ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 70 6 Number 1, 2022

DETERMINATION OF PATHOPHYSIOLOGICAL ANTI-DIARRHOEAL ACTIVITY OF THE AQUEOUS STEM BARK EXTRACT OF FICUS PLATYPHYLLA

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Link to this article: https://doi.org/10.11118/actaun.2022.006
Received: 16. 8. 2021, Accepted: 21. 2. 2022

Abstract

This study was motivated by the report of a survey of herbal remedies used by Fulani herdsmen in the management of cow diarrhoea in Plateau State, Nigeria. The report identified *F. platyphylla* stem bark amongst major herbs. Activity of aqueous stem extract of *F. platyphylla* against diarrhoea caused by physiological changes and by pathological infections was herein determined. Thirty *albino* rats were fasted for 18 hours. They were grouped into six and induced with diarrhoea using castor oil. The first two groups received distilled water (1 ml) and loperamide (5 mg/kg), while other groups were administered plant extract 50, 100, 200 and 400 mg/kg body weight respectively. The severity of diarrhoea was assessed hourly for 4 hrs. Gastrointestinal motility experiment was carried out using atropine sulphate (5 mg/kg). *In vitro* anti-diarrhoeal test was carried out against *E. coli* clinical and laboratory isolates versus antibiotics. The extract inhibited defecation moderately, and regulated frequency of wet stool better than loperamide, the lowest dose (50 mg/kg b. w.) competed favourably with the loperamide. This dose produced the most remarkable activity considering the latent period, total stool frequency, purging indices and percentage inhibition of defecation. The dose showed remarkable activity in the propulsion of charcoal meal through gastrointestinal tract as displayed in the inhibition of diarrhoea. The results of *in vitro* anti-diarrhoeal test against *E. coli* showed that the plant extract was inactive on the microorganism. The results from the present study validated the anti-diarrhoeal potential of *F. platyphylla* via its physiological action.

Keywords: diarrhoea, herbal remedy, castor oil, loperamide, gastrointestinal motility, *E. coli*

INTRODUCTION

Diarrhoea has been described in many ways by various authors; according to (Hirchhorn, 1980; Fuller, Long and Davis, 2004), diarrhoea is an intestinal disorder characterized by passage of loose watery stools more frequent than is normal for the individual; the overall weight and volume of the stool is increased (more than 200 g or ml/day), and the water content is increased to 60 or 90%. Ezekwesili *et al.* (2004) characterized diarrhoea by an increase in the frequency of bowel movements, wet stool and abdominal pains. Diarrhoea is commonly contacted when various types of bacteria, virus, and parasites infect the intestine. These microbial agents: viral (adenovirus, enterovirus and norovirus), bacterial (Shigella species, *Escherichia coli*, *Vibrio cholera* and Salmonella species) and parasitic (Cryptosporidium and Giardia) disturb the normal tone of gastrointestinal tract (Allen *et al.,* 2003). However, another cause of diarrhoea recently observed is the long-term use of some of gastrointestinal drugs (Kakei, Ichinose and Tsukada, 1993). A number

of different socio-economic and political factors also contribute to the constant morbidity arising from acute and persistent diarrhoea, as well as intermittent epidemics of cholera and dysentery (Hamer, 1998; Eke and Anaga, 2014). These microbial agents that increase the osmotic influx of water and ion to the intestinal lumen (Velazquez *et al*., 2006) usually spread out through food, drinking water, and unhygienic environment.

The hygienic or sanitary status of a group of people is greatly determined by their living standards (Otshudi *et al*., 2000; Venkatesan *et al*., 2005). People of the third world are prone to various gastrointestinal diseases especially diarrhoea due to their low level of hygiene. Diarrhoea remains one of the major health threats to infant populations in the tropical and sub-tropical countries. It is rated as the world's third highest killer disease, contributing substantially to paediatric morbidity and mortality (Havagiray, Ramesh and Sadhana, 2004; World Health Organization, 2009). The incidence of diarrhoea is still high (about 7.1 million per year), despite the efforts of international organizations to control this disease (Kouitcheu *et al*., 2006). Hence there is need to continuously research on the discovery of therapeutic agents that could greatly inhibit the activity of the microorganisms causing diarrhoea and which should contract the intestinal smooth muscle against the physiological changes that could allow free flow of intestinal contents.

Ficus platyphylla is a plant in the mulberry family (moraceae). The genus Ficus represents one of the largest angiosperm and comprises more than 800 species which include climbers, vines, hemi epiphytes, creepers and shrubs. Many grow up to become established trees in the tropics and sub-tropic areas. Hemi epiphytic habitat is a distinctive property of species of this genus (Frodin, 2004; Rønsted, Salvo and Savolainen, 2007). However, *F. platyphylla* thrives in the African floristic regions which contain over 100 ficus species employed in African traditional medicine for the treatment of various health disorders (Balogun *et al*, 2011). It is an epiphyte which establishes itself over its host and grows to a full tree to the length of 18 m and girth of 6 m with broad large widely spreading branches, and broadly-elliptic leaves. In Nigeria *F. platyphylla* is known as *gamji* in Hausa language (Amos *et al*., 2001), *opoto pupa* in Yoruba, *dundehi* in Fulfulde and *gbagun* or *gbanchi dzurugi* in Nupe.

The plant has been employed in the traditional medicine in many African countries. In Nigeria, herbalists have employed *F. platyphylla* in cases like insomnia, depression, pain and psychosis (Chindo *et al*., 2003). In the north eastern part of Nigeria where tuberculosis is endemic *F. platyphylla* is being employed in the effective management of tuberculosis and cough (Kubmarawa *et al*., 2007). The plant is indigenously used in the treatment of trypanosomiasis (Atawodi *et al*., 2001), convulsive disorder and mental illness (Adeshina *et al*., 2010). Herbal remedies used to promote fertility involves decoction of seeds, leaves and stem bark of *F. platyphylla* along with other herbs, while some other formulations are made purely of the stem bark of the plant for specific diseases. A survey of herbal remedies used by some herdsmen "*the Fulanis*" in the north central part of Nigeria for the management of livestock diseases highlighted *F. platyphylla* and *F. ingens* for the treatment of diarrhoea in cows (Offiah *et al*., 2012).

Other pharmacological screenings that have also confirmed some of the traditional uses of *F. platyphylla* include: The evaluation of the activity of *F. platyphylla* on the central nervous system (CNS) reported by Chindo *et al.* (2003), gastrointestinal activity of the stem bark in rodents (Amos *et al*., 2001) and anti-fungal and anti-bacterial activities of the stem bark and root extracts against *Staphylococcus aureus, Pseudosomonas aeruginosa* and *Salmonella species* (Kubmarawa *et al*., 2007) among others. Majority of the available antibiotics are resisted by microorganisms, while some of these antibiotics, on the other hand produced adverse effects on the patients (Soberon *et al*., 2007). Based on these occurrences, the search for more effective, safer and natural anti-diarrhoeal agents is very imperative. Hence, this research chose to evaluate the anti-diarrhoeal activity of stem bark of *F. platyphylla*.

MATERIALS AND METHODS

Leaf sample of *Ficus platyphylla* was collected from Borgu area, behind Ibrahim Badamasi Babangida (IBB) University Quarters, Niger State, Nigeria. The plant was identified as *F. platyphylla* and authenticated at the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. A voucher specimen (No: UIH001/1194) of the plant was deposited in the herbarium of the Institution. The stem bark of the plant was thereafter collected from the same tree for this study.

Preparation of the Plant Extract

The stem bark of *F. platyphylla* was cleaned of extraneous matters, air-dried under shade and pulverized into a coarse powder. The powder was macerated in distilled water (100 g of powder in 1 L of distilled water) with intermittent swirling and mixing at ambient temperature (29 ± 2 °C). The solution of the water soluble constituents was drained and filtered through Whatman filter paper (No. 1). The filtrate was evaporated to dryness using rotary evaporator.

Preliminary Phytochemical Screening

Phytochemical screening was carried out using methods described by Trease and Evans (1999). The phytochemicals determined include: Alkaloids with Dragendorff's reagent; cardiac glycosides, Keller-Killani test; flavonoids, alkaline reagent test; phlobatannins, HCl test; saponnins, froth's test; steroids, H_2SO_4 test; tannins, $FeCl_3$ test; and terpenoids, Salkowski test.

Test for alkaloids: An aliquot of the extract reconstituted in chloroform was filtered and spotted on a TLC plate. The plate was developed and dipped inside a solution of Dragendorff's reagent. Formation of a yellowish coloured spots indicated the presence of alkaloids.

Test for cardiac glycosides (Keller-Killani Test): 5 g of the extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. The appearance of brown ring at the interface upon addition of 1 ml of concentrated sulphuric acid indicated the deoxy sugar characteristic of cardenolides. Appearance of a violet ring below the brown ring, and a greenish ring in the acetic acid layer confirmed the presence of cardiac glycosides.

Test for flavonoids (Alkaline reagent test): A portion of the aqueous filtrate of the extract was treated with 5 ml of diluted ammonia solution, followed by addition of concentrated sulphuric acid. Formations of yellow colour at the interface, which became colourless on further addition of sulphuric acid indicated the presence of flavonoids.

Test for phlobatannins: A portion of the aqueous filtrate of the extract was boiled with a few drops of 2% aqueous hydrochloric acid. The solution was allowed to stand for some times and was observed for the formation of red precipitate.

Test for saponnins: 2 g of the powdered sample was shaken with 10 ml of distilled water and warmed in a water bath. 10 ml of distilled water was added and the solution was vigorously shaken for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and was observed for the formation of emulsion.

Test for steroids: 1 ml of acetic anhydride was added to ethanolic extract of the sample, followed by 1 ml of sulphuric acid from the side of the test tube. The content was observed for the formation of blue to blood red colour.

Test for tannins: 1.0 g of each plant extract was dissolved in 20 ml of distilled water, then boiled gently and cooled. 2 ml of this solution was put in a test tube and few drops of 0.1% ferric chloride solution were added. Development of a brownish-green or a blue-black colouration was considered for the presence of tannins.

Test for terpenoids (Salkowski Test): 5 g of the sample solution was mixed with 2 ml of chloroform, and 2 ml concentrated sulphuric acid was carefully added. The solution was allowed to stand to form a layer. Formation of a reddish brown colouration at the interface indicated the presence of terpenoids.

In Vivo Anti-diarrhoeal Test

The Animal

Female Albino Wistar rats (weighing 120–150 g) were housed in plastic cages (60 × 30 × 30 cm), five per cage, at ambient temperature (29 ± 2 °C) with light/darkness in 12h/12h. The rats were provided with food and water *ad libitum*, and allowed a two-week acclimatization period prior to the study. The handling and sacrificing of the animals were in accordance with the University of Ilorin – ethical committee guidelines for animal experiment.

Drug and Chemicals

Atropine sulphate and loperamide (Vixa Pharmaceutical Co., Ltd, Lagos Nigeria; manufactured by Jiangsu Ruinian Qianjin Pharmaceutical Co., Ltd, Jiangsu province, China), castor oil (Bell Sons & Co., (Druggists) Ltd, Southport PR9 9AL, England), distilled water and charcoal meal (50 g charcoal in 100 ml of 5 g Agar-agar) were used.

Castor Oil-Induced Diarrhoea

The experiment was carried out according to the method of Teke *et al.* (2012) with minor modification. Thirty (30) rats were fasted for 18 hours and divided into six groups of five animals each. The first group received distilled water (1 ml), the second group received the standard drug, loperamide (5 mg/kg b. w.), while the third, fourth, fifth and sixth groups were administered with plant extract 50, 100, 200 and 400 mg/kg b. w. respectively. After an hour, all the animals received castor oil (1 ml) orally by gavages. The animals were then kept in separate metabolic cages with a pre-weighed A4 paper beneath the cage to collect faeces. The severity of diarrhoea was assessed hourly for 4 hours. The total number of faeces (both diarrhoeal and non-diarrhoeal) expelled were compared with the control group. The total score of diarrhoeal faeces for the control group was taken as 100% and results from other groups were expressed as a percentage of inhibition of diarrhoea.

Gastrointestinal Motility Test

Another set of rats was provided for gastrointestinal motility experiment and treated as described by Akah *et al.* (1998). The rats were only allowed access to water for 18 hrs prior to the day of experiment and grouped into six as described above (n = 5). The control group received distilled water orally (1 ml each), the reference group received the standard drug, atropine sulphate (5 mg/kg b. w.), while the remaining groups received the plant extract in doses of 50, 100, 200 and 400 mg/kg b. w., respectively. Fifty (50) min later, each animal was given charcoal meal (10% charcoal in 5% agar) 1 ml each via oral route. Animals were sacrificed after 40 min using diethyl ether, and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum was measured in centimetres and expressed as percentage of distance moved.

In Vitro Anti-diarrhoeal Test

For further investigation of the anti-diarrhoeal activity of *F. platyphylla* stem bark, the extract was tested against *E. coli* clinical (wild) and laboratory

(typed) isolates. The wild was obtained from the Department of Microbiology, University of Ilorin Teaching Hospital (UITH), while the typed *E. coli* ST2747 was sub-cultured from American Type Culture Collection (ATCC) stored in Refrigerator at 4 °C in the Department of Microbiology, University of Ilorin where the experiment was conducted.

Preparation of Media, Drug Solutions and Antibiotics

Sterile Mueller-Hinton Agar (MHA) poured into sterile dish was prepared as solid medium. The vehicle 3% dimethyl sulphoxide (DMSO), the plant extracts 50, 100, 200 and 400 (mg/mL) and an antibiotic filter paper disc comprising: 300 µg/mL nitrofuratoin (NIT), 5 µg/mL ofloxacin (OFL), 30 µg/mL augmentin (AUG), 5 µg/mL cefiximin (CXM), 10 µg/mL gentamicin (GEN), 30 µg/mL cefuroxime (CRX), 30 µg/mL ceftazidime (CAZ) and 5 µg/mL ciprofloxacin (CPR) respectively, were made available for incorporation into the solid medium (Nutrient broth). The test organisms were sub cultured from standardised organisms to a turbidity equivalent of 0.5 McFarland standards which corresponds to 1.5×10^8 CFU/mL (Ortez, 2005).

Pathogens Susceptibility Test by Disk Diffusion Method

The activity of the *F. platyphylla* extract against the test organism, *E. coli* was determined by agar well diffusion assay. The experiment was carried out in triplicates, and as described by Zakariyah *et al.* (2017). A sterile cotton swab dipped into the suspension was streaked back and forth from edge to edge to cover the entire plate. A 6 mm sterile cork borer was used to bore wells in each quadrant of MHA plates A, B and C for the 50, 100, 200 and 400 (mg/mL) concentrations of the extract and a centre well for the negative control (3% DMSO) introduced aseptically into the wells using micropipette. The plates were allowed to remain in the position for some minutes for proper diffusion of the extracts and then incubated for 24 hours at 35 °C. The same procedure was repeated for antibiotic filter paper disc (6 mm in diameter) comprising NIT (300 µg/mL), OFL (5 µg/mL), AUG (30 µg/mL), CXM (5 µg/mL), GEN (10 µg/mL), CRX (30 µg/mL), CAZ (30 µg/mL) and CPR (5 µg/mL) as positive controls. After the incubation period, inhibition zones were observed and measured in millimetres. An inhibitory zone with diameter less than or equal to the diameter of the spot was taken as lack of activity (Kujumgiev *et al.*, 1999).

RESULTS AND DISCUSSION

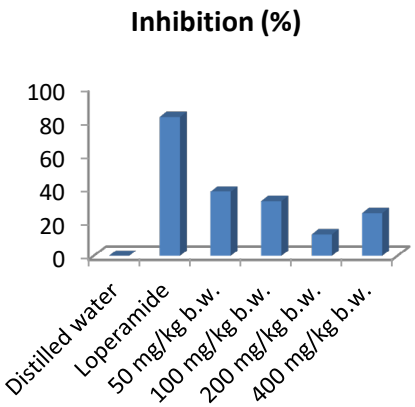
Phytochemical analysis of aqueous stem extract of *F. platyphylla* showed the presence of flavonoids, phlobatannins, saponins, steroids, tannins and terpenoids (Tab. I).

I: *Phytochemicals found in aqueous extract of F. platyphylla (stem)*

Phytochemical	Test	Qualitative
Alkaloids	Dragendorff's	-
Cardiac glycosides	Keller-Killani	-
Flavonoids	Alkaline reagent test	+
Phlobatannins	HCl test	+
Saponins	Froth's test	+
Steroids	H_2SO_4 test	+
Tannins	$FeCl_3$ test	+
Terpenoids	Salkowski test	+

The analysis of phyto constituents of *F. platyphylla* stem bark revealed the presence of tannins, saponnins, flavonoids, terpenoids and steroids, while alkaloids and cardiac glycosides were not detected (Tab. I). In the castor oil induced diarrhoeal experiment, the lowest dose (50 mg/kg b. w.) of *F. platyphylla* stem bark delayed the onset of diarrhoea (50 min) better than higher doses, it competes favourably with the reference drug, loperamide (60 min) and produced the shortest range of the onset time amongst the animals in the group. In addition, the dose gave very low frequency of wet stool and high inhibition of wet stool. Although, the plant extracts couldn't inhibit stools as much as the reference drug but the lowest dose produced most remarkable activity (Fig. 1) considering the latent period, total stool frequency, purging indices and percentage inhibition of defecation (Tab. II).

The extract of *F. platyphylla* reduced gastrointestinal distance (cm) travelled by the charcoal meal in the rats intestine. Although the activity was not dose dependent, but the dose 200 mg/kg produced same effect as the reference drug (2.5 ml) (Fig. 2). Atropine sulphate injection produced a moderate decrease in the propulsion (78.98%) of charcoal meal through gastrointestinal tract (Tab. III).

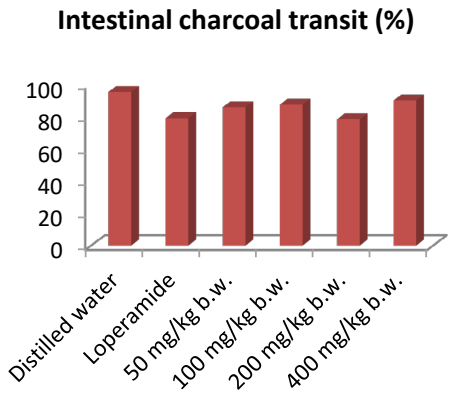


1: *Inhibitory effect of extract of F. platyphylla on castor oil-induced diarrhoea*

II: *Effects of Ficus platyphylla stem bark on castor oil-induced diarrhoea in Albino rats*

Test material (mg/kg b. w.)	Average weight (g)	Respondent (%)	Range of latent period (min)	Total stool frequency	Purging indices	Inhibition of defecation (%)	Frequency of wet stool	Water content (mL)	Inhibition of wet stool (%)
Distilled water (mL)	123.20	100	18–135	12.6 ± 2.69	23.68	0.00	8.0 ± 1.61	1.6 ± 0.49	0.00
Loperamide									
5	133.60	80	60–240	2.2 ± 3.80	1.85	82.54	7.6 ± 2.87	0.65 ± 0.66	61.41
50	128.00	80	50–68	7.8 ± 0.66	12.58	38.10	2.8 ± 0.86	0.82 ± 0.26	82.06
100	144.80	40	16–98	8.5 ± 3.30	14.91	32.54	1.75 ± 0.03	0.96 ± 0.55	86.88
200	138.20	80	26–170	11.0 ± 0.92	15.66	12.70	5.0 ± 1.71	1.48 ± 0.29	42.18
400	149.60	80	48–120	9.4 ± 2.29	13.06	25.40	7.4 ± 1.96	1.6 ± 0.38	7.50

Values are expressed as mean ± S.E.M. (n = 5); Percentage respondent is the percentage of number of animals that were diarrhoeal per group; Latent period is the waiting time for diarrhoeal faeces; purging indices is (% respondent x average number of stool/average latent period); Inhibition of defecation (%) = (Mc - Md × 100/Mc); Mc: mean number of defecation caused by castor oil; Md: mean number of defecation caused by drug or extract. Inhibition of diarrhoeic drops = (Mo - Md × 100/Mo); Mo: mean number of wet stool caused by castor oil; Md: mean number of wet stool caused by drug or extract.



2: *Effect of extract of F. platyphylla on gastrointestinal motility*

III: *Result of gastrointestinal motility experiment*

Group	Treatment	Dose (mg/kg b. w.)	Average intestinal charcoal transit (%)
1	Distilled water	1 ml	95.25
2	Atropine sulphate inj.	2.5	78.98
3	Extract	50	85.76
4	Extract	100	87.36
5	Extract	200	78.29
6	Extract	400	90.00

However, the lowest dose (50 mg/kg) showed similar activity in the propulsion of charcoal meal through gastrointestinal tract as it was found in the inhibition of diarrhoea (Tab. II). The contraction effect of this dose has been so consistent to confirm the behaviour of the plant material at low dose.

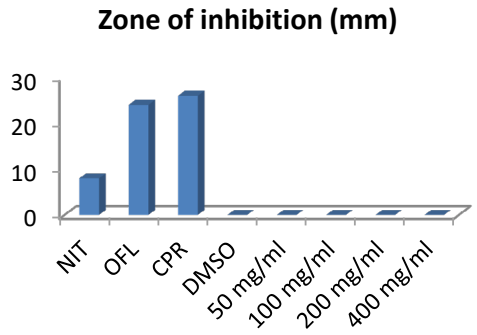
Castor oil is reported to contain up to 90% ricinoleic acid, 4% linoleic, 3% oleic, 1% stearic, and less than 1% α-linolenic (Patel *et al.*, 2016). Its ability to cause purging has been attributed mainly to the presence of ricinoleate. After oral intake of castor oil, hydrolysis of the triglyceride by intestinal lipases produces ricinoleic acid which directly influences intestinal motility and induces diarrhoea in animals (Franke, Scholl and Aigner, 2019). In addition, some ricinoleic acid derivatives have been observed to possess anti-inflammatory and analgesic properties (Pabis and Kula, 2016). Diarrhoea is caused in various mechanistic ways, this include increased electrolyte secretion, decreased absorption, changes in mucosal permeability, increased luminal osmolality, and disordered motor activity (Yakubu *et al.*, 2015).

In this study, the *F. platyphylla* extract doses inhibited defecation moderately (38%, 32%, 12% and 24%), and regulated frequency of wet stool better than the reference drug, this suggested that the plant may be milder in handling the situation than loperamide which nearly completely hindered defecation and tending towards constipation. It is a known fact that

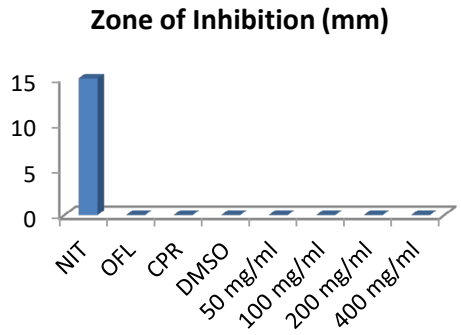
IV: *Effects of Ficus platyphylla stem bark on E. coli (typed and wild)*

Organism	Plant extract (mg/mL)	Diameter of zone of inhibition (mm)	Reference drug (5 µg)	Diameter of zone of inhibition (mm)
Typed	50	0	DMSO (3%)	0
	100	0	OFL	24
	200	0	CPR	26
	400	0	NIT (x 60)	08
Wild	50	0	DMSO (3%)	0
	100	0	OFL	0
	200	0	CPR	0
	400	0	NIT (x 60)	15

Dose: 100 µl of each concentration (mg/mL)
Key: Typed, labelled organism isolate; wild, human organism isolate; NIT, Nitrofuratoin; OFL, Ofloxacin; CPR, Ciprofloxacin and DMSO, Dimethyl sulphoxide.



3: *Inhibitory effect of extract of F. platyphylla against E. coli (Typed)*



4: *Inhibitory effect of extract of F. platyphylla against E. coli (Wild)*

every plant material that exhibit anti-diarrhoeal effect must allow or promote water absorption in the intestine. The lower doses of the extract showed least frequency of wet stool and moderate water contents indicating that they promote re-absorption of body electrolyte better than loperamide.

Studies have shown that anti-diarrhoeal properties of many plants are due to the presence of plant chemicals like alkaloids and/or tannins (Unaeze *et al*., 2017) and flavonoids and/or phenolics (Dawurung *et al*., 2019). We may plausibly attribute the anti-diarrhoeal effect observed in this plant to tannins (Unaeze *et al*., 2017). Evidently, tannins are known to reduce secretion and reduce the permeability of the intestinal mucosa to water and electrolytes due to their astrigency (Dawurung *et al*., 2019). Although the activity may be due to the presence of other phytochemicals in the extract, especially flavonoids but, the astringent actions of tannins do give ability to precipitate small encoded proteins on inflamed mucous membranes, thereby forming a protective layer over the mucosal lining and protect the underlying mucosa from irritants and toxins (Dawurung *et al*., 2019). Flavonoids have been reported to inhibit prostaglandins and autacoids release resulting in reduction of motility and secretion induced by castor oil (Derebe *et al*., 2018). It is most likely that either tannins or flavonoids present in this plant extract was responsible for inhibition of autacoids and prostaglandins release thereby inhibiting motility and secretion induced by castor oil.

Pre-treatment of the animals with *F. platyphylla* stem extract perhaps ameliorated the irritation and inflammation of intestinal mucosa induced by ricinoleic acid liberated from castor oil, thus leading to decrease in intestinal motility and secretion for an overall reduction in the rate of passage of watery stool. It is also possible that *F. platyphylla* stem extract activated the intestinal Na^+, K^+, ATPase activity to enhance normal fluid absorption, thereby inhibiting the diarrhoea.

Diarrhoea is an effect of numerous physiological and pathological changes. Pathologically, diarrhoea often resulted when some infectious microorganisms are ingested (Raj, Rai and Singh, 2017). The results of *in vitro* anti-diarrhoeal test against *E. coli* (Fig. 3) showed that the plant extract is not active at any dose on the micro organism either typed or wild (Tab. IV). Only NIT, OFL and CPR (Fig. 4) were active among the antibiotics, others could not produce any activity against the laboratory isolate (Tab. IV). The effect of NIT on the clinical isolate was however, remarkable.

CONCLUSION

The results from the present study established a scientific basis for the traditional use of *F. platyphylla* in the treatment of diarrhoea and thus present the mechanism of its action to be physiological. It is therefore recommended that, future research should centre on the isolation and characterization of the physiologically active substances that could be responsible for the anti-diarrhoeal activity of the plant.

Acknowledgements

The authors acknowledged Tertiary Education Trust Fund (TETFund), Nigeria for the financial support. We are thankful to Mr. Dele P., and Mr Thaoban U. U. (PhD student) of the Department of Biochemistry, University of Ilorin for their technical supports during the experiment. We also thank Dr. (Mrs.) Ayanniyi, Department of Pharmacology, University of Ilorin, Ilorin, Nigeria, for consultative supports.

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