http://dx.doi.org/10.4314/jpb.v12i1.5
Vol. 12 no. 1, pp. 30-37 (March 2015)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Evaluation of the antidiarrhoeal activity of the aqueous leaf extract of *Dialium guineense* in mice

Zuleikha A.M. Nworgu* and Azuka Ifebuna

Department of Pharmacology, Faculty of Pharmacy, University of Benin, Benin City. Nigeria.

Received 19th February 2015; Accepted 31st March 2015

Abstract
Diarrhoea has been recognized as being responsible for high mortality rate among the population especially in infants. Many cases of diarrhoea are fortunately, acute in nature and respond well to a number of well tried remedies including traditional remedies. The leaves of *Dialium guineense* have been used traditionally in the treatment of diarrhoea. The aim of this study was to evaluate the antidiarrhoeal activity of *D. guineense* leaf extract in mice using different experimental model. The results showed that the aqueous leaf extract of *D. guineense* caused a significant ($P< 0.001$) reduction in the number of stools and frequency of diarrhoea in castor oil induced diarrhoea in mice. The extract produced significant ($P< 0.01$) inhibition of intestinal transit with the dose of 400 mg/kg having the highest effect. The extract also produced a dose dependent and significant ($P< 0.01$) reduction in volume and weight of fluid accumulated in the GIT compared to the control. It was thus concluded that the aqueous leaf extract of *D. guineense* possesses significant anti-diarrhoeal activity probably due to its effect of decreasing GIT motility and fluid accumulation.

Keywords: Diarrhoea; Rats; Mice; *Dialium guineense*, Castor oil

INTRODUCTION

Dialium guineense is a tree 30 m high, with a densely leafy crown, but often shrubby bole without buttress. The bark is smooth, grey, slash reddish, yielding a little red gum. Leaves sometimes finely hairy, with a common stalk of 5-13 cm long and also has an odd terminal leaflet. The leaflet usually consists of 2 pairs of opposite or alternate leaflets, the lower pair being somewhat smaller (Hong *et al.*, 1996) *Dialium guineense* is used traditionally for the treatment of different ailment. The roots are used for the treatment of heart disease which might be attributed to the presence of tannins found in it (Lawrence *et al.*, 1997; Shi *et al.*, 2010). It has also been shown that they inhibited the growth of *Plasmodium falciparum* which is the causative agent of malaria (Hermans *et al.*, 2010). The roots and leaves of *D. guineense* has been used traditionally for the prevention of dental plague and studies have shown that the presence of flavonoids is responsible for this activity (Ammar *et al.*, 1990). *D. guineense* is used also as chewing stick and aids the cleaning of the teeth as well as preventing decay (Ammar *et al.*, 1990).

The leaf extract has also exhibited significant antimicrobial properties (Akinpelu *et al.*, 2004). The leaves boiled in water or palm wine have been used for the treatment of

* Corresponding author. *E-mail*: zamnworgu@gmail.com *Tel*: +234 (0) 8055158952
ISSN 0189-8442

diarrhoea, dysentery, hemorrhoid's, inflamed or ulcerated tissues (Dharmanda, 2003). The aim of this study is to investigate the anti diarrhoeal activity of the leaves of this plant so as to justify its use for the treatment of diarrhoea.

EXPERIMENTAL

Plant material. The fresh leaves of *D. guineense* were collected from the environs of the University of Benin Ugbowo campus on the 5th of June 2012. The plant was identified by Mr. Sunny Nweke of the Department of Pharmacognosy, University of Benin. The leaves were dried in the shade and then transferred into the drying oven set at 50º C. The dried leaves were pulverized into fine powder using the impact mill. The fine powder was weighed and kept for further analysis.

Animals. Mature female albino rats weighing between 250-300 g and male mice weighing between 25-40 g were obtained from the animal house of the Department of Pharmacology, University of Ibadan. The animals were maintained under standard laboratory condition with free access to feed and water. Animals were acclimatized for two weeks before use and fasted overnight with free access to water prior to experiments.

Extraction of plant material. Dried powdered leaves of *D. guineense* weighing 800 g were placed in a 5 litre flat bottom flask and 2.5 litres of hot distilled water poured into it. The mixture was soaked for 24 hours, it was decanted and filtered The filtrate was then concentrated by evaporation to dryness over a hot water bath to form a paste and dried using Gallenkamp hot oven set at 50º C. The percentage yield of the extract was determined. The dried extract was stored in the refrigerator prior to use.

Pharmacological screening (Antidiarrhoeal studies).

Effect of extract on faecal matter in mice. Twenty five mice were allotted to five groups of five mice each. The mice were administered orally the extract (100 mg/kg, 200 mg/kg, and 400 mg/kg), loperamide (5 mg/kg) & distilled water (0.2 ml) which served as the control. Each mouse was placed in a metal cage, the floor of which was lined with blotting paper and observed for 4 hours. The time taken for the first stool and number of stools produced were recorded.

Castor oil induced diarrhoea test model. Twenty five mice were allotted to five groups of five mice each. The mice were administered orally the extract (100 mg/kg, 200 mg/kg, and 400 mg/kg), loperamide (5 mg/kg) and distilled water (0.2 ml). One hour later, castor oil (0.3 ml) was administered orally to each mouse. Each mouse was placed in a metal cage, the floor of which was lined with plain blotting paper and observed for 4hours. The parameters observed were; the onset of diarrhoea, number of wet stools, and total weight of faecal matter.

Gastrointestinal motility test model. Twenty five mice were allotted to groups of five animals each. Animals were treated orally with the extract (100 mg/kg, 200 mg/kg, and 400 mg/kg), loperamide (5 mg/kg) and distilled water (0.2 ml)). After 30 minutes, each mouse was given 0.3 ml of 10 % charcoal suspension orally. All animals were sacrificed an hour after the administration of the charcoal meal and the small intestine immediately isolated. The distance travelled by the charcoal meal from pylorus to the ileo-caecal junction was measured and expressed as the percentage of the total length of the intestine.

Isolated tissue preparation test model. Mature rats were used in this experiment. The rats were sacrificed by cervical dislocation, their abdomens were cut opened and the ileum isolated. About 2 cm piece of the ileum were cut, the fat was trimmed and the tissue set in a 40ml organ bath containing tyrode

solution. The tissue was connected to the isometric transducer and the contractions were recorded in a single channel Ugo Basile recorder. The tyrode solution was bubbled with a mixture of 95 % oxygen and 5 % carbon (IV) oxide and maintained at a temperature of 37◦C. Each tissue used was allowed to equilibrate for 20 minutes in the tyrode solution before the addition of any drug. When the tissue was equilibrated, the effects of 7.5 mg/ml and 15 mg/ml of the aqueous extract on the graded doses of acetylcholine induced contraction were examined. These were compared with the effect of 5 µg/ml of atropine on acetylcholine induced contractions.

Castor oil induced enteropooling test model. Twenty five mice weighing between 25-40 g were allotted into five groups of five mice. Group 1 received (0.2 ml) of distil water, groups 2, 3 and 4 rats, received the extract 100 mg/kg, 200 mg/kg and 400 mg/kg respectively, while group 5 rats received loperamide (5 mg/kg) per oral. Thirty minutes after the administration of the above treatments each mouse received castor oil (0.3 ml) intragastrically. The animals were sacrificed an hour later; the entire small intestine was removed after ligation at the pyloric end and ileo-caecal junction respectively and weighed using an electronic balance. The intestinal content was expelled into a graduated tube and the volume measured. The intestine was reweighed and the difference between full and empty intestine was calculated to give the weight of the intestinal content.

Statistical analysis. Statistical analysis for animal experiments were carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the control group. $P<0.05$ was considered to be statistically significant.

RESULTS

Yield determination. The percentage yield for the aqueous extract of *D. guineense* was 31.25 %

Effect of the aqueous leaf extract of *Dialium guineense* on faecal matter. There was no significant reduction in the number of stools produced on treatment with 100 mg/kg of the extract of *Dialium guineense* as compared to distilled water (control). On increasing the dose to 200 mg/kg and 400 mg/kg respectively there was a marked decrease in the number of stools produced which was dose dependent. The dose of 200 mg/kg extract reduced the stools from an average of 3.5 ± 0.7 to 1.5 ± 0.3. Increasing the dose to 400 mg/kg extract further reduced the number of stool to an average of 1.0 ± 0.1. Loperamide the positive control agent completely inhibited the formation of stools (Table1).

Effect of the aqueous leaf extract of *Dialium guineense* on castor oil induced diarrhea. There was a marked and dose dependent delay in the time of onset of diarrhoea on treatment with the three doses of the extract of *D. guineense*. 100 mg/kg of extract increased time of onset of diarrhoea from 45.0 ± 4.3 min to 65.0 ± 0.3 min as compared to the control while the dose to 400 mg/kg increased the time of onset to 100.0 ± 4.2 min. Onset of diarrhea with loperamide, the standard was 120 $\pm$ 2.0 min which was significant ($P< 0.001$) Table 2. There was a significant ($P < 0.001$) dose dependent reduction in the number of stools produced after 4 hours on treatment with the three doses of the extract of *Dialium guineense* when compared to the control. 100 mg/kg of extract reduced the number of stools produced after 4hours from an average of 8.2 ± 0.4 to 6.0 ± 0.3, increase in the dose of extract to 200 mg/kg produced a reduction in the number of stools to 4.5 ± 1.0 while the dose of 400 mg/kg further reduced the number of stools to

an average of 0.5 ± 0.0. Loperamide, the standard inhibited production of stools completely. (Figure 1)

Effect of the aqueous leaf extract of *Dialium guineense* on enteropooling in mice. There was a significant and dose dependent decrease in weight and volume of GIT on treatment with three doses of the extract of *D. guineense* as compared with distilled water (control). A dose of 100 mg/kg extract reduced the weight of the GIT content from an average of 0.74 ± 0.36 g to 0.40 ± 0.05 g and the volume of GIT content from 0.13 ± 0.04 ml to 0.12 ± 0.08 ml. The dose of 200 mg/kg extract reduced the weight of GIT content from an average of 0.74 ± 0.36 g to 0.26 ± 0.08 g and the volume of GIT content to 0.13 ± 0.04ml to 0.11 ± 0.02 ml. At the dose of 400 mg/kg there was a significant ($P < 0.01$) decrease in the weight of the GIT content from 0.74 ± 0.36 g to 0.19 ± 0.01 g and volume of GIT content from 0.13 ± 0.04 ml to 0.01 ± 0.10 ml. Loperamide the positive control agent caused a significant ($P < 0.001$) decrease in the weight of the GIT content from 0.74 ± 0.36 g to 0.10 ± 0.01 g and the volume of GIT content from an average of 0.13 ± 0.04 ml to 0.003 ± 0.002 ml. (Table 3)

Effect of the aqueous leaf extract of *Dialium guineense* on intestinal transit time in mice. In the control group the charcoal meal traversed 84.8 ± 1.9 % of the total length of the intestine. Treatment with the three doses of the extract of *D. guineense* decreased the distance traversed by the charcoal meal and their effects were dose dependent. A dose of 100 mg/kg extract significantly ($P < 0.001$) reduced the percentage distance traversed by the charcoal meal from 84.8 ± 1.9 cm to 37.6 ± 0.7 cm (54 % inhibition), while 200 mg/kg caused significant ($P < 0.001$) reduction from 84.8 ± 1.9% to 33.6 ± 0.2 % (58.9 % inhibition). Increasing the dose to 400 mg/kg produced further reduction in the percentage traversed by the charcoal from 84.8 ± 1.9% to 32.2 ± 1.3% (60.6 % inhibition). Loperamide, the positive control produced the highest decrease in the percentage traversed by the charcoal 84.8 ± 1.9 % to 8.8 ± 0.9 % (89.3 % inhibition) Fig. 2.

Effect of aqueous leaf extract of *Dialium guineense* on the isolated ileum of a rat. The aqueous extract of *Dialium guineense* showed a dose dependent inhibitory effect on acetylcholine induced contractions. 7.5 mg/ml (final bath concentration) of the extract did not significantly ($P > 0.05$) caused an inhibition on the graded doses of acetylcholine induced contraction but increasing the dose to 15 mg/ml (final bath concentration) produced a significant ($P < 0.001$) inhibitory effect on the graded doses of acetylcholine induced contraction of the rat ileum. The graded doses of acetylcholine used ranged from 5 µg/ml to 5000 µg/ml. Atropine; the positive control (25 µg/ml) produced a much greater decrease in acetylcholine contraction. The extract gave a shift to the right of acetylcholine curve similar to atropine (Fig 3).

Table 1: Effect of the aqueous leaf extract of *Dialium guineense* on the faecal matter of mice

Treatment	Number of stool
Control (2 ml)	3.5 ± 0.7
Loperamide (5 mg/kg)	0
Dialium guineense (100 mg/kg)	3.5 ± 0.3
Dialium guineense (200 mg/kg)	1.5 ± 0.3[a]
Dialium guineense (400 mg/kg)	1.0 ± 0.1[a]

[a]$P < 0.01$ significantly different from the control

Table 2: Effect of the aqueous leaf extract of *Dialium guineense* on onset of diarrhoea in castor oil induced diarrhoea model.

Treatment	Onset of diarrhoea (min)
Control (2 ml)	45.0 ± 4.2
Loperamide (5 mg/kg)	120.0 ± 2.0[a]
Dialium guineense (100 mg/kg)	65.0 ± 0.3 [a]
Dialium guineense (200 mg/kg)	73.1 ± 5.3 [a]
Dialium guineense (400 mg/kg)	100 ± 4.2 [a]

[a]P < 0.01 significantly different from the control

Table 3: Effect of the aqueous leaf extract of *D. guineense* on castor oil induced enteropooling in mice

Treatment	Weight of G.I.T content (g)	Volume of G.I.T content (ml)
Control (2 ml)	0.74 ± 0.36	0.13 ± 0.04
Loperamide (5 mg/kg)	0.10 ± 0.01	0.003 ± 0.02[a]
Dialium guineense (400 mg/kg)	0.19 ± 0.01	0.01 ± 0.10[b]
Dialium guineense (200 mg/kg)	0.26 ± 0.08	0.11 ± 0.02[b]
Dialium guineense (100 mg/kg)	0.40 ± 0.05	0.12 ± 0.08

[a]P< 0.001 and [b]P< 0.01 significantly different from the control. G.I.T --Gastrointestinal Tract

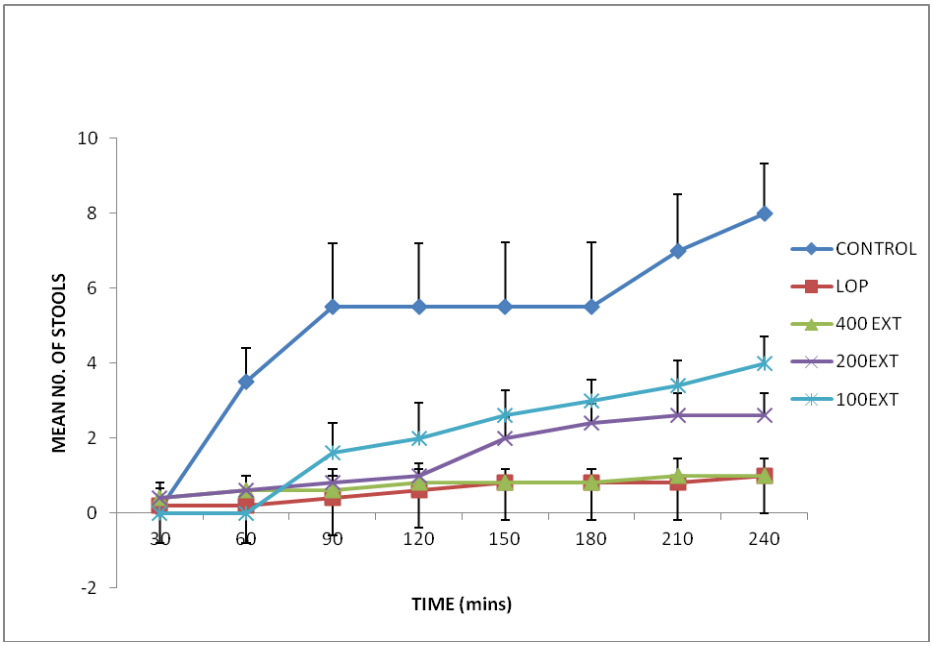

Figure 1: Effect of the aqueous extract of *D. guineense* on castor oil induced diarrhea against time in mice . n=5, Lop - Loperamide (Standard), Control-Distilled water, EXT –Extract, P < 0.01

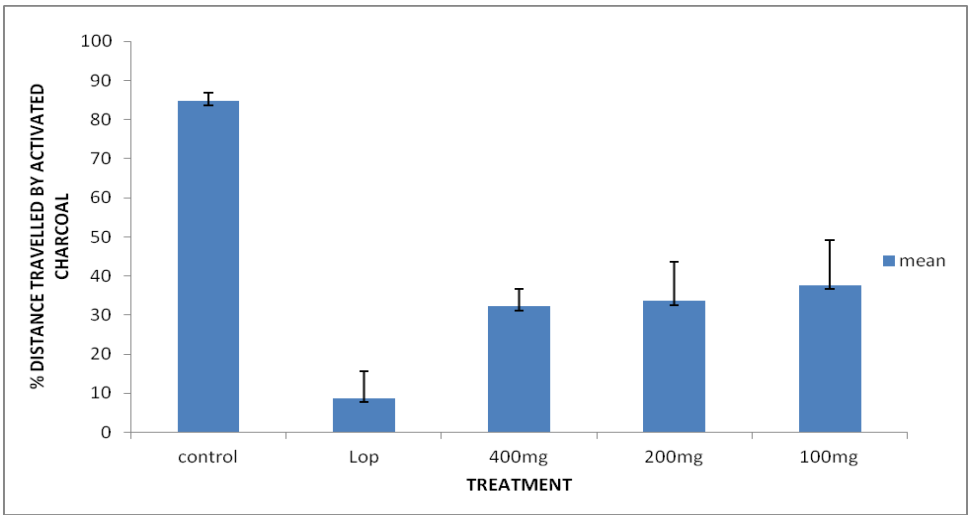

Figure 2: Effect of the aqueous extract of *Dialium guineense* on gastrointestinal motility of mice. n = 5, Control - Distilled water, Lop -Loperamide , P < 0.001.

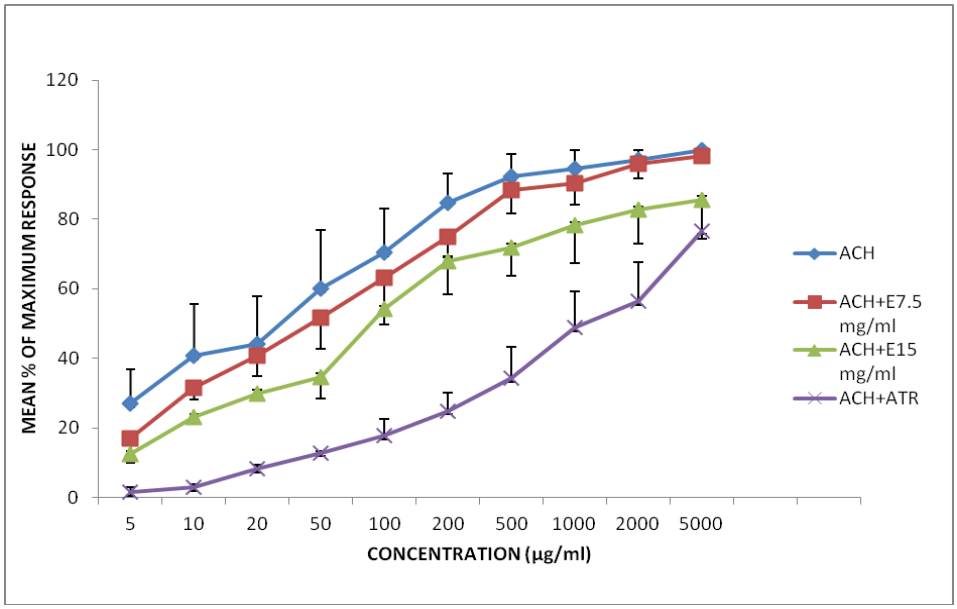

Figure 3: Effect of aqueous leaf extract (EXT) of *Dialium guineense* on the acetycholine (ACH) contractions of the ileum of the rat. This was compared with effect of atropine (ATR) on ach contractions N =5, (P < 0.001) significantly different from the control

DISCUSSION

Diarrhoea is a condition of having three or more loose or liquid bowel movements per day (WHO, 2005). Diarrhoea is also the frequent passage of liquid faeces (Rang *et al*., 2003). Diarrhea has two basic pathophysiological mechanisms; (a) Increase in the motility of the gastrointestinal tract, along with increased secretion (Fields *et al*., 1989) (b) Decrease in the absorption of fluid that leads to a loss of electrolytes (particularly sodium ion) and water (Rang *et al*., 2003). These result from alterations in gastrointestinal functions brought about by different causative agents such as; bacterial, viral enteritis, genetic disorders, metabolic disorders, environmental factors etc (Gabriel, 2000). The increased secretion and decreased

absorption of fluid causes an increase in stool volume and weight which is increase in bulk that cause increase in peristalsis and decreased transit time as seen in patients with diarrhea.

The aqueous leaf extract of *D. guineense* produced a significant ($P < 0.001$) reduction in the severity of diarrhea (frequency in stooling) produced by castor oil. There was a delay in the onset of diarrhea, a significant reduction in the total number of wet stools and total weight of stools; which suggest that the aqueous extract of *D. guineense* has a potent anti-diarrheal activity.

The extract produced a significant decrease ($P < 0.001$) in the percentage distance traversed by the charcoal meal (i.e. there was an inhibited propulsive movement) in the small intestine. This suggests that this aqueous extract works by decreasing motility which allows more time for water to be absorbed from the faecal matter thereby leading to the formation of firmer faecal matter. Experiments carried out by Murugesan *et al*. (2000) on the evaluation of antidiarrheal activity of *Jussiaea suffruticosa* Linn extract in rats showed that there a significant reduction in gastrointestinal motility following a charcoal meal in rats. It exhibited significant antidiarrhoeal potentials at doses of 100 mg/kg, 200 mg/kg and 300 mg/kg in all the animal models carried out. Comparison between the extracts shows that *J. suffruticosa* is more potent than *Dialium guineense* because it exerts its maximum response at a dose of 300 mg/kg which is less than 400 mg/kg of *D. guineense* (Murugesan *et al.,* 2000).

The aqueous extract significantly ($P < 0.01$) inhibited intestinal fluid accumulation and reduced both weight and volume of the intestinal content. This suggests that it had antisecretory activity. Studies on the antisecretory effects of dichloromethane fraction of the stem bark of *Piliostigma reticulatum* showed that it had a very potent antisecretory property at a dose of 300 mg/kg when compared to loperamide which was used as a positive control (Dosso *et al*., 2012). Comparing this to the aqueous extract of *Dialium guineense*, it can be deduced that *Piliostigma reticulatum* has a higher antisecretory property than *Dialium guineense* making it a better antidiarrheal agent.

The extract inhibited the acetylcholine induced contraction on the isolated rat ileum at a dose of 15 mg/ml but had no significant ($P > 0.05$) effect at a dose of 7.5 mg/ml. The extract at 15 mg/kg shifted the dose response curve of acetylcholine to the right indicating a competitive antagonism. The extract in all the above test models carried out showed a significantly greater effect than distilled water (Negative control) but less than the effect produced by loperamide (the positive control). This indicated that the extract was less potent than loperamide.

Drugs that are known to affect motility and frequency of diarrhea also affect secretion . Therefore when there is a decrease in secretion it would lead to reduction in the passage of watery stool. The inhibition of experimental diarrhea and reduction in faecal output by a substance are basis of the pharmacological evaluation of a potential anti-diarrhea agent (Akah *et al.,* 1999).

The inhibitory effect on castor oil induced diarrhea, intestinal transit, fluid accumulation (enteropooling) and acetylcholine activity shows that it has a high ability to decrease propulsive gut motility and secretion, it also has the capacity to cause an increase in the absorption of fluid which normally results in diarrhea.

Conclusion

The results of the entire test in this study suggest that the extract could be useful as an anti-diarrhea agent. This justifies the basis of its use as a folkloric remedy in the treatment of diarrhea in Tropical countries and Sub-Saharan Africa.

REFERENCES

Akah P.A., Aguwa C.N. and Agu R.U. (1999). Studies on antidiarrheal properties of pentaclethra macrophylla leaf extracts. *Phy Res.* 13: 292-295

Akinpelu D.A., Kolawole D.O (2004). Phytochemistry and antimicrobial activity of leaf extract of *Piliostigma thonningii* (Schum). *Sci. Foc. 7:64-70*

Ammar N., Ahmed E., Nagwa O., Soheir G., Nagwa A. (1990). Flavonoids as a possible preventive of dental plague. *Arch. Pharm . Res. 13(2): 211-213*

Dharmanda S. (2003). Gallnuts and the Uses of Tannins in Chinese Medicine Director. Institute for Traditional Medicine, Portland. Oregon.

Dosso, K., N'guessan, B.B., Bidie, A.P., Gnangoran, B.N., Méité, S., N'guessan, D., Yapo, A.P., Ehilé, E.E. (2012). Antidiarrhoeal activity of an ethanol extract of the stem bark of Piliostigma reticulatum (Caesalpiniaceae) in rats. *Afr. J. Trad. Complement. Altern. Med.* 9(2):242-249.

Field M., Rao, M.C. and Chang, (2003). Intestinal electrolyte transport and diarrheal disease. Parts I and II. *N. Eng. J. Med. 806: b79-199*

Gabriel G. (2000). Gastrointestinal Disorders. In: Clinical Pharmacology Basic Principle 4th ed. (Eds: Carruthers S.G., Hoffman, B.R. Melmon, K.I. and Nierenberg, D.W.). McGraw Hill. N.Y, pp: 303-315

Hermans M., Akpovi A., Vander M.L. (2010). Medicinal Plants Used to treat Malaria in southern Benin. *Eco. Bot. 56:239-252*

Hong TD, Linington S, Ellis RH. 1996. Seed storage behaviour: a compendium. Handbooks for Gene banks: No.4.IPGRI.

Lawrence D.R., Bennett P.N., Brown M.J., (1997). Clinical pharmacology Churchill Livingstone, USA Mensah J.K., Okoli R.I., Turay A.A., Ogie-Odia E.A. (2009).Phytochemical analysis of Medicinal Plants used in the Management of Hypertension by Esan people of Edo state, Nig. *Ethnol leaf 13:1273-1287*

Rang H.P., Dale M.M., Ritter J.M. and Moore P.K. (2003). "The Gastrointestinal Tract" Pharmacology Textbook Fifth edition pp 24:367-376

Shi L., Liao Y., Su M., Lee A., Kuo P., Damu A.G., Kuo S., Sun H., Lee K., Wu T., (2010). Cardiac glycosides from *Antiaris toxicaria* with cardiotonic activity. *J. Nat Prod 73: 1214-1222*

WHO (2005). The Treatment of Diarrhea, A manual for physicians and other senior health workers. WHO Library Cataloging in Anti-secretory effect of *P. reticulatum* in rats Publication Data.