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Evaluation of the anti-diarrhoeal activity of *Ficus pachyneura* ethanol leaf extract in mice

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Abstract

This study evaluated the anti-diarrhoeal activity of ethanol leaf extract of *Ficus pachyneura* (ELEFP), using laboratory mice. Extraction was by cold maceration using ethanol. Qualitative and quantitative phytochemical screening and acute toxicity study on the extract were done following standard procedures. The castor oil-induced diarrhoea, gastrointestinal motility and entero-pooling test models were used to evaluate the extract's anti-diarrhoeal activity. The extent of protection from diarrhoea and its impacts on the onset, number of wet faeces, motility and intestinal volume were evaluated by administering to mice graded doses (200, 400 and 800 mg/kg) of ELEFP; this was compared with a negative control group (that received 5 ml/kg of the distilled water placebo) and a positive control group (given either 5 mg/kg loperamide or 3 mg/kg atropine). Results obtained showed that ELEFP contains very high concentrations of saponins, alkaloids, cardiac glycosides, phenolics, tannins, terpenoids and flavonoids. The LD_{50} was greater than 5000 mg/kg. The 400 and 800 mg/kg doses of the extract significantly ($p < 0.05$) inhibited castor oil induced diarrhoea, reduced the charcoal meal transit and intestinal fluid accumulation, very much like the reference drugs used. It was concluded that ELEFP exhibited both anti-secretory and anti-motility/antispasmodic activities in controlling diarrhoea, hence could be a potential source of alternative anti-diarrhoeal agent.

Keywords: *Ficus pachyneura;* Ethanol leaf extract; Castor oil-induced diarrhoea; Anti-diarrhoeal activity; Phytochemicals.

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Introduction

Diarrhoea has remained a great concern and a challenge in most countries of the world because of its numerous effects on humans (especially children) and animals (Ezeigbo *et al*., 2011). The United Nations International Children's Emergency Fund (UNICEF) and World Health Organization (WHO) defined diarrhoea as the passage of three or more loose or liquid stools per day (UNICEF/WHO, 2009). Diarrhoea causes death in patients through loss of water and electrolytes (mainly sodium), which are required for normal body function (WHO, 2017). Globally, diarrhoea is the second-leading cause of death among under-five children, accounting for 90% of all under-five deaths (UNICEF, 2019; GAPPD, 2020).

The prevalence of diarrhoea among under-five children in Nigeria as documented by National Population Commission between 2018 and 2021, increased from 10% to 58.8% within 3 years n(NPC, 2021), and this was regionally distributed according to a national study conducted by Mohammed and Tamiru (2014) as reviewed by Demissie *et al.* (2021). In combating this global health problem, several conventional anti-diarrhoeal drugs have been developed and many are undergoing clinical trials. The use of most orthodox medication in treating disease conditions such as diarrhoea is associated with some negative implications such as adverse drug reactions and undesirable side effects. Due to these negative attributes, much focus has been placed on medicinal plants, which are perceived to be safer and easily accessible (Umaru *et al.,* 2015).

Ficus is a genus of the family *Moraceae* and consists of about 850 species, with about 2000 different varieties occurring as woody trees, shrubs and vines in the forests of tropical and subtropical regions (Awad *et al.,* 2011). About 500 species of Ficus are found in Africa, Asia, and Australia (Al-Aboudi and Afifi, 2011). Parts of some species of Ficus plants such as *F*icus *glomerata*, *Ficus glumosa*, *F*icus *religiosa*, *Ficus racemose*, *F*icus *carica* and *F*icus *benghalensis* have been reported to possess anti-cancer (Mousa *et al.,* 1994; Salem *et al.,* 2013); anti-tumor and antioxidant (Khan and Sultana, 2005; Aswar *et al.,* 2008); antimicrobial and anti-parasitic activities (Kirana *et al.,* 2009), hypolipidemic and hypoglycemic properties (Joseph and Raj, 2010); hepatoprotective effect (Wilson and Wilson, 2013); anthelmintic and analgesic effects (Deepa *et al.,* 2018), but there are no research reports in available literature on *Ficus pachyneura*. A preliminary study on *Ficus pachyneura* in our laboratory showed that the plant has an appreciable anti-diarrheal activity on a castor oil-induced diarrhoea model in mice. The present study comprehensively investigated the anti-diarrhoeal activity of *Ficus pachyneura* ethanol extract in albino mice.

Materials and Methods

Chemicals and Drugs used for the Study: Analytical grades of ethanol (Honeywell 1953, Harvey St. France.), acacia gum (BDH Chemicals Ltd, Poole, England) and activated charcoal (BDH Chemicals Ltd, Poole, England) used for the study were purchased from Joechem Company Ltd., Nsukka, Enugu State, Nigeria, while generic brands of loperamide, atropine sulphate (Epil Chinese company; distributed by Pavco Pharmaceutical Industry, Nigeria Ltd) and castor oil (Bell's B Label Castor oil, Bell Sons and Co. Ltd) used for the study were purchased from Blessed Pharmaceuticals Ltd., Umuahia, Abia State, Nigeria.

Study Location: The study was carried out in the Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The study design and use of the animals for research was approved by the College Research Ethics Committee (Approval number – CVM/REC/202224). All the

experimental procedures were carried out in strict compliance to the Institutional Ethical Committee Guidelines for animal studies, as well as in consultation with the Experimental Ethics Committee (EEC) guidelines for laboratory animal care and use (Zimmerman, 1983; Ward and Elsea, 1997; Louhimies, 2002).

Plant Collection and Identification: Fresh leaves of *Ficus pachyneura* used for the stidy were obtained from Ameke Afarata Ibeku, in Umuahia North Local Government Area of Abia State, Nigeria. The leaves were identified and authenticated by Mr. S. C. Ibe, of the Department of Forestry, Michael Okpara University of Agriculture Umudike (MOUAU), Nigeria. A voucher specimen (MOUAU/CVM/VPP/2022/023) was kept in the departmental herbarium.

Extract Preparation: The leaves of *Ficus pachyneura* collected for the study were washed under running water immediately after collection and air-dried to constant weight, then pulverized into fine powder using a contact mill machine. The quantity of the fine powder was weighed with an electronic digital balance and was macerated in 1: 5 weight per volume of analytical grade ethanol for 72 hours, with vigorous agitation every 2 hours. The mixture was filtered through a Whatman filter paper into an already measured beaker at room temperature. The filtrate obtained was concentrated under reduced pressure using a rotary evaporator (Cole-Parmer type N-1110, China). The percentage yield (w/w) of the plant extract was calculated as described by Sofowara (1993). The final product (dried extract of *Ficus pachyneura*) was labelled ethanol leaf extract of *Ficus pachyneura* (ELEFP) and stored in a refrigerator (4°C) until time of use.

Qualitative and Quantitative Phytochemical Screening of ELEFP: The methods of Trease and Evans (2004) and Harborne (2008) were used for evaluating the extract for its phytochemical constituents (quantitatively and qualitatively).

Experimental Animals: Adult mice (males) weighing between 29 – 30 grammes were procured from the Laboratory Animal House of the Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike (MOUAU), Nigeria. They were acclimatized for a period of three weeks before the commencement of the study.

Acute Toxicity Testing of ELEFP: A total of 35 adult mice, randomly assigned to seven groups (A – G) were used for the acute toxicity study, based on a modification of the two-step/phase acute toxicity study procedure earlier described by Lorke (1983). In the first phase, three groups (A – C) of five mice each received (orally), 10 mg/kg, 100 mg/kg and 1000mg/kg of ELEFP, respectively. In the second phase, three groups (D – F) with five mice in each group received (orally), 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of ELEFP, respectively. The five mice in group G were given distilled water at 5 ml/kg and served as the normal control. All the mice were allowed free access to feed and water. They were observed for signs of toxicity and mortality over a period of 72 hours post administration and for another 14 days for any delayed toxicity. The median lethal dose (LD_{50}) of the leaf extract was calculated using the formula adopted by Khan *et al.* (2013).

Effects of ELEFP on Castor Oil induced Diarrhoea in Mice: Twenty-five (25) mice were used for this experiment and the method modified by Ezekwesili *et al.* (2004) was adopted. The mice were fasted for 18 hours prior to the commencement of the experiment and were randomly assigned to five groups of five mice each (labeled Groups 1 – 5). Mice in Group 1 received 5 ml/kg of distilled water and served as the negative control, while those in Group 2 received the standard anti-diarrhoeal drug, Loperamide (5

mg/kg, orally) and served as the positive control. Mice in Groups 3 – 5 were orally given ELEFP at 200 mg/kg, 400 mg/kg, and 800 mg/kg body weight, respectively.

After 60 minutes of extract administration, each mouse was orally gavaged with 0.5 ml of castor oil and was placed in separate cage, over a clean blotting paper for easy spotting of the diarrhoeic faeces, according to the method of Awouters *et al*. (1978) as modified by Gricilda and Molly (2001). The papers were changed every hour to make the faecal droppings visible for counting and to check stool consistency. During the observation period of 4 hours, the onset of diarrhoea, the number and weight of both dry and wet stools excreted by each mouse were recorded and comparisons were made between the treated groups and the untreated (negative control) group. The percentage inhibition of diarrhea was calculated using the formula: PI = (MW_1 – MW_2) / MW_1 × 100, where; PI = Percentage inhibition of diarrhea; MW_1 = Mean number of wet stools of control group; MW_2 = Mean number of wet stools of treated group.

Effect of ELEFP on Gastrointestinal Motility (Transit Time): The method described by Akah *et al*. (1998) was used in this experiment. Twenty-five mice were fasted for 18 hours and assigned to five groups (V, W, X, Y and Z) of five mice each. Group V received distilled water (negative control); Group W received Loperamide via oral route at 5 mg/kg (as positive control), while Groups X, Y and Z received 200 mg/kg, 400 mg/kg and 800 mg/kg of ELEFP orally, respectively. Thirty minutes post-administration of the treatments, all the mice were given a charcoal meal 0.5 ml (10% suspension of activated charcoal in 5% aqueous Acacia gum), and 30 minutes later, each mouse was sacrificed by cervical dislocation and the abdomen opened and the small intestine immediately isolated. The length of the small intestine from pylorus to the caeca junction (LSI) and the distance travelled by the charcoal (DC) was measured and recorded. The peristaltic index (PI) for each mouse was calculated, and was expressed as percentage of the distance travelled by the charcoal meal relative to the total length of the small intestine. The percentage inhibition relative to the control was also calculated as described by Akah *et al*. (1999): PI = DC / LSI × 100, where, PI = Peristaltic Index; DC = Distance travelled by the charcoal meal; and LSI = Length of small intestine.

Effect of ELEFP on Castor Oil induced Enteropooling: Enteropooling (Intraluminal fluid accumulation) was determined by the method of Roberts *et al*. (2015). In this model, 25 mice deprived of food for 18 hours were randomly assigned to five groups (J, K, L, M and N) of five mice in each group. Group J mice received oral administration of 5 ml/kg of distilled water (negative control); Group K mice received 3 mg/kg Atropine sulphate (standard drug), while Groups L, M and N mice received 200, 400 and 800 mg/kg of ELEFP respectively. Sixty (60) minutes later, castor oil was orally administered to all the mice at 0.5 ml/mouse, and thirty (30) minutes post-castor administration, each mouse was sacrificed via cervical dislocation and the small intestine dissected out and ligated at the pyloric sphincter and at the ileo-ceacal junctions. The intestinal content was collected by milking into pre-weighed graduated test tubes and the new weight was recorded. The volume of the intestinal content was read directly from the calibrated test tube and was recorded for each mouse.

Statistical Analysis: Data obtained from this study were subjected to descriptive analysis and a summary of the results were presented as means ± standard errors. One-way analysis of variance (ANOVA) was used to compare anti-diarrhoeal activity of the graded doses of the extract with the controls, while appropriate post-hoc statistics were used to separate the means. Statistical confidence was set at 95 % ($p < 0.05$).

Results

Yield and Nature of the Extract: The ethanol leaf extract of *Ficus pachyneura* (ELEFP), obtained after extraction was dark brown coloured, almond scented, and sparingly soluble in distilled water. The extraction yielded 99.89 grams extract out of 968.2 grams of the plant material (10.32%).

Phytochemistry: The result of the phytochemical screening showed the presence of alkaloids, glycosides, phenolics, tannins, flavonoids, terpenoids, steroids, cardiac glycoside, anthraquinones and carbohydrate. The predominant phytoconstituents were: saponins (24.31%), alkaloids (19.72%), cardiac glycosides (14.94%) and phenols (14.11) (Table 1).

Acute Toxicity Testing: There was no sign of acute toxicity and no death in all the groups treated with the extract, even at the highest dose of 5000 mg/kg body weight of ELEFP. The LD_{50} was therefore assumed to be above 5000 mg/kg.

Effect of ELEFP on Castor Oil-Induced Diarrhoea in Mouse: The mean number of wet faeces collected from mice in Groups 2, 3, 4 and 5 and their purging index were significantly ($p < 0.05$) lower than that of the Group 1 mice; and for the extract treated groups 3, 4 and 5, the effects were dose dependent, and the results recorded for Groups 4 and 5 mice were comparable and better than that obtained for the Group 2 (positive control) mice given Loperamide (Table 2). The onset of diarrhoea was however significantly ($p < 0.05$) shorter in the Group 1 mice when compared to the treated groups (Groups 2, 3, 4 and 5) with the mice in Groups 4 and 5 having a comparably longer time before onset of diarrhoea than the Loperamide treated positive control (Table 2). The percentage inhibition of diarrhoea followed the same pattern (Table 2). The extract, at the doses of 400 and 800 mg/kg, exhibited significant ($p < 0.05$) dose dependent anti-diarrhoeal activity, comparable and even better than the Loperamide treated positive control group (Table 2).

Table 1: Qualitative and quantitative phytochemical constituents of the of ethanol leaf extract of *Ficus pachyneura.*

Constituents	Qualitative	Quantitative (mg/g RE)	%
Alkaloids	**++**	35.14	19.72
Saponins	**+++**	43.31	24.31
Flavonoid	**++**	12.46	6.99
Tannin	**++**	15.05	8.45
Carbohydrate	**+**	6.34	3.36
Cardiac Glycoside	**++**	26.61	14.94
Steroid	**+**	2.31	1.29
Terpenoids	**++**	9.30	5.22
Anthraquinones	**+**	2.50	1.40
Phenolics	**+++**	25.14	14.11

+++ – High; ++ – Moderate; + – Trace; RE – Relative Equivalent.

Table 2: Effect of ethanol leaf extract of *Ficus pachyneura* (ELEFP) on castor oil induced diarrhoea in mice.

Treatment groups	Mean number of wet faeces	Onset of diarrhoea (minutes)	Purging index	Percentage inhibition of diarrhoea
Group 1 (5 ml/kg Distilled water + 0.5 ml castor oil)	9.60 ± 0.50 [a]	31.80 ± 1.65 [b]	100 ± 0.00 [c]	0.00
Group 2 (5 mg/kg Loperamide + 0.5 ml castor oil)	3.00 ± 0.89 [bc]	126.60 ± 29.96 [a]	30.08 ± 0.30 [ab]	69.91
Group 3 (200 mg/kg ELEFP + 0.5 ml castor oil)	4.20 ± 0.86 [b]	109.20 ± 3.92 [a]	44.24 ± 0.21 [b]	55.75
Group 4 (400 mg/kg ELEFP + 0.5 ml castor oil)	2.80 ± 0.86 [bc]	143.60 ± 26.76 [a]	28.75 ± 1.10 [ab]	71.24
Group 5 (800 mg/kg ELEFP + 0.5 ml castor oil)	1.60 ± 0.67 [c]	173.60 ± 27.82 [a]	15.63 ± 0.31[a]	84.36

Values are presented as mean ± standard error of mean.

Different superscripts in a column indicate significant difference at $p < 0.05$.

Effect of ELEFP on Gastrointestinal Motility in Mice: At all the doses tested, ELEFP significantly ($p < 0.05$) reduced the distance travelled by the charcoal meal as well as the peristaltic index in the extract treated groups, when compared to the distilled water treated negative control group (Table 3). The percentage anti-motility of the ELEFP-treated mice groups were dose-related, and at 800 mg/kg dose was comparable with 5 mg/kg of Loperamide used as standard (Table 3).

Effect of ELEFP on Castor Oil induced Intestinal Fluid Accumulation in Mice: The effect of ELEFP on castor oil-induce intestinal fluid accumulation is presented in Table 4. The volume of intestinal fluid accumulation in the extract-treated groups were significantly ($p < 0.05$) lower when compared to that of the distilled water treated negative control group, and was comparable to the Atropine sulphate-treated positive control group (Table 4). The percentage inhibition in the extract treated groups was dose-dependent, though none of the extract treated groups was as good as Atropine sulphate-treated positive control (Table 4).

Discussion

Diarrhoea usually results from the inability of the intestine to absorb water or from an increase in the secretion of water/fluid into the intestinal lumen. It occurs because the balance between fluid secretion into, and fluid absorption from the intestinal lumen is altered resulting in a net increase in fluidity of the faeces. Diarrhoea has remained a global concern and the search for possible alternative remedy is in high demand (Ejeh *et al.,* 2017).

Table 3: Effect of ethanol leaf extract of *Ficus pachyneura* (ELEFP) on gastrointestinal motility in mice.

Treatment groups	Distance travelled by charcoal meal (cm)	Peristaltic index	Percentage anti-motility (%)
Group V (5 ml/kg Distilled water + 0.5 ml castor oil)	35.34 ± 1.20 [a]	64.42 ± 3.33 [a]	0.00
Group W (5 mg/kg Loperamide + 0.5 ml castor oil)	8.14 ± 1.65 [c]	14.24 ± 2.46 [c]	77.42
Group X (200 mg/kg ELEFP + 0.5 ml castor oil)	16.04 ± 1.35 [b]	31.09 ± 2.90 [b]	54.15
Group Y (400 mg/kg ELEFP + 0.5 ml castor oil)	13.02 ± 2.17 [b]	23.15 ± 3.72 [b]	62.48
Group Z (800 mg/kg ELEFP + 0.5 ml castor oil)	7.70 ± 1.01 [c]	15.00 ± 2.21 [c]	77.80

Values are presented as mean ± standard error of mean.

Different alphabetical superscripts in a column indicate significant difference at $p < 0.05$.

Table 4: Effect of ethanol leaf extract of *Ficus pachyneura* (ELEFP) on castor oil induced intestinal fluid accumulation in mice.

Treatment groups	Volume of intestinal fluid (ml)	Percent inhibition
Group J (5 ml/kg Distilled water + 0.5 ml castor oil)	1.02 ± 0.08 [a]	0.00
Group K (3 mg/kg Atropine sulphate + 0.5 ml castor oil)	0.43 ± 0.03 [b]	55.75
Group L (200 mg/kg ELEFP + 0.5 ml castor oil)	0.48 ± 0.04 [b]	71.24
Group M (400 mg/kg ELEFP + 0.5 ml castor oil)	0.42 ± 0.06 [b]	84.36
Group N (800 mg/kg ELEFP + 0.5 ml castor oil)	0.30 ± 0.04 [b]	69.91

Values are presented as mean ± standard error of mean.

Different alphabetical superscripts in a column indicate significant difference at $p < 0.05$.

The dark brown colored appearance and almond scent of the extract obtained in this present study is similar to that reported for other species of the genus Ficus. The percentage yield (10.32%), of the *Ficus pachyneura* plant obtained by the cold maceration extraction technique was within the range reported for other species of Ficus using varied extraction methods.: 7.14% in *F. carica* leaf (Aref *et al.,* 2010), 10.69% in *F. auriculata* leaf (EL-Fishway *et al*., 2011), 9.9% in *F. religiosa* leaf (Rajiv and Sivaraj, 2012), 11.06% in *F. retusa* leaf (Aly *et al*. (2013), and 8.33% in *F. capensis* leaf (Salem *et al*. (2013).

It is common knowledge that medicinal plants contain several secondary phytochemicals and metabolites, and these phytochemicals and metabolites are the bioactive components of plants, which have great value and application in pharmaceutical and medicinal fields (Nawaz *et al.,* 2019). These phytochemicals have been reported to have protective and disease preventive properties (Syed *et al.,* 2011; Vaghasiva *et al*., 2011). The ethanol leaf extract of *Ficus pachyneura* (ELEFP) used for this study contained very high concentrations of saponins (24.31%), alkaloids (19.72%), cardiac glycoside (14.94%), phenolics (14.11%), tannins (8.45%), terpenoids (5.22%) and flavonoids (6.99%), amongst others, and the presence of these phytochemicals suggests that this plant leaf extract has the potential for diverse biological activities.

Most of the natural plant extracts used in traditional medicine are believed to be safe, compared with synthetic drugs (Wanger and Farnsworth, 1993), and extracts of some other species of Ficus plants such as *Ficus glomerata*, *Ficus* glumosa, *Ficus religiosa*, *Ficus racemose*, *Ficus carica* and *F*icus *benghalensis* have been reported to have wide safety margin (Channabasavaraji *et al.,* 2008; Khan and Sultana, 2005; Poudel *et al.*, 2015; Ahmed and Urooj, 2010; Caliskan and Polat, 2011; Patil and Pimprikar, 2009). The finding in this study that the LD_{50} of the extract was greater than 5000 mg/kg body weight in the mice implies that the extract is acutely safe and not toxic. Several earlier reports on LD_{50} of extracts of plants in the Ficus genus have shown that most of them have an LD_{50} greater than 5000 mg/kg (Mousa *et al.*, 1994; Khan and Sultana, 2005; Aswar *et al.,* 2008; Kirana *et al.,* 2009; Joseph and Raj, 2010; Salem *et al.,* 2013; Wilson and Wilson, 2013; Deepa *et al.,* 2018).

Castor oil induces diarrhoea through its active metabolite (ricinoleic acid), which increases nitric oxide synthesis, leading to increased secretion in the gut (Sisay *et al.,* 2017), inhibition of fluid absorption (Imam *et al*., 2013), and promotion of motility of the small intestinal peristaltic activity (Degu *et al.,* 2016), resulting in altered electrolyte permeability of the intestinal mucosal membrane with resultant watery stool (Sahoo *et al.,* 2014). Prostaglandins are known to stimulate the secretion of fluid and electrolytes in the small intestine and also decrease the absorption of glucose (Desta *et al.,* 2021), and this spontaneous release of prostaglandin E2 by the ricinoleic acid induces intestinal secretion (Dosso *et al.,* 2011).

The graded doses of ELEFP used in this study significantly decreased the castor oil-induced increased peristaltic movements, allowing the intestinal contents to stay for a longer time and become exposed to the absorptive surface of the intestinal tract, increasing absorption, and thereby controlling diarrhoea (Sahoo *et al.,* 2014; Ejeh *et al.,* 2017; Oteiza *et al.,* 2018). Some anti-diarrhoeals such as Loperamide function by slowing down intestinal contractions, increasing the time it takes for the contents of the bowel to be excreted, and this allows more water to be absorbed from the bowel back into the body, reducing the water content of the stool (Ezekwesili *et al.,* 2004; Dipiro *et al.,* 2008; Balaji *et al.,* 2012). The non-steroidal anti-inflammatory drugs such as Loperamide, apart from its inhibitory effect on the prostaglandin synthesis, also prolong the onset of diarrhoea in the castor oil-induced

diarrhoea model (Awouter *et al.,* 1978). It is thought that the delayed onset of diarrhoea associated with ELEFP administration in this study, may be due to its earlier reported anti-inflammatory activity exhibited by the phytochemicals contained in the plant (Moreira *et al.,* 2013; Madubuike and Onyeacholam, 2015).

The significant suppression of the propulsion of charcoal meal in the extract-treated mice, especially at the 800 mg/kg treatment dose, could be due to the ability of ELEFP to significantly increase the segmental contractions within the gastro-intestinal tract, which decreases the time it takes for the intestinal contents to be evacuated, thus allowing ample time for absorption/re-absorption of intestinal fluids and other vital nutrients, much like the action of Loperamide (standard drug) used as control in this study. This suggests that the mechanism of action of the anti-diarrhoeal activity of ELEFP as recorded in this study may be similar to that of Loperamide.

The reduction of castor oil-induced entero-pooling by ELEFP in this study (in a dose dependent manner) could be due to the ability of the extract to reduce the weight gain of intestinal contents by preventing fluid and electrolyte secretion into the intestine.

The anti-diarrhoeal activity of ELEFP as recorded in this study is thought to possibly be as a result of the presence in the extract of phytochemicals such as alkaloids which acts as smooth muscle relaxants (Gong *et al.,* 1986), with analgesic, antispasmodic and bactericidal effects (Agu and Thomas, 2012; Epidi *et al.,* 2016). Flavonoids, which were found to be present in higher amounts are known to activate alpha 2 receptors of luminal absorptive cells which stimulate absorption of fluid (Oteiza *et al.,* 2018) and inhibit both the production of prostaglandin E2 and expression of COX-2, (Hamalainen *et al.,* 2011), thus increasing the intestinal absorption of water, and electrolytes which may have accounted for decreased volume of the intestinal content (wet faeces) in the extract treated groups. It is also thought that the presence of tannins in the extract may have played a role, as earlier reports by Seow *et al.* (1988), showed that tannins inhibit the release of PGE-2 and PGI-2 from macrophages (Awad *et al.,* 2004), act as an astringent and anti-inflammatory (either by binding to precipitate or shrinking of proteins), and also modulate cystic fibrosis transmembrane conductance regulator protein (CFTR), a membrane protein that acts as a channel to transport chloride ions from epithelial cells to the lumen in a way that reduces secretion in the small intestine and colon (Ashok and Upadhyaya, 2012), thereby reducing intestinal secretion by inhibiting intracellular Ca^{2+} inward current (Yacob *et al.,* 2016). Anthraquinones and terpenoids have been reported to act as an anti-inflammatory agents (Chien *et al.,* 2015; Prakash, 2017), by their ability to inhibit the production of prostaglandin E2 (Awad *et al.,* 2004). Saponins has also been reported to have suppressant, antimicrobial and strong anti-inflammatory effects (Hazem *et al.,* 2012; Brusotti *et al.*, 2014), which could have also played vital role, while phenols are widely known to have antioxidant activity by neutralizing free radicals which causes inflammation, and the activation of the inflammatory cascade in turn may have caused the synthesis of prostaglandins (Moreira *et al.,* 2013). The presence of these important phytochemicals in ELEFP which may have contributed (synergistic effect) to the significant decrease in peristaltic movements (reduced intestinal motility), through inhibition of intracellular Ca^{2+} inward current, preventing fluid and electrolyte secretion into the intestine (Yacob *et al.,* 2016), and inhibiting intestinal secretion, and thus, the reduction in the passage of wet stool as observed in the extract treated mice. This finding is in agreement with the earlier reports by Longanga-Otshudi, (1999); Umer *et al.* (2013); Madubuike and Onyeacholam, 2015; Onoja *et al.* (2019) and

Ifenkwe *et al.* (2023), who showed that most of these secondary metabolites such as tannins, alkaloids, saponins, flavonoids, steroids, phenolics and reducing sugars, have been shown to possess very strong anti-diarrhoeal property.

Lomasney and Hyland (2013) grouped various anti-diarrhoeal drugs into several categories, such as anti-motility or anti-spasmodic agents, anti-secretory compounds, adsorbents and antibiotics (which are occasionally used to treat diarrhoea caused by specific infections, such as Campylobacter, Giardia, etc.), bile acid sequestrants, enzymes, and probiotics (Brunton *et al.,* 2006), based on their mechanisms, such as effects on intestinal motility (μ receptors), intestinal secretion (δ receptors), or absorption (μ and δ receptors), which are mediated principally through either μ- or δ-opioid receptors on enteric nerves, epithelial cells, and muscle, although most anti-peristaltic agents act by altering intestinal motility (Brunton *et al.,* 2006; Breslin, 2017). From the results of this study, we speculate and believe that the ELEFP used in this study acted as both anti-secretory/anti-spasmodic and anti-motility agent, much like the standard drug (Loperamide).

Conclusion: It was concluded from the findings of this study that ethanol leaf extract of *Ficus pachyneura* as used in the study (especially at the higher doses of 400 and 800 mg/kg) exhibited significant anti-diarrhoeal activity in the mice model, via reduction of intestinal hyper-motility and hyper-secretion, hence could be a potential alternative source of anti-diarrhoeal agent.

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Conflict of Interest

The authors declare no conflict of interest.

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