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Antidiarrhoeal Activities of Lime (*Citrus aurantiifolia*) Extract in Experimentally-Induced Diarrhoea Model

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Abstract

This study investigated the effects of Lime on Diarrhoea in Wistar rats. A total of 60 Wistar rats were procured and randomly divided into 3 groups of 20 animals each for each of the three t-test models. The twenty healthy Wistar rats for each diarrhoea model were fasted for 6 hours prior to the experiment but allowed free access to water. The twenty animals were randomly divided into 5 groups of 4 animals each for each experiment. Established antidiarrhea models were followed. The test groups received various doses (97.65mg/kg, 195.3mg/kg, and 390.6mg/kg) of *Citrus aurantiifolia* juice extract; whereas positive controls received Loperamide (2.5mg/kg) and negative controls received distilled water (1ml/kg). The administration was done once daily for 15 days, and the faeces of each animal was collected on the 5th, 10th and 15th day. The result of this study showed that medium and high dose *Citrus aurantiifolia* has an anti-diarrhoeal effect on castor oil-induced diarrhoea over repeated administration for a minimum of 15 days as it prolonged the onset of diarrhoea, decreased the frequency of defecation and gastrointestinal transit time in Wistar rats. This study shows that *Citrus aurantiifolia* demonstrates significant anti-diarrhoeal activity and can be used as an anti-diarrhoea agent.

Keywords: Diarrhoea; *Citrus aurantiifolia*; Castor Oil; Lime juice extract; Gastrointestinal Tract; Enteropooling; Diarrhoea model.

INTRODUCTION

Limes (*Citrus aurantifolia*) are a small citrus fruit either sour or sweet. Sour limes possess a greater sugar and citric acid content than lemons and feature an acidic and tart taste. Lime contains unique flavonoid compounds that have antioxidant and anticancer properties. While these flavonoids have been shown to stop cell division in many cancer lines, they are perhaps most interesting for their antibiotic effects (Bina et al., 2010).

Citric acid the major organic acid‑in these juices has been found to be responsible for inhibiting the growth of vibrio (gram-negative bacteria of the family Vibronaceae). The natural biocidal activity of lime juice was studied in order to explore its possible use as a disinfectant and inhibitor of vibrio cholera in drinking water for areas lacking water treatment plants (D'Aquino et al., 1994). Owing to the support of the national and international organizations for the studies on the treatment and prevention of these diseases based on traditional practices, medicinal plants are becoming a hopeful source of antidiarrhoeal drugs (Lin et al., 2002).

According to Ekwawati and Darmanto (2019), medicinal plants are one of the natural resources that can be explored by humans. Various secondary metabolites from plants can be used as medicines, agrochemicals, flavours, fragrances, dyes, biopesticides and food addictive. Citrus fruits contain nutrients and phytochemicals that are beneficial to health. Citrus juice contains various substances including carbohydrates, fibre, vitamin C, potassium, folate, calcium, thiamine, niacin, vitamin B6, vitamin A, phosphorus, magnesium, copper, riboflavin, pantothenic acid and various phytochemicals. These substances are needed by the body. Some compounds in citrus fruit can provide additional protection against chronic disease and basic nutrition. Citrus fruits also contain lots of phytochemicals, essential oils, alkaloids, flavonoids, psoralens, carotenoids, limonoids, tannins, and terpenoids.

Previous pharmacological studies revealed that citrus fruits have antimicrobial, anthelminthic, antioxidants, anticancer and many other pharmacological effects (Prastiwi and Ferdiansyah, 2013).

Diarrhoea is an alteration in the normal bowel movement characterized by an increase in the volume, fluidity, frequency, and passage of loose or watery stool at least three times a day (UNICEF/WHO, 2009). It is a common symptom of gastrointestinal infection due to the ingestion of many bacteria, viruses, or parasites that may be transmitted by water, food, utensils, hands, and flies (UNICEF/WHO, 2009). It is the mechanism by which

the body rids itself of pathogenic organisms, with excessive stimulation of intestinal motility, leaving insufficient time for the absorption of intestinal fluid (Keusch et al., 2006).

Diarrhoea is one of the most important health problems in developing countries affecting people of all ages (Snyder and Merson, 1982) and results in electrolyte loss, dehydration, shock, and sometimes death (UNICEF/WHO, 2009). Diarrhoeal diseases account for 1 in 9 child deaths worldwide, making diarrhoea the second leading cause of death among children under the age of 5 (Hutton et al., 2007), while Lakshminarayana et al. noted that diarrhoea is a leading cause of mortality and morbidity in children under 5 years old, and it accounts for 5–8 million deaths worldwide each year (Lakshminarayana et al., 2011; World Health Organization, 2006). A report in 2015 revealed that diarrhoea is one of the main killers of children that accounts for 9% of all deaths among kids below the age of 5 years worldwide (UNICEF, 2019). According to this report, sub-Saharan Africa and southern Asia were recorded as the regions that experienced the highest child death toll because of diarrhoea (Sow et al., 2016).

In Nigeria, the prevalence of diarrhoeal infection is as high as 18.8%, above the average of 16%, making it one of the worst in Sub-Saharan Africa (Olawuyi et al., 2004). It accounts for an annual estimated 300,000 deaths mainly amongst children under five in Nigeria (Olawuyi et al., 2004), while 7-to-12-month-old babies continue to be the most susceptible (Audu et al., 2000) caused mainly by poor sanitation and hygiene practices.

The disease may be caused by a wide array of agents such as enteropathogenic microorganisms (*Shigella flexneri*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Candida albicans*) (Jouret-Mourin and Geboes, 2002), alcohol, irritable bowel syndrome, bile salts and hormones (Teke et al., 2007), secretory tumours, and intoxication (Brijesh et al., 2011), whereas Susan & Mays said the causes of diarrhoea include infectious agents, gastrointestinal disorders such as inflammatory or dysmotility problems, and substances that increase gastrointestinal tract secretions (Susan and Mays, 2005).

Despite different pathophysiological changes in different types of diarrhoeas, there are four major mechanisms responsible for pathophysiology in electrolyte and water transport that is, increased luminal osmolarity, increased electrolyte secretion, decreased electrolyte absorption and accelerated intestinal motility causing decreased transit time (Lutterodt, 1992).

Management of diarrhoea comprises both non-pharmacological and pharmacological interventions. In general, the treatment is aimed at reducing the discomfort and inconvenience of frequent bowel mobility and frequency of faecal passage (Suleiman et al., 2008). Several options employed in the management of the diseases include oral agents such as metronidazole, antibiotics, and oral rehydration therapy (ORT) (Hardman et al., 2001; Sastry and Burgard, 2005). With over a decade of the practice and promotion of ORT, diarrhoea is still the second the cause of child death (Grant, 1993). In addition, these management options which often fail during high stool output state are also associated with undesirable side effects such as headache, convulsion, stomach cramps, vomiting, constipation, and hallucination.

Consequently, attention is now being shifted to alternatives in medicinal plants for the management of the disease. Medicinal plants represent a promising source for the discovery of new antidiarrhoeal agents. These plants are cheaper and more easily available than conventional medicines. Even the World Health Organization has encouraged studies for the treatment and prevention of diarrhoeal disease using traditional medicinal practices (Atta et al., 2004). Various plants are being investigated for their possible antidiarrhoeal activities to provide safe and inexpensive alternatives to standard drug therapies.

MATERIALS AND METHODS

Animals Procurement

Animals were procured from the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt and were acclimatized for a period of two weeks with their weights checked constantly during this period. All the animals (160±0.02g) were housed in clean plastic cages that were placed in a well-ventilated house (temperature: 22 ± 3^{o}C; photoperiod: 12 hours; humidity: 45–50%). The animals were fed on rat pellets (Premier Feed Mill Co. Ltd., Ibadan, Nigeria) and clean tap water, except when fasting was required during the experiment. The cages and the animal house were cleaned on daily basis.

Plant Collection

Fresh lime fruits were purchased from Choba Market and were identified and authenticated at the Department of Plant Science and Biotechnology, University of Port Harcourt, Herbarium.

Extraction of Plant

The extraction method by Amita and Shalini (2014) with slight modifications was used. The Lime was sliced and the juice was squeezed out into a beaker and after which the extract was filtered. The filtrate was then centrifuged for clarification of the extract (Das et al., 2010) and stored in the refrigerator for use.

Drugs and Chemicals

Loperamide hydrochloride was products of Laborate Pharmaceuticals (India) and Richy Gold International Limited (Nigeria), respectively. Castor oil was a product

of Bills Sons and Co. (Druggist) Limited, Southport, England.

Experimental Protocols

Castor Oil-Induced Diarrhoea in Rats

Twenty (20) healthy Wistar rats were fasted for 6 hours prior to the experiment but allowed free access to water. The experimental rats were completely randomized into five groups of four animals each. The procedure described by Bajad et al. (2001) was adopted with slight modification. Animals in group A which received 1.0ml of distilled water served as a negative control, while those in groups B (positive control), C, D, and E (test groups) received 1.0ml each corresponding to 2.5mg/kg body weight of loperamide (a reference drug), 0.25, 0.5, and 1.0ml of the extract, respectively. Thirty minutes after administration, all the animals were orally administered 1ml of castor oil and thereafter placed in cages lined with a pre-weighed transparent paper. During the 6-hour observation period, the time of onset of diarrhoea, the total number of faeces, diarrhoeal faeces, total weight of faeces, and percentage inhibition of diarrhoeal defecation in each group were computed. The weight of the faeces was obtained from the difference in the pre-weighed transparent paper and the fresh weight of the stool. The dry weight of the faeces was obtained by drying the fresh faeces in the laboratory oven (Uniscope Laboratory oven, SM9053, Surgifriend Medicals, England) at 100^0C until a constant weight was obtained. The difference in the fresh weight of the faeces and the dry weight of the faeces gives the water content of the faeces. At the end of the 6-hour exposure period, the animals were sacrificed under the effect of diethyl ether anaesthesia and the small intestine of the animals was dissected and removed. Thereafter, the contents of the small intestines were squeezed out.

Castor Oil-Induced Enteropooling

The procedure described by Chitme et al. (2004) was adopted for the castor oil-induced enteropooling study. The animals were fasted without food for 6h prior to the experiment but were allowed free access to water. Four animals were randomly selected for each group and placed in their respective cages. Animals in the negative control group (group A) received 1.0ml of distilled water, while those in the positive control group (group B) received 1.0ml of loperamide (2.5mg/kg body weight). Rats in groups C, D, and E (test groups) were orally administered 0.25, 0.50, and 1.0ml of the sap, respectively. Immediately afterwards, 1.0ml of castor oil was administered orally to each of the rats in all the groups. After 30 minutes, each rat was sacrificed according to the method described by Akanji and Yakubu (2000) and the ends of the pylorus and caecum of the small intestine were tied. The small intestine was dissected and its intestinal content was squeezed into a measuring cylinder. The volumes and the masses of the intestinal contents were noted and used to compute the percentage of inhibition of intestinal content.

Gastrointestinal Motility

The method described by Teke et al. (2007) was adopted for the evaluation of the effect of the sap on gastrointestinal transit in rats. Twenty, healthy Wistar rats were fasted for 6 hours prior to the experiment but were allowed free access to water. The experimental rats were completely randomized into five groups of four animals each. The negative control group (group A) received 1.0ml of distilled water while the positive control group (group B) received 1.0ml of loperamide (0.6mg/ml) intramuscularly. Animals in groups C, D, and E (test groups) were orally administered 0.25, 0.50, and 1.0ml of the sap, respectively. Charcoal meal (10% charcoal suspension in 5% agarose agar, prepared by weighing 10 g of charcoal powder and 5 g of agarose agar into 100ml distilled water and mixed thoroughly) was administered orally, 30 minutes after the administration of atropine sulphate and the *Citrus aurantiifolia*. The animals were then sacrificed after 45 minutes of charcoal administration, using the diethyl ether as anaesthesia as described by Akanji and Yakubu (2000). The small intestine was removed very carefully and the length of the intestine as well as the distance travelled by the charcoal meal through the intestine was measured. The percentage of gastrointestinal motility was computed as the ratio of the distance moved by the charcoal meal to the length of the small intestine.

Methods of Data Analysis

The experimental results were analyzed using the Statistical Package for the Social Sciences (IBM SPSS), version 25.0. Results were expressed as a mean ± Standard Error Mean (SEM), and statistical analyses were carried out by employing a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to compare the results of controls and the groups. In all cases, statistical significance was set at $p<0.05$.

RESULTS

Analysis of the effect of the extract on Castor oil-induced diarrhoea in Wistar rats

Below is a table showing the observed effect of *Citrus aurantiifolia* on Castor oil-induced diarrhoea and enteropooling respectively in Wistar rats. The low dose, medium dose and high dose of the extract were administered and the effect of these substances on the Wistar rats was observed and analysed alongside that of the well-known drug and the control substance.

Table 1. Effects of *Citrus aurantiifolia* (lime juice extract) on castor oil-induced diarrhoea in Wistar rats.

Group	MOT	MND	MNWS	MNDS	MFWS	MWCS
Control	63.25±0.47	6.00±0.41	2.50±0.29	2.00±0.41	4.75±0.25	2.20±0.41
Loperamide	121.75±1.37	2.50±0.29	1.25±0.25	1.25±0.25	0.83±0.025	0.48±0.025
Low Dose	93.50±1.55	2.75±0.48	1.50±0.29	1.50±0.29	0.93±0.03	0.55±0.03
Medium Dose	108.00±0.91	2.50±0.29	1.00±0.41	1.50±0.29	0.78±0.03	0.45±0.03
High Dose	140.00±1.08	1.75±0.48	0.75±0.25	1.70±0.25	0.65±0.00	0.30±0.41

MOT = Mean Onset Time (Min); **MND** = Mean Number of Defecations; **MNWS** = Mean Number of Wet stool; **MNDS** = Mean Number of Dry stool; **MFWS** = Mean Fresh Weight of stool; **MWCS** = Mean Water Content of stool

Table 2. Effects of *Citrus aurantiifolia* (lime juice extract) on Castor oil-induced enteropooling in Wistar rats.

Group	MWIC	MVIC
Control	2.88±0.14	2.60±0.09
Loperamide	1.63±0.09	1.60±0.07
Low Dose	2.15±0.10	1.90±0.27
Medium Dose	1.65±0.25	1.33±0.11
High Dose	0.98±0.17	1.10±0.04

MWIC = Mean weight of intestinal content; **MVIC** = Mean volume of intestinal content

Table 3. Effects of *Citrus aurantiifolia* (lime juice extract) on castor oil-induced transit time in Wistar rats.

Group	MDTCM
Control	77.25±0.63
Loperamide	35.50±0.29
Low Dose	40.25±0.25
Medium Dose	31.50±0.64
High Dose	27.75±1.11

MDTCM = Mean Distance travelled by Charcoal Meal

Table 4. Effects of *Citrus aurantiifolia* (lime juice extract) on the onset of diarrhoea in castor oil-induced diarrhoea in Wistar rats.

Parameter	Distilled water (ml)	Loperamide (mg/kg body weight)	*Citrus aurantiifolia* (mg/kg)		
	1	2.5	97.65	195.3	390.6
MOT	63.25±0.47	121.75±0.53	93.50±1.55	108.00±0.91	140.00±1.08
D_{freq}		92.49±0.09	47.83±1.08	70.75±0.24	121.34±0.64

$$D_{freq} = \frac{MODTG - MODNG}{MODNG}\ x\ 100 \qquad (1)$$

Where, **MOT** = Mean onset time, **D_{freq}**= Percentage inhibition in terms of control of the onset of diarrhoeal stool, **MODTG** = Mean onset of diarrhoea in the treated group and **MODNG** = Mean onset of diarrhoea in the negative control (Schuster, 2001).

Table 5. Effects of *Citrus aurantiifolia* (lime juice extract) on the mean number of defecations in castor oil-induced diarrhoea in Wistar rats.

Parameter	Distilled water (ml)	Loperamide (mg/kg body weight)	*Citrus aurantiifolia* (mg/kg)		
	1	2.5	97.65	195.3	390.6
MND	6.00±0.41	2.50±0.29	2.75±0.48	2.50±0.27	1.75±0.48
PID		58.33±0.41	54.17±0.18	58.33±0.43	70.83±0.07

$$PID = \frac{MNDCC - MNDCD/E}{MNDCC}\ x\ 100 \qquad (2)$$

Where, *MND* = Mean number of defecations, *PID* = *Percentage inhibition of defecation*, *MNDCC* = Mean number of defecations caused by castor oil and M*NDCD/E* = *M*ean number of defecations caused by drug/Extract (Kola-Mustapha et al., 2019).

Table 6. Effects of *Citrus aurantiifolia* (lime juice extract) on mean number of wet faeces in castor oil-induced diarrhoea in Wistar rats.

Parameter	Distilled water (ml)	Loperamide (mg/kg body weight)	*Citrus aurantiifolia* (mg/kg)		
/Dose (ml)	1	2.5	97.65	195.3	390.6
MNWS	2.50±0.29	1.25±0.25	1.50±0.17	1.00±0.41	0.75±0.25
P_{freq}		50.00±0.03	40.00±0.12	60.00±0.14	70.00±0.04

$$P_{freq} = \frac{MNWSC - MNWST}{MNWSC} \; x \; 100 \qquad (3)$$

Where, **MNWS** = Mean number of wet stools, $\mathbf{P_{freq}}$ = Percentage inhibition in terms of the purging frequency (number of wet stools), **MNWSC** = Mean number of wet stool in control group and **MNWST** =Mean number of wet stool in treated group.

Table 7. Effects of *Citrus aurantiifolia* (lime juice extract) on number of dry faeces, fresh weight of faeces and water content of faeces in castor oil-induced diarrhoea in Wistar rats.

Parameter	Distilled water (ml)	Loperamide (mg/kg body weight)	*Citrus aurantiifolia* (mg/kg)		
	1	2.5	97.65	195.3	390.6
MNDF	2.00±0.41	1.25±0.25	1.50±0.29	1.50±0.29	1.70±0.25
MFWF	4.75±0.25	0.83±0.025	0.93±0.03	0.78±0.03	0.65±0.01
MWCF	2.20±0.41	0.48±0.025	0.55±0.03	0.45±0.03	0.30±0.41

Where, **MNDF** = Mean number of dry faeces, **MFWF** = Mean fresh weight of faeces and **MWCF** = Mean water content of faeces

Table 8. Effects of *Citrus aurantiifolia* (lime juice extract) on weight of intestinal content in castor oil-induced diarrhoea in Wistar rats.

Paramete	Distilled water (ml)	Loperamide (mg/kg body weight)	*Citrus aurantiifolia* (mg/kg)		
	1	2.5	97.65	195.3	390.6
MWIC	2.88±0.14	1.63±0.09	2.15±0.10	1.65±0.25	0.98±0.17
PIIC		43.40±0.24	25.35±0.04	42.71±0.29	65.97±0.64

$$PIIC = \left(\frac{MWICC - MWICT}{MWICC}\right) x \; 100 \qquad (4)$$

Where, **MWIC** = Mean weight of the intestinal content, **PIIC** = Percentage inhibition of intestinal content, **MWICC** = Mean weight of the intestinal content of the control group and **MWICT** = Mean weight of the intestinal content of the test group (Giday et al., 2010).

Table 9. Effects of *Citrus aurantiifolia* (lime juice extract) on Volume of intestinal content in castor oil-induced diarrhoea in Wistar rats.

Parameter	Distilled water (ml)	Loperamide (mg/kg body weight)	*Citrus aurantiifolia* (mg/kg)		
	1	2.5	97.65	195.3	390.6
MVIC	2.60±0.09	1.60±0.07	1.90±0.27	1.33±0.11	1.10±0.04
PIIFV		38.46±0.02	26.92±0.21	48.85±0.17	57.69±0.05

$$PIIFV = \left(\frac{MVICC - MVICT}{MVICC}\right) x \; 100 \qquad (5)$$

Where, **MVIC** = Mean volume of the intestinal content, **PIIFV** = Percentage inhibition of intestinal fluid volume, **MVICC** = Mean volume of the intestinal content of the control group and **MVICT** = Mean volume of the intestinal content of the test group (Ekor, 2014).

Table 10. Effects of *Citrus aurantiifolia* (lime juice extract) on charcoal meal distance travelled in Wistar rats.

Parameter	Distilled water (ml)	Loperamide (mg/kg body weight)	*Citrus aurantiifolia* (mg/kg)		
	1	2.5	97.65	195.3	390.6
MDTCM (cm)	77.25±0.63	35.50±0.29	40.25±0.25	31.50±0.64	27.75±1.11
PI	68.97±0.13	31.70±0.21	33.04±0.11	28.13±0.12	24.78±0.45
G_{meq}		54.04±0.13	42.72±0.12	59.21±0.02	64.07±0.31

Mean length of small intestine (MLSI) = 112±0.56cm

$$PI = \frac{MDTCM}{MLSI} \; x \; 100 \qquad (6)$$

Where, **PI** = Peristaltic index, **MDTCM** = Mean distance travelled by charcoal meal and **MLSI** = Mean length of the small intestine (Agegnehu et al., 2019)

$$PIT = \frac{MDTCM}{MLSI} \; x \; 100 \qquad (7)$$

Where, **PIT** = Percentage intestinal transit, **MDTCM** = Mean distance travelled by charcoal meal, and **MLSI** =

Mean length of small intestine (Kola-Mustapha et al., 2019).

$$PI\left(G_{meg}\right) = \frac{PIC-PIT}{PIC}\ x\ 100 \qquad (8)$$

Where, $\mathbf{G_{meq}}$ = Percentage inhibition and **PIC** = Peristaltic index of control and **PIT** = peristaltic index of test group (Bern et al., 1992).

or

$$G_{meq} = \frac{PIC-PIT}{MPICG}\ x\ 100 \qquad (9)$$

Where $\mathbf{G_{meq}}$ = Percentage inhibition in terms of control in the Gut travelled distance, **MPICG** = Mean peristaltic Index of control group and **MPITG** = Mean peristaltic Index of treated group.

Table 11. The in vivo anti-diarrhoeal index ($ADI_{\text{in vivo}}$) (Hussain et al., 2009; Than et al., 1989).

Parameter	Loperamide (mg/kg body weight)	*Citrus aurantiifolia* (mg/kg)		
	2.5	97.65	195.3	390.6
D_{freq}	92.49±0.09	47.83±1.08	70.75±0.24	121.34±0.64
G_{meq}	54.04±0.13	42.72±0.12	59.21±0.02	64.07±0.31
P_{freq}	50.00±0.03	40.00±0.12	60.00±0.14	70.00±0.04
ADI in vivo	62.99±0.07	43.40±0.25	63.11±0.09	81.64±0.2

$$ADI_{invivo} = \sqrt[3]{D_{freq}\ x\ G_{meq}\ x\ P_{feq}} \qquad (10)$$

ADI in vivo for Loperamide

$$ADI_{invivo} = \sqrt[3]{(92.49x54.04x50.00)}$$
$$ADI_{invivo} = \sqrt[3]{249907.98}$$
$$ADI_{invivo} = 62.99$$
$$ADI_{invivo} = \sqrt[3]{(0.09x0.13x0.03)}$$
$$ADI_{invivo} = \sqrt[3]{0.000351}$$
$$ADI_{invivo} = 0.07$$

ADI in vivo for Low Dose

$$ADI_{invivo} = \sqrt[3]{(47.83x42.72x40.00)}$$
$$ADI_{invivo} = \sqrt[3]{81731.904}$$
$$ADI_{invivo} = 43.40$$
$$ADI_{invivo} = \sqrt[3]{(1.08x0.12x0.12)}$$
$$ADI_{invivo} = \sqrt[3]{0.015552}$$
$$ADI_{invivo} = 0.25$$

ADI in vivo for Medium Dose

$$ADI_{invivo} = \sqrt[3]{(70.75x59.21x60.00)}$$
$$ADI_{invivo} = \sqrt[3]{251346.45}$$
$$ADI_{invivo} = 63.11$$
$$ADI_{invivo} = \sqrt[3]{(0.24x0.02x0.14)}$$
$$ADI_{invivo} = \sqrt[3]{0.000672}$$
$$ADI_{invivo} = 0.09$$

ADI in vivo for High Dose

$$ADI_{invivo} = \sqrt[3]{(121.34x64.07x70.00)}$$
$$ADI_{invivo} = \sqrt[3]{544197.766}$$
$$ADI_{invivo} = 81.64$$
$$ADI_{invivo} = \sqrt[3]{(0.64x0.31x0.04)}$$
$$ADI_{invivo} = \sqrt[3]{0.007936}$$
$$ADI_{invivo} = 0.2$$

DISCUSSION

Effect of *Citrus aurantiifolia* (lime juice extract) on castor oil-induced diarrhoea in Wistar Rats.

The result of the effect of *Citrus aurantiifolia* on castor oil-induced diarrhoea in Wistar rats is presented in Table 1. It was observed that the animals had diarrhoea after treatment with castor oil. However, when loperamide (standard drug) was administered there was an observed significant reversal reduction in the mean number of defecation (MND), mean number of wet stool (MNWS), mean number of dry stool (MNDS), mean fresh weight of stool (MFWS) and mean water content of stool (MWCS) compared to the mean onset time (MOT) and the normal control group, this means that loperamide is effective in the treatment of diarrhoea. This reversal is not shocking because loperamide is a known anti-diarrhoeal agent used in the treatment of diarrhoea that works by decreasing peristalsis and fluid secretion, resulting in longer gastrointestinal transit time and increased absorption of fluids and electrolytes from the gastrointestinal tract (Baker, 2007). Therefore, since castor oil works by stimulating prostaglandin synthesis thereby increasing fluid and electrolyte into the lumen of the bowels, it is expected that loperamide will reverse the

process. Furthermore, when the low dose of *Citrus aurantiifolia* was administered there was also an observed significant reversal and reduction in the mean number of defecation (MND), mean number of wet stool (MNWS), mean number of dry stool (MNDS), mean fresh weight of stool (MFWS) and mean water content of stool (MWCS) when compared to the mean onset time (MOT) and the normal control group. Also, when the medium dose of *Citrus aurantiifolia* was administered to the Wistar rats, there was an observed significant reversal and reduction in the mean number of defecation (MND), mean number of wet stool (MNWS), mean number of dry stool (MNDS), mean fresh weight of stool (MFWS) and mean water content of stool (MWCS) when compared to the mean onset time (MOT) and the normal control group. Since the medium dose of *Citrus aurantiifolia* caused a reversal of the diarrhoea caused by the castor oil, it could mean that it works with the same mechanism with Loperamide. Therefore, since castor oil works by stimulating prostaglandin synthesis thereby increasing fluid and electrolyte into the lumen of the bowels, it is expected that Loperamide will reverse the process. Furthermore, when the high dose of *Citrus aurantiifolia* was administered to the Wistar rats, there was an observed significant reversal reduction in the mean number of defecation (MND), mean number of wet stool (MNWS), mean number of dry stool (MNDS), mean fresh weight of stool (MFWS) and mean water content of stool (MWCS) when compared to the mean onset time (MOT) and the normal control group. Generally, the *Citrus aurantiifolia* is observed to be effective in the treatment of diarrhoea just like Loperamide, with the high dose seen to be more effective than other doses.

Effect of *Citrus aurantiifolia* (lime juice extract) on castor oil-induced enteropooling in Wistar rats.

The result of the effects of *Citrus aurantiifolia* on Castor oil-induced enteropooling in Wistar rats is presented in table 2. There was an increase in the enteropooling level of the Wistar rats when exposed to *Citrus aurantiifolia*, but when the standard anti-diarrhoeal was administered, there was an observed reduction in the mean weight of intestinal content (MWIC) and the mean volume of content (MVIC) when compared with the normal control group. This means that Loperamide can reverse reduction in accumulation of fluids in the small intestine.

This reversal is possible because Loperamide is a known anti-diarrhoeal agent used in the treatment of diarrhoea that works by decreasing peristalsis and fluid secretion, resulting in longer gastrointestinal transit time and increased absorption of fluids and electrolytes from the gastrointestinal tract (Baker, 2007). Therefore, since castor oil works by stimulating prostaglandin synthesis thereby increasing fluid and electrolyte into the lumen of the bowel. However, when the standard anti-diarrhoeal was administered, there was an observed reduction in the mean weight of intestinal content (MWIC) and the mean volume of content (MVIC) when compared with the normal control group. This means that Loperamide can reverse reduction in accumulation of fluids in the small intestine.

Furthermore, when the medium dose of *Citrus aurantiifolia* was administered there was an observed significant reduction in the mean weight of intestinal content (MWIC) and the mean volume of content (MVIC) when compared with the normal control group. This means that the medium dose of *Citrus aurantiifolia* the ability to cause a reversal reduction in accumulation of fluids in the small intestine. Since the medium dose of *Citrus aurantiifolia* caused a significant reduction in the enteropooling level of the Wistar rats. Also, when the high dose of *Citrus aurantiifolia* was administered there was an observed significant reduction in the mean weight of intestinal content (MWIC) and the mean volume of content (MVIC) when compared with the normal control group. It indicates that the high dose of *Citrus aurantiifolia* could cause a reversal reduction in accumulation of fluids in the small intestine. Hence, the Loperamide is effective for the enteropooling reversal *and Citrus aurantiifolia* is also effective for enteropooling reversal with the high dose seen to be more significant followed by the medium dose and then the low dose.

Effects of *Citrus aurantiifolia* (lime juice extract) on castor oil-induced transit time in Wistar rats

The result of the effect of *Citrus aurantiifolia* on castor oil-induced transit time in Wistar rats is presented in table 3. During the study, it was observed that there was an increase in the transit time of the Wistar rats when exposed to castor oil but when loperamide was administered, there was an observed reversal reduction in the mean distance travelled by charcoal (MDTCM) meal when compared with the normal control group. This means that loperamide is effective in the reversal of the inability of the ingested food to travel on time. Loperamide is a known anti-diarrhoeal agent used in the treatment of diarrhoea that works by decreasing peristalsis and fluid secretion, resulting in longer gastrointestinal transit time and increased absorption of fluids and electrolytes from the gastrointestinal tract (Baker, 2007). And castor oil works by stimulating prostaglandin synthesis thereby increasing fluid and electrolyte into the lumen of the bowels, therefore loperamide could reverse the process. Furthermore, when the low dose of *Citrus aurantiifolia* was administered there was an observed significant reduction in the mean distance travelled by charcoal meal (MDTCM) when compared with the normal control group. When the medium dose of *Citrus aurantiifolia* was administered there was a significant reduction in the mean distance travelled by charcoal meal (MDTCM) when compared with the normal control group. When the high dose of *Citrus aurantiifolia* was administered there was an observed significant reduction in the mean distance

travelled by charcoal meal (MDTCM) when compared with the normal control group. The high dose of *Citrus aurantiifolia* caused a significant reduction in the time it takes for ingested food to travel through the gut of the Wistar rats, Hence, loperamide is effective in the treatment of delay in transit time and *Citrus aurantiifolia* is effective with high dose seen to be more effective followed by medium dose and then the low dose. This finding is similar to a previous study on the antidiarrheal properties of aqueous leaf extract of *Cyathula prostrata* by Uahomo and Isirima (2022).

In conclusion, the results of this study have shown that *Citrus aurantiifolia* demonstrates significant anti-diarrhoeal activity and may be working through anti-secretory and anti-motility mechanisms or through inhibition of prostaglandin activities and/or synthesis.

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REFERENCES

Agegnehu MD, Zeleke LB, Goshu YA,Ortibo YL, Mehretie AY. (2019). Diarrhea prevention practice and associated factors among caregivers of under-five children in Enemay district, Northwest Ethiopia. Journal of Environmental Public Health; 5490716.

Akanji MA, Yakubu MT (2000). α-tocopherol protects against metabisulphite-induced tissue damage in rats. *Nigerian Journal of Biochemistry & Molecular Biology*; 15(2):179–183.

Amita P, Shalini T (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry; 2 (5): 115-119

Atta AH, Mouneir SM (2004) Antidiarrhoeal activity of some Egyptian medicinal plant extracts *Journal of Ethnopharmacology* 92 (2-3), 303-309, 2004

Audu R., Umilabug S. A., Renner J. K., Awodiji J. A. (2000). Diarrhoea management. *Journal of Nigerian Infection Control Association*; 3:15–17.

Bajad S., Bedi K. L., Singla A. K., Johri R. K. Antidiarrhoeal activity of piperine in mice. *Planta Medica.* 2001;67(3):284–287. https://doi.org/10.1055/s-2001-11999

Baker DE (2007). Loperamide: a pharmacological review. Rev Gastroenterol Disord.; 7 Suppl 3: S11–S18.

Bern C, Martines J, De Zoysa I, Glass RI (1992). The magnitude of the global problem of diarrhoeal disease: A ten-year update. Bulletin of World Health Organization,70:705-714.

Bina LJ, Tista P, Anjana S and Kayo DY. (2010). Study of antimicrobial activity of lime juice against *Vibrio cholerae*. Scientific World, 8(8), 44-46

Brijesh S., Tetali P., Birdi T. J. (2011). Study of effect of anti-diarrhoeal medicinal plants on enteropathogenic Escherichia coli induced interleukin-8 secretion by intestinal epithelial cells. *Alternative Medicine Studies*; 1(1, article e16) doi: https://doi.org/10.4081/ams.2011.e16.

Chitme HR, Chandra R, (2004). Kaushik S. Studies on anti-diarrhoeal activity of Calotropis gigantean R. Br. in experimental animals. *Journal of Pharmacy & Pharmaceutical Sciences*; 7(1):70–75.

D'Aquino, Miguel T, Sergio, A (1994). Lemon juice as a natural biocide for disinfecting drinking water Data

Das K, Tiwari RKS and Shrivastava DK (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research, 4(2):104-111. https://doi.org/10.5897/JMPR09.030

Ekawati ER, Darmanto W (2019). Lemon (Citrus limon) Juice Has Antibacterial Potential against Diarrhoea-Causing Pathogen. IOP Conf. Series: Earth and Environmental Science, 217 012023. https://www.doi.org/10.1088/1755-1315/217/1/012023

Ekor M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacology, 4:177.

Giday, M., Asfaw, Z., & Woldu, Z. (2010). Ethnomedicinal study of plants used bySheko ethnic group of Ethiopia. Journal ofethnopharmacology, 132(1), 75–85. https://doi.org/10.1016/j.jep.2010.07.046

Grant J. P. (1993). *The State of the World Children*. UNICEF; Diarrhoeal disease: a strategy for the 90s; pp. 22–23.

Hardman J. G., Limbird L. E., Goodman F., Gilman M. (2001). *The Pharmacological Basis of Therapeutics*. 10th. New York, NY, USA: McGraw-Hill.

Hutton G., Haller L., Bartram J. (2007). Global cost-benefit analysis of water supply and sanitation interventions. *Journal of Water and Health*.; 5(4):481–501.

Hussain Z, Amresh G, Singh S, Rao CV.2009. Antidiarrhoeal and antiulcer activity of *Amaranthus spinosus* in experimental animals. Pharmaceutical Biology; 47(10):932-939

Jouret-Mourin, K. and Geboes, A. (2002) Infectious Colitis. Acta Endoscopica, 32, 167-183. https://doi.org/10.1007/BF03016654

Keusch GT, Joan W, Arthur K. (2006). Stigma and global health: developing a research agenda. *The Lancet*, 367 (9509), 525-527

Kola-Mustapha AT, Ghazali YO, Ayotunde HT, Atunwa SA and Usman SO (2019). Evaluation of the antidiarrheal activity of the leaf extract of Parquetinanigrescens and formulation into oral suspensions: Journal of Experimental Pharmacology, 11; 65-72

Lin J, Puckree T, Mvelase TP (2002). Anti-diarrhoeal evaluation of some medicinal plants used by Zulu traditional healers. *Journal of Ethnopharmacology* 79 (1), 53-56

Lutterodt G. D. (1992). Inhibition of Microlax-induced experimental diarrhoea with narcotic-like extracts of Psidium guajava leaf in rats. *Journal of ethnopharmacology*, *37*(2), 151–157. https://doi.org/10.1016/0378-8741(92)90073-z

Olawuyi JF, Egbewale BE, Anifalaje LA, Okochi EA. (2004). Care seeking practices on diarrhoea in a rural community in

Nigeria. *African Journal of clinical and experimental microbiology* 5 (1), 119-125

Prastiwi SS and Ferdiansyah F (2017). Kandungan Dan Aktivitas Farmakologi Jeruk Nipis (*Citrus aurantifolia* Swing), Farmaka, 15(2), 1–8. Tersedia pada: http://jurnal.unpad.ac.id/farmaka/article/view/12964.

Susan EA, Mays A. (2005). Pharmacology. In: The Merck veterinary manual. 9th ed. USA: Merck and Co. Inc; p. 1638.

Suleiman MM, Dzenda T, Sani CA (2008). Antidiarrhoeal activity of the methanol stem-bark extract of Annona senegalensis Pers. (Annonaceae) *Journal of Ethnopharmacology*, 116(1), 125-130

Snyder, J.D. & Merson, M. H. (1982). The magnitude of the global problem of acute diarrhoeal disease: a review of the active surveillance data. Bulletin of the World Health Organization, 60: 605-613

Sow, S. O., Muhsen, K., Nasrin, D., Blackwelder, W. C., Wu, Y., Farag, T. H., Panchalingam, S., Sur, D., Zaidi, A. K., Faruque, A. S., Saha, D., Adegbola, R., Alonso, P. L., Breiman, R. F., Bassat, Q., Tamboura, B., Sanogo, D., Onwuchekwa, U., Manna, B., Ramamurthy, T., … Levine, M. M. (2016). The Burden of Cryptosporidium Diarrhoeal Disease among Children < 24 Months of Age in Moderate/High Mortality Regions of Sub-Saharan Africa and South Asia, Utilizing Data from the Global Enteric Multicenter Study (GEMS). *PLoS neglected tropical diseases*, *10*(5), e0004729. https://doi.org/10.1371/journal.pntd.0004729

Sastry N., Burgard S. (2005). The prevalence of diarrhoeal disease among Brazilian children: trends and differentials from 1986 to 1996. *Social Science & Medicine*; 60(5):923–935.

Schuster BG (2001). A new integrated program for natural product development and the value of an ethnomedical approach. Journal of Alternative and Complementary Medicine, 7(1):61-72.

Teke GN, Kuiate JR, Ngouateu OB, Gatsing D (2007). Antidiarrhoeal and antimicrobial activities of Emilia coccinea (Sims) G. Don extracts. Journal of ethnopharmacology 112 (2), 278-283

Than A, Kulkarni HJ, Hmone W, Tha SJ.1989. Anti-diarrhoeal efficacy of some Burmese indigenous drug formulations in experimental diarrhoeal test models. International Journal of Crude Drug Research, 27:195–200

Uahomo PO and Isirima JC (2022). Antidiarrheal Properties of Aqueous leaf extract of Cyathula prostrata on Castor oil-induced Diarrhoea in Wistar Rats. International Journal of Pharmaceutical Research and Applications, 7(4),1679-1692

UNICEF/WHO (2009). *Final Report – Diarrhoea.* Geneva, Switzerland: United Nations Children's Fund/World Health Organization. Why children are still dying and what can be done.

UNICEF (2019). Diarrhoea—UNICEF DATA. [(accessed on 29 October 2021)]. Available online: https://data.unicef.org/topic/child-health/diarrhoeal-disease/

World Health Organization (2006). Country Health System Fact Sheet. Available via: https://books.google.com.ng/books?id=a4cYeKpbXH8C&lpg=PP2&ots=JnbUJot0HV&dq=World%20Health%20Organization%20(2006).%20Country%20Health%20System%20Fact%20Sheet&lr&pg=PP2#v=onepage&q=World%20Health%20Organization%20(2006).%20Country%20Health%20System%20Fact%20Sheet&f=false. Accessed 20th December, 2022.

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