EFFECTS OF *PHYLLANTHUS AMARUS* ON FAECAL LOADS OF *SALMONELLA ENTERITIDIS* AND CASTOR-OIL INDUCED DIARRHOEA IN BROILER CHICKENS

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ABSTRACT

A 21 day study on the antibacterial and antidiarrhoeal potentials of methanol extract of Phyllanthus amarus leaf (PAL) in Salmonella enteritidis (SE) and castor oil (CO) induced diarrhoea in broiler chickens was conducted. Seventy-five, 5 week old broiler chickens were randomly allotted to seven treatments; T_1= distilled water, T_2= SE inoculum, T_3= SE + PAL, T_4 = SE + Enrofloxacin, T_5 = CO, T_6 = CO + PAL and T_7 = CO + Loperamide. $T_1 – T_4$ were replicated thrice whereas T_5 to T_7 had 5 birds each. $T_2 – T_4$ received SE orally with T_3 and T_4 continued on PAL for another 4 days. Faeces were collected weekly post infection from $T_1 – T_4$. CO was administered to T_1, T_5, T_6 and T_7 18 hours post-fasting. PAL reduced faecal Salmonella counts as the weeks progressed. PAL inhibited diarrhea better than Loperamide. Therefore, PAL could be used as an antibacterial and antidiarrhoeal in broiler chickens.

Keywords: Broiler chickens, Castor oil, Diarrhoea, *Phyllathus amarus*, *Salmonella enteritidis*

INTRODUCTION

One of the factors affecting poultry production is disease, which causes deviation on state of health and hinders growth and production. Among these diseases is salmonellosis which causes early mortality in young birds and reduces egg production in laying birds (Kabir, 2010). *Salmonella* belongs to the group of enteropathogens that are commercial barriers to poultry products and present extremely important serovars to public health (Marcus *et al*., 2007). In these serovars, *Salmonella enteritidis* stands out as a source of feed toxicant in humans and animals (Fernandes *et al*., 2006).

However, synthetic antibiotic may leave resistance and is also becoming unaffordable for livestock farmers (Ologhobo and Adejumo, 2015). This then necessitates the need to explore plant based alternatives that could effectively replace synthetic antibiotics for food animal use. Medicinal plants are important due to their ability to prevent and treat several human and animal diseases with little or no trail of microbial resistance. Plant extracts are some of the most attractive sources of new drugs and have shown promising results in the treatment of diarrhoea (Regassa, 2013; Mishra *et al*.,

2017). One of such medicinal plant species used widely is *Phyllanthus amarus* (Schumach. and Thonn.). It is widely distributed in tropical and subtropical regions of the planet with about 800 species (Tahseen and Mishra, 2013). Because of its efficacy in the field of gastrointestinal disorders, it is used in the treatment of disorders like dyspepsia, colic, diarrhoea, constipation and dysentery (Devi *et al*., 2017). Lignans like phyllanthin and hypophyllanthin, flavonoid like quercetin were isolated from the leaves of *P. amarus* (Meena *et al*., 2018).

Studies on hexane, chloroform, ethyl acetate, acetone and methanol extracts of *P. amarus* bark demonstrated the antimicrobial activities against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Candida albican* and *Aspergillus flavus* (Ushie *et al*., 2013). The antimicrobial activity of the methanolic extract of *P. amarus* studied by agar dilution method and disc diffusion showed significant concentration-dependent antibacterial activity specifically for gram-negative microbes. It was also observed that antibacterial action was mainly due to the isolated phyllanthin (Mazumder *et al*., 2006).

Castor oil plant (*Ricinus communis* Linn.) is a robust perennial shrub of Euphorbiaceae family and is widespread throughout the tropical regions (Lal and Harini, 2017). It is valuable due to the high content of ricinoleic acid, which is used in induction of diarrhoea (Dunford, 2012; Patel *et al*., 2016). The pharmacological effects of castor oil are mediated by activation of EP_3 receptors on smooth-muscle cells (Tunaru *et al*., 2012).

P. amarus was selected for the study after earlier researches and reviews provided evidences of repeated efficacies of antibacterial and antidiarrhoeal activities in diseased animals. Methanolic extraction of the plant had also demonstrated high quality yields and efficacious extracts in previous researches. The quantity chosen for investigation in this present study has been established as being sub-lethal and therapeutic in animals. More so, earlier studies were conducted on other animals than birds therefore a paradigm shift has been established in this present study. Therefore, this study was aimed at evaluating the antimicrobial and antidiarrhoeal potentials of methanol extract of *P. amarus* in *S. enteritidis*-infected and castor oil induced diarrhoea in broiler chickens.

MATERIALS AND METHODS

Experimental Site and Ethical Consideration: The experiment was carried out at the student's project site of the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Oyo State, Nigeria. Ibadan is located approximately on longitude 3° 5′ to 4° 36′ E and latitude 7° 23′ to 7° 55′ N (Oladele and Oladimeji, 2011). Ibadan has a tropical wet and dry climate, with a lengthy wet season. It has total rainfall of 9,233.60 mm, maximum and minimum temperatures of 39.82 °C and 22.5 °C respectively (Egbinola and Amobichukwu, 2013) and relative humidity of 74.55 %.

Ethical conditions governing the conduct of experiments with life animals were strictly observed as stipulated by Ward and Elsea (1997) and NIH guide for care and use of laboratory animals (NIH, 1985).

***Phyllanthus amarus* Extract:** *P. amarus* leaves were collected from the Federal College of Animal Health and Production Technology Botanical Garden. The plant was identified (Akobundu *et al.,* 2016) and authenticated by a plant taxonomist and voucher specimen (FCAH&PT/SLT/2019/021) was kept in the institutional herbarium for referral purposes.

The harvested fresh leaves were washed, rinsed with distilled water and air dried under shade to a constant weight. The dried leaves were ground with domestic electric grinding machine (Sonik Model SB-464) to produce *P. amarus* leaf meal (PALM). The PALM was subjected to 80 % methanol extract maceration technique by putting one hundred grams (100 g) of the PALM in 500 mL of 80% methanol at room temperature for 72 hours while shaking intermittently with rotary shaker. The extract was filtered through a muslin cloth and Whatman filter paper No. 1. The extract was placed in a beaker and evaporated by placing it inside the water bath at 45 °C for 3 days to obtain a thick concentration. The

resulting dry hydro-alcoholic extract was weighed and percentage yield calculated.

Toxicity and Phytochemical Assay of *Phyllanthus amarus:* The lethal toxicity test of *P. amarus* was adopted from Adomi *et al*. (2017), while the phytochemical screening of *P. amarus* extract was adopted from Obianime and Uche (2008).

***Salmonella enteritidis*:** *S. enteritidis* was obtained from Fish and Wild Life Laboratory, Department of Veterinary Medicine, University of Ibadan. *S. enteritidis* strain was got by overnight culture on modified Luria-Bertani (LB) agar plates at 37 °C. *Salmonella* strains from LB plates were inoculated into 50 ml of LB broth and grown standing overnight at 37°C. Cultures were harvested by centrifugation at 16,000 rpm for 10 minutes at room temperature and washed once with phosphate-buffered saline (PBS), pH 7.4 (Day *et al*., 2009).

Castor oil, Loperamide and Enrofloxacin: These were sourced from Danax Pharmacy, Mokola, Ibadan.

Birds, Management and Design: Seventy five (75) day-old unsexed Arbor acre broiler chicks were used for the study. Prior to the arrival of the birds, the pens were cleaned and washed with detergent solutions. Disinfection of the pen was done using saponated cresol (Lysol), rested for one week and the floor litter laid to 5 cm^3 with wood shavings. On arrival of the chicks, anti-stress solution (mixture of water, glucose and multivitamin) was served as well as normal feed (Starter Top Feed, 22 % CP, 2800 kcal/kg) and borehole water *ad libitum*. Routine vaccinations (Newcastle disease vaccine (NDV) i/o, Lasota and Infectious bursal disease (IBD) were administered accordingly during the two weeks of acclimatization. IBD vaccine was repeated on day fourteen. After acclimatization, the birds were allocated to seven treatments in a completely randomized design. Groups T_1 – T_4 were replicated thrice with 5 birds per replicate (60 birds) whereas T_5 – T_7 had 5 birds per treatment (15 birds). Measured quantity of Starter Top Feed (0 – 4 weeks old, 22 % CP and 2800 kcal/kg metabolizable energy) and Finisher Top Feed (>4 weeks, 19 % CP, 3200 kcal/kg) were given by 7 am and 5 pm daily whereas clean borehole water was supplied *ad libitum* throughout the experimental duration of 8 weeks under standard environmental conditions (12 our light/dark cycle). Also, coccidiostat was given when the birds showed signs of coccidiosis at week four. The experimental dosing and grouping were as stated hereunder: T_1 = Distilled water (control), T_2 = *S. enteritidis* inoculated (1 x 10^7 cfu, PO), T_3 = *S. enteritidis* inoculated + *P. amarus* (150 mg/kg), T_4 = *S. enteritidis* inoculated + Enrofloxacin (10 mg/kg), T_5 = Castor oil only (2 mL/bird), T_6 = Castor oil + *P. amarus* (150 mg/kg) and T_7 = Castor oil + Loperamide (1 mg/kg).

In T_2 – T_4, inoculation of *S. enteritidis* was done at 5 weeks of age. One hour prior to the inoculation, T_3 and T_4 received *P. amarus* (150 mg/kg) and Enrofloxacin (10 mg/kg) respectively. There was continued administration of *P. amarus* and Enrofloxacin for another 4 days (i.e. 5 days in all) in T_3 and T_4. The sub lethal dosage of *P. amarus* used in this study was derived by dividing the non-acute toxicity value of 8,000 mg/kg (Adomi *et al*., 2017) by a factor (53.33).

Faecal samples were collected via the cloacae at weeks 1, 2 and 3 post-infection.

Data Collection and Laboratory Analyses

a) Faecal Sample Microbiology: One (1 g) of faecal sample was put into test-tube and 9 ml of sterile distilled water was added, serial dilution was then done on the next four test-tube to reduce the microbial load in which fifth test-tube was used to culture *S. enteritidis* on the Xylose lysine deoxycholate (XLD) agar and the plates were incubated in an incubator at 37 ^oC for 18 to 24 hours (ISO, 2018).

b) Castor Oil Induced Diarrhoea Model: The study was carried out according to the method described by Yadav and Tangpu (2007). Birds were fasted for 18 hours with free access to water before the antidiarrhoeal test. After 1 hour of treatment with PALM (T_6) and

Loperamide (T_7), diarrhoea was induced by the administration of 2 mL of castor oil orally to each bird while T_5 served as positive control (2 mL of castor oil given orally/bird) and T_1 as negative control (given distilled water). The birds were housed individually in battery cages, the bottom of which was lined with white polythene sheet for observation of the number and consistency of faecal droppings. The polythene was changed every hour to make the faecal droppings visible for counting and to check stool consistency. During the 4 hours observation period, the onset of diarrhoea, the number and weight of both wet and dry stools excreted by the birds were recorded and compared with the control for assessment of antidiarrhoeal activity. The onset was measured as the interval (minutes) between the administration of castor oil and the appearance of the first watery stool (Akter *et al.*, 2009). The percentage (%) inhibition of defecation was measured using the following formula:

$$\% \text{ inhibition of defecation} = (A-B)/A \times 100$$

where A is the mean number of faecal droppings caused by castor oil and B the mean number of faecal droppings caused by drug or extract. Percentage inhibition of fluid accumulation was calculated using the understated formula:

$$\% \text{ inhibition of fluid accumulation} = (C-D)/C \times 100$$

where C is the mean wet weight of faeces produced due to castor oil induction of diarrhoea and D is the mean wet weight of faeces produced by test ingredients (PALM or Loperamide).

Data Analysis: All data obtained were subjected to analysis of variance (ANOVA) using a Statistical Package for Social Sciences (SPSS) version 20.0. Significantly different means were separated using Duncan's New Multiple Range Test (DNMRT) as described by Obi (2002). Whereas the data on castor oil induced diarrhoea model were further subjected to inferential statistics as well.

RESULTS AND DISCUSSIONS

Toxicity and Phytochemical Contents of *Phyllanthus amarus:* The acute toxicity study (Adomi *et al.*, 2017) revealed that the oral administration of the extract was safe up to the dose level of 8,000 mg/kg. The dosage of 150 mg/kg used in this study was non-toxic to the birds administered. The phytochemical contents of *P. amarus* (Obianime and Uche, 2008) indicated the presence of alkaloids, flavonoids, terpenoids, saponins, tannins, steroid and cardiac glycosides (Table 1).

Table 1: Phytochemical composition of *Phyllanthus amarus*

Phytochemicals	*P. amarus*
Alkaloids	+
Flavonoids	+
Terpenoids	+
Saponins	+
Tannins	+
Steroid	+
Resins	-
Cardiac glycosides	+

Legend: + = present, - = absent, Source: Obianime and Uche (2008)

These phytochemicals conferred on the extract the antioxidant and antimicrobial potencies (Adegoke *et al.*, 2010; Eldeen *et al.*, 2011).

***Salmonella enteritidis* Loads in the Faeces of Broiler Chickens on *Phyllanthus amarus* Leaf Extract:** The faecal titres of *S. enteritidis* in the broiler chickens during 1st, 2nd and 3rd weeks post administration of *P. amarus,* enrofloxacin and *S. enteritidis* indicated a significant rise ($p<0.05$) in *S. enteritidis* load of T_2 compared to other groups (T_1, T_3 and T_4) throughout the study period (Table 2). The *S. enteritidis* titre of T_2 decreased during the 2nd week possibly due to initial immune reaction against the inoculum which was probably overwhelmed in the 3rd week no wonder a spike in titre. However, in the 3rd week, group T_1 differed ($p<0.05$) from T_3 and T_4 suggesting a probable unmitigated growth of *S. enteritidis* in T_1 since it had no antibacterial supplement, though less than observed in T_2 ($p<0.05$).

Table 2: *Salmonella enteritidis* loads in the faeces of broiler chickens on *Phyllanthus amarus* leaf extract

Weekly Collection	*Salmonella enteritidis* load (x 10^7 cfu/g)			
	T_1	T_2	T_3	T_4
1st week	27.67 ± 11.31[b]	91.33 ± 1.41[a]	46.67 ± 10.61[b]	47.00 ± 16.97[b]
2nd week	42.33 ± 20.51[b]	84.00 ± 7.07[a]	38.33 ± 12.73[b]	25.67 ± 9.90[b]
3rd week	52.67 ± 13.40[c]	95.67 ± 6.18[a]	26.33 ± 8.65[b]	15.00 ± 3.14[b]

T_1= Distilled water, T_2= SE inoculum, T_3= SE + PAL, T_4, = SE + Enrofloxacin, SE = Salmonella enteritidis, PAL = Phyllanthus amarus leaf extract, means on the same row with different letter superscripts are significantly different ($p<0.05$)

Numerically, there were progressive increases in the titre of *S. enteritidis* in T_1 as the weeks progressed possibly because there was no medicinal intervention unlike T_3 and T_4 where gradual decreases in titre were recorded as the weeks progressed from 1st to the 3rd possibly due to anti-microbial activity of *P. amarus* and enrofloxacin. Many authors have demonstrated the antimicrobial properties of *P. amarus* (Adegoke *et al*., 2010; Eldeen *et al*., 2011; Njoroge *et al*., 2012; Saranraj and Sivasakthivelan, 2012; Ushie *et al*., 2013; Babatunde *et al*., 2014; Meena *et al*., 2018). However, there was no statistical difference ($p>0.05$) in the inhibition of the growth of *S. enteritidis* between *P. amarus* and enrofloxacin throughout the study period although numerically, *P. amarus* was marginally better in the 1st week whereas enrofloxacin had greater inhibition in the last two weeks. Previous studies reported that the presence of alkaloids, terpenoids, glycosides steroids and proteins may be responsible for the antibacterial properties of plant extracts (Nazemiyeh *et al*., 2008; Al Akeel *et al*., 2014). Indeed, most of the metabolites detected in the *P. amarus* extract (saponins, flavonoids, tannins, alkaloids and terpenoids) are well known to have significant inhibitory action against bacteria and fungi (Hayek *et al*., 2013). Although the mechanisms of action for natural products are distinct, the cytoplasmic membrane ranks as the most common site of action for secondary metabolites. They usually act through cell lysis, triggering the leakage of cellular contents and consequently cell death (Da Silva *et al*., 2013). The interaction with genetic material and protein synthesis is also a possible factor regarded to the promotion of the therapeutic action.

In this case, when there is a contact with the genetic material, the compound is able to promote changes in the genetic machinery, which result in ineffective transcription and disturbance of vital functions for the cell (Hayek *et al*., 2013; Gyawali and Ibrahim, 2014). The phenolic compounds (polyphenols, tannins and flavonoid) can act at two different levels: the cell membrane and cell wall of the microorganisms (Taguri *et al*., 2006). They can also penetrate into bacterial cells and coagulate cell content (Tian *et al*., 2009). The antimicrobial property of saponins is due to a lipophilic portion into its structure (aglycon or sapogenin) and a hydrophilic core comprising one or more sugars (Costa *et al*., 2010). Tannin mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins, and so forth (Cowan, 1999). Vrieze *et al*. (2010) showed that the gastrointestinal tract microflora play an important role in the health status of host as it contributes to the overall metabolism and physiology and plays a role in converting food into nutrients and energy.

Inhibition of Diarrhoea by *Phyllanthus amarus* Leaf Extract on Castor oil-induced Diarrhoea in Broiler Chickens: The number of times, intervals of defecation and percentage inhibition of diarrhoea in different treatments after the administration of castor oil are presented in Table 3. With respect to number of droppings, T_5 was significantly higher ($p<0.05$) than T_6 and T_7 and further differed ($p<0.05$) from T_1 which was the least. There was statistical similarity ($p>0.05$) among T_5, T_6 and T_7 in terms of mean defecation intervals although T_6 had the longest interval, suggesting that *P. amarus* had more potential to stop diarrhoea in chicken relative to Loperamide.

Table 3: Inhibition of diarrhea by *Phyllanthus amarus* leaf extract on castor oil-induced diarrhoea in broiler chickens

Treatments	Number of droppings in 4 hours	Mean defecation intervals in 4 hours	% inhibition of diarrhea
T_1	2^c	56.00 ± 0.00^d	0^a
T_5	14^a	17.14 ± 4.31^a	0^a
T_6	8^b	30.00 ± 8.32^c	42.86^c
T_7	10^b	20.80 ± 3.31^b	28.57^b

T_1= Distilled water, T_5 = CO, T_6 = CO + PAL, T_7 = CO + Loperamide, CO = Castor oil, SE = Salmonella enteritidis, PAL = Phyllanthus amarus leaf extract, means on the same row with different letter superscripts are significantly different ($p<0.05$)

Moreso, the percentage inhibition of diarrhoea for T_6 (42.86 %) was higher than T_7 (28.57 %). This further lent credence to the above assertion that *P. amarus* had greater ability to stop diarrhoea than the standard drug (Loperamide). The results equally demonstrated that castor oil actually induced diarrhoea in the broiler chickens no wonder T_5 had the highest frequency of defecation than other treatments. Castor oil is diarrhoeagenic in animals (Patel *et al*., 2016). With respect to interval of defecation, it was evidenced that T_6 took longer mean time than T_7 for defecation to occur, corroborating a more anti-diarrhoeic potential of *P. amarus*. Antidiarrhoeal properties of medicinal plants were found to be due to tannins, flavonoids, alkaloids, saponins, reducing sugar, sterols and/or terpenes (Otshudi *et al*., 2000; Venkatesan *et al*., 2005).

Tannins present in plant, denature proteins in the intestinal mucosa forming protein tannate complex. The complex forms a coat over the intestinal mucosa and makes the intestinal mucosa more resistant to chemical alteration and reduces secretion (Israili and Lyoussi, 2009; Pandey *et al*., 2012). Studies on the functional role of tannins also revealed that they can also reduce the peristaltic movements and intestinal secretions by reducing the intracellular Ca^{2+} inward current or by activation of the calcium pumping system (which induces the muscle relaxation) (Al-Taher, 2008) attributed to spasmolytic and calcium channel blocking (CCB) activities of tannins present in the plant extract (Gilani *et al*., 2013). In addition, flavonoids present antioxidant properties which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism (Agbon *et al*., 2013), thus, reducing prostaglandin induced fluid secretion. Again it is likely that the enhanced electrolyte absorption by the extract may have encouraged the absorption of other intestinal solute contents like nutrients that in turn may have created an osmotic gradient across enterocytes which stimulated water absorption (Ezenwali *et al*., 2010). The present finding is in consonance with the results of Saranraj and Sirasakthivelan (2012) who reported susceptibility of many bacteria to *P. amarus* as well as Njoroge *et al*. (2012) and Meena *et al*. (2018) whose studies revealed inhibitory activities of *P. amarus* and *Phyllanthus niruri* extracts against some *E. coli* isolates.

Effect of *Phyllanthus amarus* Leaf Extract on Enteropooling in Broiler Chickens: The effect of *P. amarus* leaf extract on enteropooling in castor oil-induced diarrhoea in broiler chickens indicated that Group T_6 produced better inhibition of fluid accumulation (31.95 %) compared to the standard drug (30.38 %) (Table 4). There was numerical reduction in faecal wet weight of T_6 (69.72 g) compared to T_7 (71.33 g), whereas the volume of fluid lost due to diarrhoea was greater in T_7 (25.35 mL) than T_6 (25.10 mL). Inference can therefore be drawn that *P. amarus* exhibited a more profound anti-diarrhoeic potential than Loperamide by possibly suppressing the peristaltic movement of the intestine and/or influenced the Na^+ -K^+ ion pump to reduce water influx into the intestinal lumen. Thus one possible anti-diarrhoeic activity of the extract against castor oil induced diarrhoea may be attributed to its anti-electrolyte permeability action (Rode *et al*., 2013).

Table 4: Enteropooling caused by *Phyllanthus amarus* leaf extract in castor oil-induced diarrhoea in broiler chickens (4 hour interval)

Treatments	Number of droppings in 4 hours	Wet weight (g)	Dry weight (g)	Water loss (mL)	% inhibition of fluid accumulation
T_1	2^a	56.20 ± 0.40^a	42.30 ± 0.38	13.90 ± 0.73^a	0^a
T_5	14^c	102.46 ± 0.57^b	46.70 ± 0.38	55.76 ± 0.66^c	0^a
T_6	8^b	69.72 ± 0.40^a	44.62 ± 0.56	25.10 ± 0.93^b	31.95^b
T_7	10^b	71.33 ± 0.56^a	45.98 ± 0.63	25.35 ± 0.40^b	30.38^b

T_1= Distilled water, T_5 = CO, T_6 = CO + PAL, T_7 = CO + Loperamide, CO = Castor oil, SE = Salmonella enteritidis, PAL = Phyllanthus amarus leaf extract, means on the same row with different letter superscripts are significantly different ($p<0.05$)

Usually castor oil is metabolized into ricinoleic acid in the gut, which causes irritation and inflammation in the intestinal mucosa and results in the release of inflammatory mediators (e.g. prostaglandins and histamine). The released prostaglandins initiate vasodilatation, smooth muscle contraction, and mucus secretion in the small intestines. In animals as well as human beings, prostaglandins of the E series (prostaglandin E_1 also known as alprostadil and prostaglandin E_2 also known as dinoprostone) are considered to be good diarrhoeagenic agents (Rahman *et al*. 2015). It has also been established that Loperamide inhibits diarrhoea even when induced by castor oil (Bahekar and Kale, 2015). It was reported that flavonoids and polyphenols were responsible for the antidiarrhoeic properties of *P. amarus* (Dosso *et al*., 2011). Moreover, *in vivo* and *in vitro* tests have shown that flavonoids are able to inhibit prostaglandin E_2 induced intestinal secretion; spasmogens induce contraction and also inhibit release of prostaglandins and autocoids (Dosso *et al*., 2011). The antidiarrhoeal activity of the leaves of *P. amarus* could therefore be ascribed to the presence of flavonoids. Several studies have validated the use of antidiarrhoeic medicinal plants by investigating the biological activities of plant extracts, which include antispasmodic effects, delay intestinal transit, suppress gut motility, stimulate water reabsorption and reduce intraluminal fluid accumulation (Ezeja *et al*., 2012; Pérez-Gutiérrez *et al*., 2013). Similar to *P. amarus* used in this study, Tadesse *et al.* (2014) used methanolic leaf extract of *Zehneria scabra* in mice and achieved a reduced fecal output produced by castor oil.

Conversely, Yacob *et al*. (2016) had 42.40 % inhibition in intestinal fluid accumulation using 400 mg/kg of extract of the aerial part of *Ajuga remota* Benth and 66.6 % with Loperamide HCl (5 mg/kg) probably because a different leaf (*P. amarus*) was used that might not have equal quantity of flavonoid and other antidiarrhoeic constituents. Similar to the present study, Chauhan and Sharma (2018) used 400 mg/kg of *P. amarus* to achieve a statistically significant reduction in the severity and frequency of diarrhoea produced by castor oil in Wistar rat.

Conclusion: It can be concluded that methanolic extract of *P. amarus* at 150 mg/kg was able to inhibit the growth of gut *S. enteritidis* similar to enrofloxacin and as well suppressed diarrhoea in castor oil induced diarrhoea even greater than the standard drug Loperamide. Since natural products have little or no side effect on biological systems, *P. amarus* leaf is recommended as a potent alternative to synthetic chemotherapeutics with respect to inhibition of growth of gut SE and remediation of diarrhoea in broiler chickens.

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