solution plus 2.0 mL of methanol will be used as the negative control. Ascorbic acid (vitamin C) will be used as reference standard (Iwalewa *et al.*, 2008).

FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

The ferric reducing antioxidant power was carried out as described by Benzie & Strain (1999). The following three reagents were procured;

Acetate buffer (300 mM), pH 3.6 (3.1 g sodium acetate.$3H_2O$ and 16 mL glacial acetic acid in 1000 mL buffer solution), 2, 4, 6-triphridyl-s-triazine (TPTZ) (10 mM) in 40 mM HCL and $FeCl_3$ $6H_2O$ (20 mM) in distilled water.

The FRAP working solution will be prepared by mixing the three aforementioned solutions in the ratio of 10:1:1, respectively. The working solution was usually freshly prepared.

The FRAP reagent (3 mL) and 100 µl sample solution at concentrations of 25, 50, 100, 200 and 400 µg/mL were mixed and allowed to stand for 4 minutes. The absorbance was recorded at 593 nm, at 37°C. The ascorbic acid was tested in a parallel process. The absorbance (abs) of each test tube was read taken at 0 and 4 minutes after addition of sample.

FRAP value = abs 4 minutes $-$ abs 0 $minute$

GAS CHROMATOGRAPHY – MASS SPECTRA (GC-MS) ANALYSIS OF MEES

The GC–MS analysis of bioactive compounds in MEES was done using the procedure adopted by Anekwe *et al*., (2023). Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film), was used. Spectroscopic detection by GC–MS involved an electron ionization system which utilizes high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 –150 °C with increasing rate of 3°C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10°C/min. One microliter of the prepared 1% MEES diluted with respective solvents was injected in a split-less mode. Relative quantity of the chemical compounds present in the MEES was expressed as percentage based on peak area produced in the chromatogram. The bioactive compounds were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC–MS systems).

BRINE SHRIMP LETHALITY TEST

The shrimp's eggs stored in refrigerator at 4 °C was hatched with sea water under environmental conditions (temperature, light and humidity). The shrimp's eggs were poured into sea water (at ratio 1:1000) and allowed to hatch after 24 hours. After hatching, actively swimming larva/nauplii were seen in the water (Sarah *et al.*, 2017). Clean test tubes were taken and labelled. 10 mg of MEES was weighed using an analytical balance and stock solution was prepared by dissolving 10 mg of MEES in 1 mL of water. Concentrations of 1, 10, 100, and 1000 µg/mL of MEES was prepared by serial dilution from the stock solution. The test was done in triplicate. Then 1 mL of prepared solutions was taken into each of the test tubes containing 10 nauplii and 1 mL of sea water. The number of dead nauplii were counted after 24 hours (Sarah *et al.*, 2017). The blank control was conducted with only sea water. The lethal concentration for 50% mortality after 24 h of exposure (LC_{50}) was determined using the probate method.

$$\text{Percentage mortality (\%)} = \frac{initial\ number\ of\ nauplii - number\ of\ surviving\ nauplii}{initial\ number\ of\ nauplii} \times \frac{100}{1}$$

ANTIDIARRHEA SCREENING

EFFECT OF MEES ON CASTOR OIL-INDUCED DIARRHOEA IN MICE

The method described by Onoja & Udeh (2015) was adopted to investigate the effect of MEES on castor oil induced diarrhoea in mice. A total of twenty-five (25) mice was used for this experiment. The mice were fasted for 12 hours before the commencement of the experiment and were randomly divided into five groups (1-5; n = 5). The mice in group 1 received 5 ml/kg distilled water and served as negative control, while those in groups 2-4 received graded doses of 100, 200, and 400 mg/kg of the MEES, respectively. The mice in group 5 received loperamide at 3 mg/kg and served as positive control. After 60 minutes post treatment, all the mice received 0.5 ml of castor oil orally

and were kept in separate metabolic cages. The total number of the faeces (dry and wet diarrheal droppings) were determined at an hourly interval for up to 4 hours post castor oil administration. The total score of diarrheal faeces for the negative control group was considered as 100% and percent inhibition of total defecation was calculated using the following formulas;

$$\% \text{ Inhibition of diarrhea} = \frac{\text{Total number of feces in control} - \text{Total number of feces in treated}}{\text{Total number of feces in the negative control}} \times \frac{100}{1}$$

EFFECT OF MEES ON GASTROINTESTINAL TRANSIT TIME

The methods described by Onoja *et al.* (2018) was adopted in this study to investigate the effect of MEES on gastrointestinal transit time in mice. A total number of twenty-five (25) mice were fasted for 12 hours with free access to water. The mice will be randomly divided into five groups (1-5; n =5). The mice in group 1 received 5 ml/kg distilled water and served as negative control, while those in groups 2-4 received 100, 200, and 400 mg/kg of the MEES, respectively. The mice in group 5 received 3 mg/kg of atropine sulphate through intra peritoneal route and served as positive control. Six (60) minutes post treatment, all the mice were given 0.5 ml charcoal meal (5% suspension of activated charcoal in 5% aqueous acacia gum), and 30 minutes later, each mouse was humanely sacrificed by cervical dislocation, and abdomen was opened and the small intestines immediately removed. The length (cm) of the small intestine from pylorus to the caecum (LSI) and the distance travelled by the charcoal meal (DC) were measured and recorded. The Mean values obtained were used to calculate the peristaltic index (PI) of each mouse, which was expressed as the percentage of the distance travelled by the charcoal meal to the total length of the small intestine. Also, the percentage inhibition of movement was calculated as described by (Akah *et al.*, 1999).

$$\text{PI} = \frac{DC}{LSI} \times \frac{100}{1}$$

Where: PI = Peristaltic index; DC = Distance travelled by charcoal meal; LSI = Length of small intestine

$$\% \text{ inhibition} = \frac{PI \text{ of negative control} - PI \text{ of test}}{PI \text{ of negative control}} \times \frac{100}{1}$$

Effect of MEES on Castor-oil Induced Enteropooling

The method of Mahmud (2020) was adopted for this study to investigate the effect of MEES on castor oil induced enteropooling in mice. Twenty-five (25) mice were fasted for 12 hours and randomly divided into five groups (1-5; n =5). The mice in group 1 received 5 ml/kg distilled water and served as negative control, while those in groups 2-4 received 100, 200, and 400 mg/kg of the MEES, respectively. The mice in group 5 received loperamide at 3 mg/kg and served as positive control. Six (60) minutes post treatment, castor oil was administered orally to all the mice at a dose of 0.5 ml/mouse and 30 minutes later, each mouse was sacrificed via cervical dislocation. The abdomen was opened and the small intestine was ligated at the pyloric sphincter and the ileo-caecal junctions and dissected out. The intestinal content was collected by milking into pre-weighed graduated test tubes and the new weight measured and recorded. The volume of the intestinal content was read directly from the graduation and recorded.

$$\% \text{ inhibition} = \frac{mean\ Volume\ of\ negative\ control - Mean\ volume\ of\ test}{mean\ Volume\ of\ negative\ control} \times \frac{100}{1}$$

STATISTICAL ANALYSIS

Data obtained from the study was analyzed using Statistical Package for Social Science (SPSS 2008), version 22. One-way analysis of variance (ANOVA) coupled with appropriate post-hoc statistics was conducted to compare the means. The statistical confidence was set at 95 % ($P < 0.05$).

RESULT

QUANTITATIVE ANALYSIS OF PHYTOCHEMICAL CONSTITUENT OF MEES

The result of the quantitative estimation of the total flavonoid concentration and total phenol concentration of MEES is presented in Table I. The total flavonoid concentration and total phenol concentration of MEES are 252.33 ± 0.67 mg RE/g MEES and 36.05 ± 0.35 mg GAE/g MEES, respectively.

Table I: Total flavonoid and total phenol concentrations of MEES leaf

Sample	Total flavonoid (mg RE/g MEES)	Total phenol (mg GAE/g MEES)
MEES	252.33 ± 0.67	36.05 ± 0.35

THE GCMS IDENTIFIED COMPOUNDS

The GCMS analysis identified the presence of 76 bioactive compounds. Amongst the 76 identified compounds; caryophyllene (6.22%), alpha selinene (3.15%), E-beta famesene (4.30%), beta cubebene (3.98%), beta bisabolene (10.89%), beta sesquiphellandrene (7.58%), 1-piperonyl-3,5-diamino-1,2,4-triazole (4.68%) and piperanin (9.18%) were the major constituents. The percentage composition of 55 of the identified compounds were less than one percent. Only 25 of the identified compounds had percentage composition of greater than one percent.

DPPH RADICAL SCAVENGING ASSAY OF MEES LEAF

The result of the DPPH radical scavenging activity of MEES is presented in Figure I. The MEES produced concentration-dependent increase in free radical scavenging activity, which was significantly ($p < 0.05$) lower than that of ascorbic acid.

Table II The GCMS result of MEES leaf

S/N	% Comp.	RT	Chemical name	MW (g/mol)	MF
1	0.71	6.492	Decane	142. 29	$C_{10}H_{22}$
2	0.34	6.84	1,4-dichloro-benzene	147	$C_6H_4Cl_2$
3	0.40	8.112	Oxalic acid, isobutyl nonyl ester	272.38	$C_{15}H_{28}O_4$
4	0.54	8.379	2,6,10-trimethyl-Dodecane	212.41	$C_{15}H_{32}$
5	1.07	8.641	Dodecane	I70.34	$C_{12}H_{26}$
6	0.20	8.697	3,7-dimethyl-Undecane	184.36	$C_{13}H_{28}$
7	1.53	8.958	2,6-dimethyl-Undecane	184.36	$C_{13}H_{28}$
8	0.38	9.119	2,7-dimethyl-Octane	142.28	$C_{10}H_{22}$
9	0.54	9.274	2,6,10,14-tetramethyl-Heptadecane	296.57	$C_{21}H_{44}$
10	0.70	9.339	Hexadecane	226.44	$C_{16}H_{34}$
11	2.44	9.444	Linalool	154.25	$C_{10}H_{18}O$
12	0.42	9.689	4-methyl-1-Decene	154.29	$C_{11}H_{22}$
13	0.23	9.733	2,6-dimethyl-Octane	142.28	$C_{10}H_{22}$
14	0.54	9.806	3-methyl-Nonane	142.29	$C_{10}H_{22}$
15	0.09	10.027	2,6-Dimethyldecane	170.33	$C_{12}H_{26}$
16	0.06	10.102	2,3,3-trimethyl-Octane	156.30	$C_{11}H_{24}$
17	0.38	15.110	Tridecane	184.4	$C_{13}H_{28}$
18	0.10	16.471	.alpha.-Cubebene	204.35	$C_{15}H_{24}$
19	1.23	17.188	.alfa.-Copaene	204.36	$C_{15}H_{24}$
20	2.71	17.651	2,5-Octadiene	110.20	C_8H_{14}
21	0.37	17.837	Tetradecane	198.39	$C_{14}H_{30}$
22	0.49	18.087	α-Gurgujene	204.35	$C_{15}H_{24}$
23	0.14	18.258	1,3,6,10-Dodecatetraene, 3,7,11-tr imethyl-, (Z,E)-	204.35	$C_{15}H_{24}$
24	6.22	18.374	Caryophyllene	204.35	$C_{15}H_{24}$
25	3.15	18.741	.alpha.-Selinene	204.35	$C_{15}H_{24}$
26	0.28	18.981	• *Guaia-6,9-diene*	204.35	$C_{15}H_{24}$
27	1.94	19.257	Humulene	204.35	$C_{15}H_{24}$
28	4.30	19.372	(E)-.beta.-Famesene	204.35	$C_{15}H_{24}$
29	0.62	19.481	• (-)-Spathulenol	220.35	$C_{15}H_{24}O$
30	0.49	19.880	.gamma.-Muurolene	204.35	$C_{15}H_{24}$
31	3.98	19.991	beta.-Cubebene	190.32	$C_{15}H_{24}$
33	2.98	20.355	(E,Z)-.alpha.-Farnesene	204.35	$C_{15}H_{24}$
34	0.43	20.486	.alpha.-Muurolene	204.35	$C_{15}H_{24}$
35	0.37	20.594	8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene	204.35	$C_{15}H_{24}$
36	10.89	20.734	.beta.-Bisabolene	204.35	$C_{15}H_{24}$
37	1.02	20.992	2,4-Di-tert-butylphenol	206.32	$C_{14}H_{22}O$
38	7.58	21.104	beta.-Sesquiphellandrene	204.35	$C_{15}H_{24}$
39	0.93	21.266	• γ-Bisabolene	204.35	$C_{15}H_{24}$

Table II The GCMS result of MEES leaf

S/N	% Comp.	RT	Chemical name	MW (g/mol)	MF
40	0.68	21.535	Bisabolene	204.35	$C_{15}H_{24}$
41	0.43	21.751	1,5-Cycloundecadiene, 9-(1-methylethylidene)-	190.32	$C_{14}H_{22}$
42	2.22	21.886	Alloaromadendrene	204.35	$C_{15}H_{24}$
43	2.83	22.082	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	222.37	$C_{15}H_{26}O$
44	0.26	22.457	(-)-Spathulenol	220.35	$C_{15}H_{24}O$
45	0.98	22.537	Caryophyllene oxide	220.35	$C_{15}H_{24}O$
46	0.98	22.693	Cyclododecane, ethyl-	196.37	$C_{14}H_{28}$
47	0.80	22.932	Guaiol	222.37	$C_{15}H_{26}O$
48	0.29	23.184	3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-	138.21	$C_9H_{14}O$
49	0.29	23.302	2-Octenoic acid, 4-isopropylidene-7-methyl-6-methylene-, methyl ester	222.32	$C_{14}H_{22}O_2$
50	0.13	23.596	Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano-	313.44	$C_{20}H_{27}NO_2$
51	0.51	23.986	• (+)-γ-Gurjunene	204.35	$C_{15}H_{24}$
53	0.85	25.066	Aromadendrane	206.37	$C_{15}H_{26}$
54	0.64	27.256	E-15-Heptadecenal	252.44	$C_{17}H_{32}O$
55	0.27	27.795	2,6-Octadienal, 3,7-dimethyl-, (Z)	152.23	$C_{10}H_{16}O$
56	0.19	28.363	Estran-3-one, 17-(acetyloxy)-2-methyl-, (2.alpha.,5.alpha.,17.beta.)	332.50	$C_{21}H_{32}O_3$
57	0.18	29.556	Pentadecanoic acid, 14-methyl-, methyl ester	270.50	$C_{17}H_{34}O_2$
58	0.90	29.759	2,3-Pentadiene, 2,4-dimethyl-	96.17	C_7H_{12}
59	0.40	30.017	Dibutyl phthalate	278.34	$C_{16}H_{22}O_4$
60	0.94	30.280	Hexadecanoic acid, ethyl ester	284.48	$C_{18}H_{36}O_2$
61	0.09	30.433	Furo[2,3-H]coumarine, 6-methyl-1-phenylamino-		
62	0.15	31.080	2-Trifluoroacetoxypentadecane	324.4	$C_{17}H_{31}F_3O_2$
63	0.18	31.191	13-Octadecenoic acid, methyl ester	296.49	$C_{19}H_{36}O_2$
64	0.13	31.304	Phytol	296.53	$C_{20}H_{40}O$
65	0.28	31.628	Linoleic acid ethyl ester	308.50	$C_{20}H_{36}O_2$
66	0.23	31.665	Ethyl Oleate	310.51	$C_{20}H_{38}O_2$
67	0.43	31.813	1-Docosene	308.59	$C_{22}H_{44}$
68	1.94	33.266	4,5-Dihydropiperlonguminine	275.34	$C_{16}H_{21}NO_3$
69	0.33	34.004	Bis(2-ethylhexyl) phthalate	390.56	$C_{24}H_{38}O_4$
70	0.14	34.145	1,4-Cyclohexanedimethanamine	142.24	$C_8H_{18}N_2$
71	4.68	34.696	1-Piperonyl-3,5-diamino-1,2,4-triazole		
72	9.18	34.907	Piperanin	287.35	$C_{17}H_{21}NO_3$
73	0.57	35.660	Bicyclo[4.1.0]heptane,-3-cyclopropyl,-7-carbmethoxy, cis-		
74	2.32	35.846	3-oxatricyclo[3.2.1.02,4]octane	110.15	$C_7H_{10}O$
75	0.82	36.007	Piperine	285.35l	$C_{17}H_{19}NO_3$
76	1.18	36.181	1-(3,4-Dimethoxyphenyl)propan-1-on		

THE FRAP MEES LEAF

The result of the ferric reducing antioxidant power is presented in Figure II. The MEES exhibited concentration-dependent increase in FRAP value, which was significantly ($p < 0.05$) lower than that of ascorbic acid.

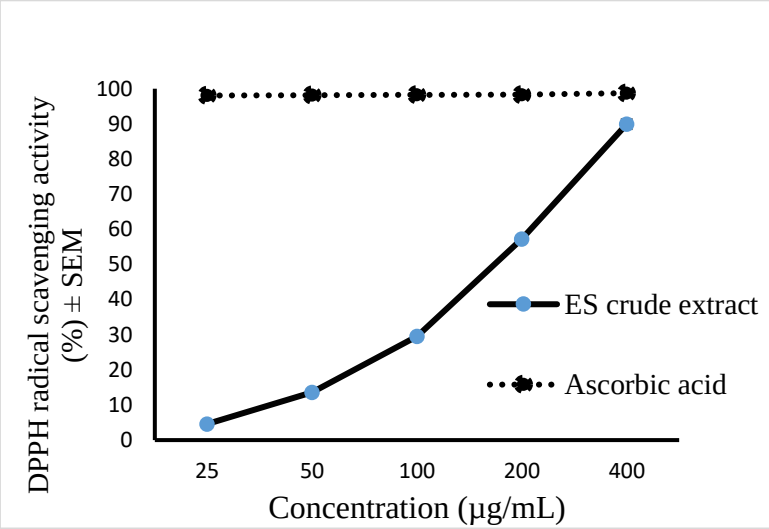

Figure I: DPPH radical scavenging activity of MEES leaf

Legend: DPPH = 2, 2-Diphenyl-1-Picrylhydrazyl, MEES = Methanol extract of *Erythrina senegalensis*, E.S = *Erythrina senegalensis*

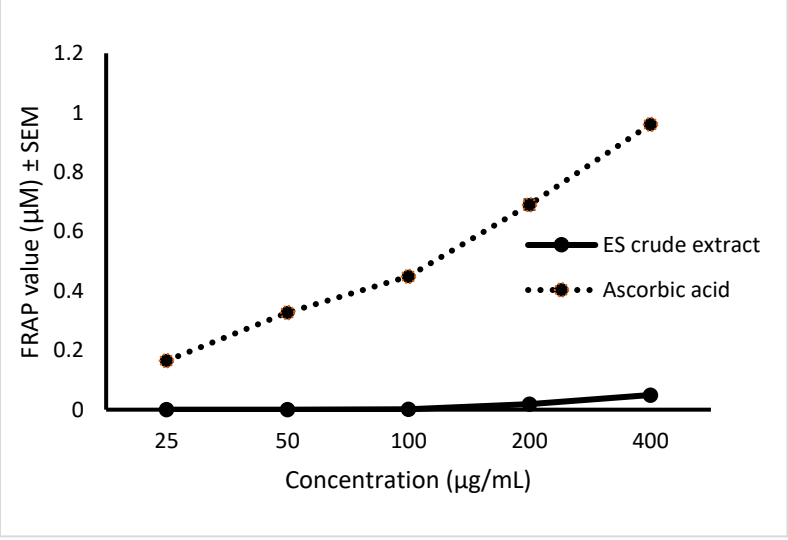

FIGURE II The FRAP value of MEES leaf

BRINE SHRIMP LETHALITY TEST OF MEES

The result of the brine shrimp lethality test of MEES is presented in Table III. The MEES was tested for cytotoxic activity by the brine shrimp lethality assay. The MEES produced concentration dependent increase in mortality of the shrimps. The mortality rate at concentrations 100 ppm and below was significantly ($P < 0.05$) low. The lethal concentration (LC_{50}) of the MEES is 1000 ppm.

EFFECT OF MEES ON TOTAL NUMBER OF FAECES (PERCENTAGE INHIBITION) IN CASTOR OIL INDUCED DIARRHOEA IN MICE

The result of the effect of MEES leaf on castor oil induced diarrhoea is presented in Table IV. The MEES produced dose- and time-dependent increase in antidiarrheal activity. At three and four hours post treatment, the total number of faeces in groups treated with MEES (100, 200 and 400 mg/kg) and loperamide (3 mg/kg) was significantly ($p < 0.05$) lower when compared to the distilled water treated group. At two hours post treatment, the total number of faeces in the MEES (400 mg/kg) and loperamide (3 mg/kg) was significantly ($p < 0.05$) lower when compared to the distilled water treated group. At two hours post treatment, the MEES (100, 200 and 400 mg/kg) and loperamide (3 mg/kg) produced 19, 27, 50% and 100% inhibition of diarrhoea respectively, when compared to the distilled water treated group. At four hours post treatment, the MEES (100, 200 and 400 mg/kg) and loperamide (3 mg/kg) produced 27, 32, 40 and 99% inhibition of diarrhoea respectively, when compared with the distilled water treated group.

EFFECT OF MEES ON CHARCOAL MEAL TRANSIT TIME IN MICE

The result of the effect of MEES on charcoal meal transit time in mice is presented in Table V. The peristaltic index in the groups treated with MEES (400 mg/kg) and loperamide (3 mg/kg) were significantly ($P < 0.05$) lower, when compared to the distilled water treated group. There was no significant ($P > 0.05$) difference in the peristaltic index of the groups treated with MEES (100 and 200 mg/kg), when compared with the distilled water treated group. The MEES (200 and 400 mg/kg) and loperamide produced 13, 25 and 52% inhibition of peristaltic index respectively, relative to the distilled water treated group.

EFFECT OF MEES ON INTRALUMINAL FLUID CONTENT (ENTEROPOOLING) IN MICE

The result of the effect of MEES on intraluminal fluid content (enteropooling) in mice is presented in Table VI. The intestinal content in the loperamide (3 mg/kg) treated group was significantly ($p < 0.05$) lower when compared with distilled water treated group. There was no significance ($P >$

0.05) in the MEES (100, 200 and 400 mg/kg) treated groups when compared with distilled water treated group. The MEES (100, 200 and 400 mg/kg) and loperamide (3 mg/kg) produced 19, 12, 15 and 28% inhibition of intraluminal fluid content accumulation respectively, when compared to the distilled water treated group.

Table III: Brine shrimp lethality test of MEES

Concentration (ppm)	Number of Nymph alive	Number of Nymph dead	Mortality (%)
0	10.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1	9.67 ± 0.33	0.33 ± 0.33	3.33 ± 0.33
10	9.33 ± 0.33	0.67 ± 0.33	6.67 ± 0.33
100	9.33 ± 0.33	0.67 ± 0.33	6.67 ± 0.33
1000	5.00 ± 0.58	5.00 ± 0.58	50.00 ± 0.58

IC_{50} = 1000 ppm

Table IV: Effect of MEES on total number of faeces (percentage protection) in castor oil induced diarrhoea in mice.

	Mean number of faeces/percentage inhibition of diarrhea			
Treatment groups	One hour	Two hours	Three hours	Four hours
Distilled water, 5 ml/kg	4.00 ± 1.23 (0%)	13.00 ± 2.59 (0%)	17.00 ± 1.38 (0%)	20.50 ± 2.29 (0%)
Loperamide, 3 mg/kg	0.00± 0.00 (100%)	0.00 ± 0.00* (100%)	0.25 ± 0.19* (99%)	0.25 ± 0.19* (99%)
MEES, 100 mg/kg	4.75 ± 1.32 (-18%)	10.50 ± 1.43 (19%)	14.00 ± 0.32* (18%)	15.00 ± 0.32* (27%)
MEES, 200 mg/kg	4.00 ± 2.12 (0%)	9.50 ± 1.43 (27%)	11.50 ± 0.67* (32%)	14.00 ± 0.84* (32%)
MEES, 400 mg/kg	2.00 ± 1.30 (50%)	6.50 ± 1.94* (50%)	10.25 ± 1.50* (40%)	12.25 ± 1.32* (40%)

*$p < 0.05$ when compared with the distilled water treated group

Table V: Effect of MEES on charcoal meal transit time in mice

Treatment groups	Peristaltic index	Percentage Inhibition
Distilled water, 5 ml/kg	84.98 ± 1.39	0%
Loperamide 3 mg/kg	40.86 ± 4.74*	52%
MEES, 100 mg/kg	83.93 ± 5.15	1%
MEES, 200 mg/kg	73.92 ± 5.67	13%
MEES, 400 mg/kg	63.73 ± 4.83*	25%

DISCUSSION

This study investigated the phytochemical composition, antioxidant and antidiarrheal activities of methanol extract of *Erythrina senegalensis* leaf. The strength of a medicinal plant lies on the secondary metabolites it contains. Plant with tannins, alkaloids, saponins, phenols, flavonoids, steroids, and terpenoids have been reported to possess antidiarrheal and antioxidant activity (Akter *et al*., 2010; Agbor *et al*., 2014; Dey *et al*., 2014). The MEES leaf exhibited mild antioxidant and antidiarrheal properties which could be linked to the presence of some potent bioactive compounds (Agbor *et al*., 2014; Dey *et al*., 2014).

The result of the study showed presence of flavonoids and phenols in the extract, although, the flavonoids and phenols content of the MEES observed in this study is lower than the values reported by Souleymane *et al.* (2021). The variation in the values could be attributed to the difference in the vegetative parts of the plant, soil type of where the plant was harvested, climate factor and other environmental factors that might influence the phytochemical composition of plants (Li *et al.,* 2020; Pant *et al.,* 2021). The phytochemical composition of plants also varies with the vegetative part of the plant and solvent used in the extract preparation (Altemimi *et al.,* 2017). The previous studies used the stem-bark and dichloromethane/methanol in the preparation of the extract, while in this study, leaf and methanol was used in extract preparation. The antidiarrheal, antioxidant, antimicrobial and antispasmodic properties of phenols and flavonoids have been documented (Dhawefi *et al.,* 2021).

Reactive oxygen species (ROS) have been reported as an important factor in many pathological processes (Akter *et al*., 2010; Agbor *et al*., 2014). The resultant effect of ROS is cell membrane disintegration, membrane protein damage and DNA mutation, which can progress to cancer, liver injury and cardiovascular disease (Akter *et al*., 2010; Agbor *et al*., 2014). Consequent upon this, plant extract with free radical scavenging activities may be a great asset in prevention and treatment of diseases originating from ROS effect. Polyphenolic compounds, like flavonoids, tannins and phenols have been reported to have multiple biological effects, including antioxidant activity (Akter *et al*., 2010; Agbor *et al*., 2014). The result of inhibition of DPPH free radical in this study shows that some components of the plant extract have the ability to donate hydrogen proton to the lone pair electron of the radical. This could suggest that the plant extracts contain compounds capable of donating protons to the free radicals (Agbor *et al*., 2014; Dey *et al*., 2014). The MEES leaf showed a concentration dependent increase in inhibition of DPPH radical, with the highest concentration having more effect which is comparable to what was obtained with ascorbic acid reference standard (Agbor *et al*., 2014). The antioxidant activity observed in the DPPH radical scavenging assay may be as a result of the of phytoconstituent content like Flavonoids in the plant extract (Agbor *et al*., 2014; Dey *et al*., 2014). The mechanism of action of FRAP assay is basically on electron transfer, owing to the fact that antioxidant have the ability to reduce the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) form (Benzie & Strains, 1996). The FRAP assay is a reasonable screening for detection of compounds with redox potential (Dey *et al*., 2014; Agbor *et al*., 2014). In this study, MEES showed a concentration dependent increase in the FRAP value, although, the value is lower than that of DPPH.

The GCMS analysis revealed the presence of some bioactive compounds that have been shown to possess antioxidant and antidiarrheal properties. The antioxidant properties of some of the identified compounds such as hexadecane, γ-bisabolene, β-sesquiphellandrene, alloaromadendrene, 2,4-Di-tert-butylphenol, alpha-muurolene, d-nerolidol, ethylcyclododecane, viridiflorol, piperine, gamma-muurolene and β-farnesene have been documented (Nwobodo *et al.,* 2022;). The antidiarrheal as well as antispasmodic property of citral, piperine and tetradecane have been documented (Sharma *et al.,* 2023; Tangpu & Yadav, 2006; Ganjewala *et al*., 2012).

Brine shrimp lethality test is a bioassay model used in the evaluation of the toxicity of variety of substances (Suryawanshi *et al*., 2020; Suneka & Manoranjan, 2021). The MEES leaf caused concentration-dependent death of the nympha with the LC_{50} of 1000 ppm. This indicates that the MEES is relatively safe and contain some bioactive components. This is in agreement with the report of Atsamo *et al*. (2011) on the acute and subacute toxicity profile of *Erythrina senegalensis* stem-bark on mice. They reported that the extract did not cause significant behavioral changes and mortality in the treated animal.

Diarrhea is due to inhibition of absorption and increased secretion of fluid and electrolyte, and abnormal increase in gastrointestinal motility (Bartel & Gau, 2012; Madubuike *et al.,* 2012). Castor oil-induced diarrhea is an extensively used model for the evaluation of the antidiarrheal potentials of druggable agents (Sisay *et al.,* 2017). Castor oil is metabolized into its active metabolite (ricinoleic acid) by the intestinal lipases which causes irritation and inflammation in the intestine with subsequent release of prostaglandins, nitric oxide and platelet activating factor (Meite *et al.,* 2009; Awe *et al.,* 2011), thus stimulating hyper-secretion of fluid and electrolyte, and hyper-motility (Sisay *et al.,* 2017). The MEES leaf produced significant ($P < 0.05$) (Figure I) dose- and time-dependent decline in total number of faecal droppings (Table IV), while the highest dose (400 mg/kg) retarded the propulsive movement of the charcoal meal (Table V) and decreased the volume of intestinal fluid content (Table VI) in the treated mice, although it was not

significant ($P > 0.05$). The MEES leaf exhibited potent antidiarrheal activity which could be attributed to its phytochemical constituents like the flavonoids and phenols, which is in line with the findings of Edward *et al.* (2020) on the phytochemical screening of *Erythrina senegalensis* root. Flavonoids have the ability to inhibit prostaglandin induced intestinal secretion while phenols, in addition to their astringent property, can also reduce intestinal secretion and transit (Rahman *et al.,* 2015). This suggests a possible mechanism of antidiarrheal activity of MEES leaf. Another possible mechanism of antidiarrheal effects of MEES may be via the deactivation of lipase and nitric oxide synthase activities (Ullah *et al.,* 2020; Onoja & Udeh, 2015). Castor oil induces nitric oxide production through the activation of inducible nitric oxide synthase leading to decreased absorption, increased secretion and motility (Gelberg, 2018). Therefore, compounds that can retard the nitric oxide synthase and intestinal lipase activities may be a potential antidiarrheal agent. Also, the antidiarrheal effect of MEES may in part be due to its nitric oxide scavenging activity because the extract has been reported to contain flavonoids which are potent nitric oxide radical scavenger (Dey *et al*., 2014; Ullah *et al*., 2020; Onoja & Udeh, 2015).

The MEES leaf may have elicited the antidiarrheal effect via the inhibition of prostaglandin biosynthesis. Ricinoleate enhances the secretion of endogenous prostaglandins and agents that impair prostaglandins biosynthesis has been implicated to delay castor oil induced diarrhea (Akter *et al*., 2010; Dey *et al*., 2014; Tadesse *et al.,* 2017). The anti-inflammatory activity of some identified compounds of MEES such as 3-methyl-nonane, linalool, tetradecane, gamma-muurolene, beta cubebene, α-bisabolene, d-nerolidol, piperine, ethyl oleate, guaiol, viridiflorol, E-15-heptadecenal and β-citral, may be another possible mechanism (Nwobodo *et al.,* 2022; Sharma *et al.,* 2023). These compounds have been reported to elicit their anti-inflammatory effect via the inhibition of cyclooxygenase which catalyzes prostaglandins production (Tadesse *et al*., 2017; Nwobodo *et al*., 2022; Sharma *et al.,* 2023).

Cator oil-induced diarrhea is attributed to the induction of contraction of intestinal smooth muscles mediated by the activation of EP3 prostanoid receptors on intestinal smooth muscles. Thus, agents that are spasmolytic in action are known to counteract castor oil induced diarrhea (Kumar *et al.,* 2010; Onoja & Udeh, 2015; Rahman *et al*., 2015; Tadesse *et al.,* 2017). The MEES leaf significantly ($p<0.05$) reduced the intestinal transit of the charcoal meal at the highest dose of 400 mg/kg (Table V). This could be linked to the presence of some phytoconstituents such as β-citral and β-sesquiphellandrene, which have been reported to exhibit antispasmodic and antihistamine properties (Ganjewala *et al*., 2012; Sharma *et al.,* 2023). The action of MEES is similar to that of loperamide and atropine (reference drugs) with potent antisecretory and antimotility effect which they exhibit through their action on the circular and longitudinal muscles of the intestine, through interaction with the μ-opioid receptors in the intestine, and this justifies their use as antidiarrheal agent (Sharkey & Wallace, 2011; Madubuike *et al.,* 2012).

In the castor oil induced enteropooling, MEES reduced intraluminal fluid accumulation and volume of intestinal content when compared with the negative although the reduction was not significant which suggest that MEES leaf possess antisecretory activity (Onoja *et al.,* 2018; Woldeyohannes *et al.,* 2022).

In conclusion, the MEES demonstrated mild antidiarrheal property which validates its use as antidiarrheal agent in folkloric medicine.

Conflict of Interest

The authors declare no conflict of interest

REFERENCES

Agbor, G. A., Longo, F., Makong, E. A. & Tarkang, P. A. (2014). Evaluation of the antidiarrheal and antioxidant properties of *Justicia hypocrateriformis*. *Pharmaceutical Biology*, *52*(9), 1128-1133.

Akter, R., Hasan, S. R., Hossain, M. M., Jamila, M., Chowdhury, S. S., Mazumder, M. E. H. & Rahman, S. (2010). Antidiarrhoeal and antioxidant properties of *Curcuma alismatifolia* leaves. *Australian Journal of Basic and Applied Sciences*, *4*(3), 450-456.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G. & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, *6*(4), 42.

Anekwe, I. I., Chikwendu, C. I., Amadi, E. S., Nwogwugwu, N. U. & Ihenetu, F. C. (2023). Gas Chromatography-Mass Spectrometry analysis of bioactive compounds of *Curcuma longa* leaves extract. *International Journal of Biological and Chemical Sciences*, *17*(3), 1199-1207.

Atsamo, A. D., Nguelefack, T. B., Datté, J. Y. & Kamanyi, A. (2011). Acute and subchronic oral toxicity assessment of the aqueous extract from the stem bark of *Erythrina senegalensis* DC (Fabaceae) in rodents. *Journal of Ethnopharmacology*, *134*(3), 697-702.

Awe, E. O., Kolawole, S. O., Wakeel, K. O. & Abiodun, O. O. (2011). Antidiarrheal activity of *Pyrenacantha staudtii* Engl. (Iccacinaceae) aqueous leaf extract in rodents. *Journal of Ethnopharmacology*, *137*(1), 148-153.

Bartel, B. & Gau, E. (2012). Nosocomial diarrhea: a review of pathophysiology, etiology, and treatment strategies. *Hospital Practice*, *40*(1), 130-138.

Benzie, I. F. & Strain, J. J. (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of

total antioxidant power and ascorbic acid concentration. *Methods in Enzymology*, *299*, 15-27.

Bilanda, D. C., Dzeufiet, P. D. D., Fouda, Y. B., Ngapout, R. F., Tcheutchoua, Y., Owona, P. E., ... & Kamtchouing, P. (2020). Antihypertensive and antidiabetic activities of *Erythrina senegalensis* DC (Fabaceae) stem bark aqueous extract on diabetic hypertensive rats. *Journal of Ethnopharmacology*, *246*, 112200.

Chu, C., Rotondo-Trivette, S. & Michail, S. (2020). Chronic diarrhea. *Current Problems in Pediatric and Adolescent Health Care*, *50*(8), 100841.

Dey, S. K., Hira, A., Howlader, M. S. I., Ahmed, A., Hossain, H. & Jahan, I. A. (2014). Antioxidant and antidiarrheal activities of ethanol extract of *Ardisia elliptica* fruits. *Pharmaceutical Biology*, *52*(2), 213-220.

Dhawefi, N., Jedidi, S., Rtibi, K., Jridi, M., Sammeri, H., Abidi, C., ... & Sebai, H. (2021). Antidiarrheal, antimicrobial, and antioxidant properties of the aqueous extract of *Tunisian persimmon* (Diospyros kaki Thunb.) Fruits. *Journal of Medicinal Food*, *24*(10), 1100-1112.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S. & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*, 22(3):296-302.

Donfack, J. H., Njayou, F. N., Rodrigue, T. K., Chuisseu, D. D. P., Tchana, N. A., Vita, F. P., ... & Moundipa, F. P. (2008). Study of a hepatoprotective and antioxidant fraction from *Erythrina Senegalensis* stem bark extract: in vitro and in vivo. *Pharmacologyonline*, *1*, 120-130.

Doughari, J. H. (2010). Evaluation of antimicrobial potentials of stem bark extracts *of Erythrina senegalensis* DC. *African Journal of Microbiology Research*, *4*(17), 1836-1841.

Edwards, C. E., Yount, B. L., Graham, R. L., Leist, S. R., Hou, Y. J., Dinnon III, K. H., ... & Baric, R. S. (2020). Swine acute diarrhea syndrome coronavirus replication in primary human cells reveals potential susceptibility to infection. *Proceedings of the National Academy of Sciences*, *117*(43), 26915-26925.

Ganjewala, D., Gupta, A. K. & Muhury, R. (2012). An update on bioactive potential of a monoterpene aldehyde citral. *Journal of Biologically Active Products from Nature*, *2*(4), 186-199.

Gelberg, H. (2018). Pathophysiological mechanisms of gastrointestinal toxicity. *Comprehensive Toxicology*, 139-178.

Iwalewa, E. O., Adewale, I. O., Aiwo, B. J., Arogundabe, T., Osinowo, A., Daniyan, O. M. & Adetogun, G. E. (2008). Effects of *Harungana Madagascariensis* stem Bark Extract on the Antiodixant Markers in Alloxan Induced Diabetic and Carrageenan Induced inflammatory Disorders in Rats. *Journal of Complementary and Integrative Medicine*, 5(1), 1-18.

Kumar, R., Sharma, R. J., Bairwa, K., Roy, R. K. & Kumar, A. (2010). Pharmacological review on natural antidiarrheal agents. *Der Pharma Chemica*, *2*(2), 66-93.

Lanata, C. F., Fischer-Walker, C. L., Olascoaga, A. C., Torres, C. X., Aryee, M. J., Black, R. E. & Child Health Epidemiology Reference Group of the World Health Organization and UNICEF. (2013). Global causes of diarrheal disease mortality in children< 5 years of age: a systematic review. *PloS one*, *8*(9), e72788.

Li, Y., Kong, D., Fu, Y., Sussman, M. R. & Wu, H. (2020). The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiology and Biochemistry*, *148*, 80-89.

Liu, J., Gratz, J., Maro, A., Kumburu, H., Kibiki, G., Taniuchi, M., ... & Houpt, E. R. (2012). Simultaneous detection of six diarrhea-causing bacterial pathogens with an in-house PCR-luminex assay. *Journal of Clinical Microbiology*, *50*(1), 98-103.

Madubuike, K. G., Ezeja, M. I. & Ezeigbo, I. I. (2012). Antidiarrhoeal activity of *Pterocarpus santalinoides* leaf extract in mice. *Continental Journal of Animal and Veterinary Research*, *4*(1), 1-6.

Mahmud, B., Ijudigal, L., Yunusa, I., Shehu, A. & Magaji, M. G. (2020). Activity of methanol root extract of *Parquetina nigrescens* (Afzel.) bullock on castor oil-induced diarrhoea in mice. *Journal of Pharmacy & Bioresources*, *17*(2), 180-188.

Meite, S., Bahi, C., Yéo, D., Datté, J. Y., Djaman, J. A. & N'guessan, D. J. (2010). Laxative activities of *Mareya micrantha* (Benth.) Müll. Arg. (Euphorbiaceae) leaf aqueous extract in rats. *BMC Complementary and Alternative Medicine*, *10*, 1-6.

Meng, C. Y., Smith, B. L., Bodhidatta, L., Richard, S. A., Vansith, K., Thy, B., ... & Mason, C. J. (2011). Etiology of diarrhea in young children and patterns of antibiotic resistance in Cambodia. *The Pediatric Infectious Disease Journal*, *30*(4), 331-335.

Mensor, L. L., Fabio, S. M., Gilda, G. L., Alexandre, S. R., Tereza, C. D., Cintia, S. C. & Suzana, G. L. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Res*earch, 15, 127-130.

Nwobodo, D. C., Eze, P. M., Okezie, U. M., Okafoanyali, J. O., Okoye, F. B. & Esimone, C. O. (2022). Bioactive Compounds Characterization and Antimicrobial Potentials of Crude Extract of *Curvularia lunata*, a Fungal Endophyte from *Elaeis guineensis*. *Tropical Journal of Natural Product Research*, *6*(3). 395-402

Obidah, W., Badung, H. L., Ajuji, J., Peter, H. & Bello, H. (2014). Effects of *Erythrina senegalensis* aqueous leaf extract in rats. *American Journal of Research Communication*, *2*(4), 179-185.

Onoja, S. O., Ihejirika, G. Q., Nwankudu, O. N., Omeh, Y. N. & Ezeja, M. I. (2018). Antidiarrheal and Antioxidant Activities of Methanol Extract of *Bryophyllum pinnatum* Leaf Harvested from South-

Eastern Nigeria in Mice. *Journal of Pharmaceutics*, (1), 6810620.

Pant, P., Pandey, S. & Dall'Acqua, S. (2021). The influence of environmental conditions on secondary metabolites in medicinal plants: A literature review. *Chemistry & Biodiversity*, *18*(11), e2100345.

Rahman, M. S., Akter, R., Mazumdar, S., Islam, F., Mouri, N. J., Nandi, N. C. & Mahmud, A. S. M. (2015). Antidiabetic and antidiarrhoeal potentials of ethanolic extracts of aerial parts of *Cynodon dactylon* Pers. *Asian Pacific Journal of Tropical Biomedicine*, *5*(8), 658-662.

Rwang, P. G., Fabiyi, J. P., Suleiman, M. & Mercy, K. P. (2016). Evaluation and phytochemical analysis of *Prosopis africana* and *Erythrina senegalensis* used against immature stages of Schistosoma haematobium. *European Journal of Medicinal Plants*, *13*(1), 1-9.

Saidu, K., Onah, J., Orisadipe, A., Olusola, A., Wambebe, C. & Gamaniel, K. (2000). Antiplasmodial, analgesic, and anti-inflammatory activities of the aqueous extract of the stem bark of *Erythrina senegalensis*. *Journal of Ethnopharmacology*, *71*(1-2), 275-280.

Sarah, Q. S., Anny, F. C. & Misbahuddin, M. (2017). Brine shrimp lethality assay. *Bangladesh Journal of Pharmacology*, 12, 186–189

Sharkey, K. A. & Wallace, J. L. (2011). Treatment of disorders of bowel motility and water flux; anti-emetics; agents used in biliary and pancreatic disease. *Goodman and Gilman's. The Pharmacological Basis of Therapeutics. 12th ed. New York: McGraw Hill*, 1323-1349.

Sharma, A., Harjai, K., Ramniwas, S. & Singh, D. (2023). Bioactivity of Citral and Its Nanoparticle in Attenuating Pathogenicity of *Pseudomonas aeruginosa* and Controlling *Drosophila melanogaster*. *Journal of Nanomaterials*, *2023*(1), 4703650.

Sisay, M., Mengistu, G., Molla, B., Amare, F. & Gabriel, T. (2017). Evaluation of rational drug use based on World Health Organization core drug use indicators in selected public hospitals of eastern Ethiopia: a cross sectional study. *BMC health services research*, *17*, 1-9.

Souleymane, F., Benjamin, K. K., Ollo, D., Serge, Y. R., Sylvain, I., Noufou, O., ... & Pierre, G. I. (2021). Acute and subchronic toxicity assessment of the dichloromethane/methanol stem bark extract from *Erythrina senegalensis* DC (Fabaceae) in Wistar rats. *Issues in Biological Sciences and Pharmaceutical Research*. 9(1), 19-26

Suneka, S. & Manoranjan, T. (2021). Brine shrimp lethality assay with selected medicinal plants extracts. *Vingnanam Journal of Science*, *16*(2), 14-17.

Suryawanshi, V., Yadav, A., Mohite, S. & Magdum, C. S. (2020). Toxicological Assessment using Brine shrimp lethality Assay and Antimicrobial activity of *Capparis grandis*. *Journal of University of Shanghai for Science and Technology*, *22*(11), 746-759.

Tadesse, E., Engidawork, E., Nedi, T. & Mengistu, G. (2017). Evaluation of the anti-diarrheal activity of the aqueous stem extract of *Lantana camara* Linn (Verbenaceae) in mice. *BMC Complementary and Alternative Medicine*, *17*, 1-8.

Tangpu, V. & Yadav, A. K. (2006). Antidiarrhoeal activity of *Cymbopogon citratus* and its main constituent, citral. *Pharmacologyonline*, *2*, 290-298.

Udeh, N. E. & Onoja, S. O. (2015). Analgesic and free radical scavenging activities of hydromethanolic extract of *Crateva adansonii* stem bark. *Journal of Intercultural Ethnopharmacology*, *4*(3), 224-227.

Udem, S. C., Obidoa, O. & Asuzu, I. U. (2010). Acute and chronic toxicity studies of *Erythrina senegalensis* DC stem bark extract in mice. *Comparative Clinical Pathology*, *19*, 275-282.

Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulson, B. G., ... & Jaremko, M. (2020). Important flavonoids and their role as a therapeutic agent. *Molecules*, *25*(22), 5243.

Woldeyohannes, M. G., Eshete, G. T., Abiye, A. A., Hailu, A. E., Huluka, S. A. & Tadesse, W. T. (2022). Antidiarrheal and antisecretory effect of 80% hydromethanolic leaf extract of *Moringa stenopetala* baker f. In mice. *Biochemistry Research International*, *2022*(1), 5768805.

World Health Organization. (2005). *The treatment of diarrhoea: a manual for physicians and other senior health workers* (No. WHO/FCH/CAH/05.1). World Health Organization. https://iris.who.int/bitstream/handle/10665/43209/9241593180.pdf