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Acute Toxicity and Antidiarrheal Activity of Aqueous Extract of Aerial Parts of *Hygrophila Auriculata in* Albino Rats

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ABSTRACT
Hygrophila auriculata (*H. auricalata*) plant extract was studied for it phytochemical constituents, acute toxicity and its anti-diarrheal activity in albino rats using standard procedure. The phytochemical screening revealed the presence of cardiac glycosides, terpenoids and saponins. The acute toxicity of the extract was above 2000 mg/kg b. wt which is slightly toxic. The result of castor oil induced diarrhea model indicates that the extract at all test doses was significant ($p < 0.05$). Similarly, the extract produced a significant ($p < 0.05$) decline in the weight and volume of intestinal contents at all tested doses. In addition, a significant ($p < 0.05$) reduction in the gastrointestinal motility in charcoal meal test was also observed in all doses of the extract administered. This activity may be attributed to the presence of the identified phytchemicals in the plant extract. The results in this study confirmed the antidiarrheal activity of the aerial part of *H. auriculata* and hence support the folkloric believe and provide the scientific basis for the traditional use of this plant in the treatment of diarrhea.

Keywords: Antidiarrheal activity; Castor oil; Gastrointestinal motility; *H.auriculata;,* Acute toxicity

INTRODUCTION

Diarrhea is the abnormal passage of watery or loose stools at least three times in a twenty-four hours (24 hours) period; it is classified as either acute or chronic based on the duration of symptoms (Medscape, 2020). Acute diarrhea is caused by bacteria e.g. *Campylobacter*, *Salmonella*, *Shigella*, and *Esch erichia coli* (Gascon *et al.,* 2000; Reda *et al.,* 2011) and viruses e.g. rotavirus and bovine viral diarrhea in cattle (Grooms, 2004). Acute diarrhea is also caused by drug medications like antibiotics, anticancer and antacids containing magnesium (Wendy, and Andrew, 2014). Chronic diarrhea is usually related to a functional disorder such as irritable bowel syndrome or any gastrointestinal disease (Sorouri *et al.*, 2010). Parasites such as *Giardia lamblia, Entamoeba histolytica*, and *Cryptosporidium* may also cause chronic or persistent diarrhea (Thapar and Sanderson, 2004). Infectious diarrhea of neonatal animals is one of the most common and economically devastating conditions encountered in the livestock industry (House, 1978). Diarrhea is also a major health problem in children under the ages of 5 years in developing countries (Thapar and Sanderson, 2004).

The high incidence of diarrhea in developing countries coupled with limitations of currently available antidiarrheal drugs and poor healthcare treatment may make traditional medicines good alternative agents for the management of diarrhea. The WHO has encouraged studies into the treatment and prevention of diarrheal using traditional medical practices (Snyder and Merson, 1982). Currently, most available drugs are linked with adverse effects and contra-indications Tsai, et al., 2012). Antibiotics resistance is a major problem in the treatment of diarrhea (Alam and Bhatnagar, 2006). Phytochemicals, commonly known as plant constituents or natural compounds are secondary metabolites found in plants that possess diverse toxicological and pharmacological activities which are mostly documented to be responsible for treating various ailments (Gnanavel *et al.,* 2018).

Hygrophila. auriculata is a herbaceous plant that belongs to the family *Acanthaceae,* it usually grow in wet places. It is a native of Asia and Africa (http://www.efloras.org/florataxon

.aspx?). In India it is commonly known as *kokilaksha* or *gok ulakanta* (www.wikipedia.org), Neermulli in Tamil, Marsh Barbel in English (Sarvananda, and Premarathna, 2018) and Zazar giwa or Kayar rakumi in Hausa language (Sodipo and Wannang, 2015). In the north eastern Nigeria, the aerial part (Stem, leaves, flowers and branches) of *H. auriculata* is known for its varied medicinal uses for the treatment of cough, anal fistula, blood disorders, jaundice, anemia, aphrodisiac, rheumatoid arthritis, and inflammation (Sarvananda and Premarathna, 2018).

In north eastern Nigeria, the aerial parts (stem, leaves, flowers and branches) of *H. auriculata* has been used for decades in the treatment of diarrheal and helminths (oral communication). Though there are no information on the empirical and scientific evidence to support the folkloric believes. Therefore, this study was design to assess the phytochemical constituents, the acute toxicity (LD_{50}) and to evaluate the antidiarrheal activity of the aqueous extract of the aerial part of *H. auriculata* in Albino rats.

MATERIALS AND METHODS

Ethical Statement

Ethical procedure according to Council for International Organization of Medical Sciences and the International Council for Laboratory Animal Science (CIOMS and ICLAS, 2012), was used.

Plant Collection and Identification

H. auriculata plant containing the stem, leaves and flowers was collected from Lassa town, Askira/Uba Local government area of Borno State Nigeria and submitted for identification and authentication by Professor Sunusi in the Department of Biological Science University of Maiduguri. The aerial part of the plant used for this research was thoroughly washed with distilled water and air dried at room temperature ($25 \pm 2\ ^0C$) for two weeks.

Plant Extraction

The dried plant material of *H. auriculata* was grounded manually using pestle and mortal into pulverized form. The cool macerated method as described by Umeh *et al.* (2005), was used for the extraction with distilled water as solvent. Two hundred grams (200 g) of the powdered plants material was mixed with 1 litre of distilled water in conical flasks and allowed to stay over a period of 48 hours. The mixture was then filtered using Whatman filter paper No. 1 to obtain an aqueous extract. The crude extract was recovered by evaporating the solvent over a rotary evaporator below the boiling point of the water, further trapped solvent was removed from the extract using a drier. After the extraction of eight hundred grams (800 g) of the dried powdered form of the plant material, the crude extract yield was calculated using the formula;

$$\textbf{Extract yield} = \frac{\mathbf{W1\ X\ 100}}{\mathbf{W2}}$$

Where;

W1 = the weight of the extract residue obtained after solvent removal

W2 = the weight of powdered form of the plant material used

Preliminary Phytochemical Screening

Preliminary phytochemical screening of *H. auriculata* was studied using the method described by Trease and Evans (2002) and Jones and Kinghorn (2006).

Experimental Animals

Adult albino rats weighing between (80-100 g) were used for the experiments. The rats were kept in plastic rat cages and allowed to acclimatize to laboratory environment for the period of two weeks before the commencement of the experiment. They were fed with growers' mash (Vital feeds Nig. Ltd) and water *ad libitum*.

Acute Toxicity Study (LD_{50})

The acute toxicity of aqueous extract of *H. auriculata* was determined by using five female Wistar rats aged between 8-12 weeks and weighed between (80- 100 g). The up and down as described by Dixon, (1991) limit test at 2000 mg/kg was used, the animals were fasted 3 hours prior to the administration of the extract according to OECD guideline 425, (2000). One animal was administered a test dose of 2000 mg/kg orally of the extract and observed for 48 hours after which the animal survived. Four additional animals were dosed with the same dose of 2000 mg/kg of the extracts using the same route of administration, the second and third animals were dosed concurrently while the fourth and fifth were dosed sequentially. All the animals were observed for 14 days for clinical signs and mortality.

Effect of Oral Administration of *H. auriculata* Extract on Gastrointestinal Transit of Charcoal Meal in Albino rats

The gastrointestinal motility test was carried out according to the principle described by Akah *et al.* (1999). Fifteen albino rats aged and weighed as same as that of the acute toxicity study were grouped into five (A-E) consisting of three rats each (n = 3). The animals were starved for 24 hours prior to commencement of the experiment. Group (A) was administered normal saline at 5 ml/kg per os and served as the negative control, the test groups (B-D) received 300, 600 and 900 mg/kg b.wt of the extract, While Group (E) was administered atropine sulphate at 3 mg/kg and serves as the positive control. After 5 minutes of the administration, 0.5 ml of 5.0 % charcoal suspension in 10 % aqueous solution of tragacanth powder was administered orally to each rat. The animals were then humanely sacrificed after 30 minutes and their abdomens dissected, the distance travelled by the charcoal plug from the pylorus to the cecum was measured and recorded in centimetre. The peristaltic index and percentage of inhibition were calculated by using the following formula as described by Than *et al*. (1989).

$$\textbf{Peristaltic index} = \frac{\textbf{Distance travelled by charcoal meal}}{\textbf{Length of small intestine}}\ \textbf{x 100}$$

$$\textbf{Percentage inhibition} = \frac{\mathbf{Dc-Dt}}{\mathbf{Dc}}\ \textbf{x 100}$$

Where,

Dc = Mean distance travelled by the negative control,

Dt = Mean distance travelled by the test group.

Effect of Oral Administration of *H. auriculata* Extract on Castor Oil-Induced Enteropooling in Albino rats

The castor oil induced antidiarrheal test was carried out using the method as described by Tagne *et al*. (2019) with fifteen (15) albino rats. The rats were randomly divided into five groups (A-E) of three rats each (n = 3). Diarrhea was induced orally in each rat using castor oil at 1 ml/kg b.wt. After an hour of inducement with castor oil, group (A) were treated with normal saline at 5ml/kg b. wt. orally and serves as a negative control, group (B) were administered 300 mg/kg b. wt. per os, group (C) were administered 600 mg/kg b. wt. per os, group (D) were administered 900 mg/kg b.wt. per os of the extract solution. Group (E) which serves as the positive control were treated with atropine sulphate at 3 mg/kg b.wt intra-muscular. The rats were housed in individual metal cages lined with white nonabsorbent paper. Fecal output was assessed by collecting the fecal material 8 hours after treatment and was dried at 50 ºC for 2 hours, weighed and then recorded. The percentage reduction in intestinal content was calculated using the formula;

$$\textbf{Percentage reduction in intestinal content} = \frac{\mathbf{VIC-VIT}}{\mathbf{VIC}}$$

Where;

VIC = Volume of intestinal content (ml) in the negative control

VIT = Volume of intestinal content (ml) in treated group

Data Analyses

Data collected were analyzed using IBM SPSS statistical software, version 25. The results were expressed as means ± SEM. Comparisons between groups were made using ANOVA followed by post hoc Tukey's multiple comparison test. At 95% confidence interval ($p < 0.05$), the difference between the compared groups were considered as statistically significant.

RESULTS

Phytochemical Screening

The preliminary phytochemical screening revealed the presence of cardiac glycosides, terpenoids and saponins, as shown in Table 1.

Acute Toxicity Study (LD_{50}

There was no mortality or any signs of toxicity noticed in the 14-days period of observation of the Wistar rats used for the test. The LD_{50} of the aqueous extract of the aerial part of *H. auriculata* was above 2000 mg/kg.

Table 1: Preliminary Phytochemical Screening of Aqueous Extract of *H. auriculata*

Phytochemical	Test	Result
Anthraquinone	Borntrager's	-
Combine anthraquinone		
Cardiac glycoside	Salkowski's	+
	Liebermann-Burchard's	+
Saponins	Frothing	+
Terpenoids		+
Tanins	Hydrochloric acid	-
Flavonoids	Shinoda	-
	Ferirc chloride	-
	Lead acetate	-
Carbohydrate		
General test	Molisch's	-

Key: += present; − = absent

Effect of Oral Administration of *H. auriculata* Extract on Gastrointestinal Transit of Charcoal Meal in Albino rats

The anti-motility of the aqueous extract of *H. auriculata* on the intestine of albino rats is presented in Table 2, as percentage reduction in movement of the charcoal meal as compared with the negative control. Atropine sulphate was the standard drug administered to rats in group E (3 mg/kg) and it has been found to significantly ($p < 0.05$) inhibit the distance travelled by the charcoal meal by 71.3% indicating its strong anti-motility activity. The group (A) rats were administered normal saline at 5mg/kg body weight per os and served as the negative control. The percentage inhibition of the extract administration on gastric motility (charcoal meal) on albino rats in the test groups (B-D) were recorded as 45.2%, 47.1% and 49.4% respectively, which indicates that the reduced gastric motility of the extract was significant ($p < 0.05$), when compared with the standard. In group B, the mean total length of the intestine was 119.8 cm and the mean movement of the charcoal meal was 65.7cm. In group C, the mean total length of the intestine was 98.4 cm and the mean distance moved by the charcoal was 51.9 cm, and in group D (900 mg/kg), the mean total length of intestine was 120.5 cm and the mean movement of charcoal was 50.8 cm. All of the values recorded above were significant ($p < 0.05$).

Table 2: Effect of Aqueous Extract of *H. auriculata* on the Gastrointestinal Motility (Charcoal meal) of Albino Rats.

Group	Treatment	Mean total length of intestine (cm)	Mean movement of Charcoal in intestine (cm)	Peristaltic Index	% Inhibition
A	Control (Normal Saline5ml/kg b. wt)	127.8±3.0	89.8±2.4[a]	70.7	00[a]
B	Extract (300 mg/kg)	119.8±12.7	65.7±1.1[b]	54.8	45.2[b]
C	Extract (600 mg/kg)	98.4±13.2	51.9±7.2 [b]	52.7	47.1[b]
D	Extract (900 mg/kg)	120.5±23.3	50.8±4.0[b]	42.2	49.4[b]
E	Atropine sulphate (3 mg/kg)	115.4±1.17	33.0±1.3 [b]	28.6	71.3[b]

Within column, mean with the same superscript are statistically significant p>0.05 when compared with control group.

Effect of Oral Administration of *H. auriculata* Extract on Castor Oil-Induced Enteropooling in Albino rats

The antidiarrheal activity of *H. auriculata* on castor oil induced diarrhea in albino rats were expressed as percentage reduction in fecal content of the intestine as shown in table 3. The extract had significantly ($p < 0.05$) decreased the fecal mass of the rats induced by castor oil as compared with the control group. The percentage reduction of the volume of intestinal contents of the rats in group E (Atropine sulphate 3mg/kg) was 61.9%. The percentage reduction of the intestinal contents of the rats in test groups (B - D) were 33.3 %, 42.1 %, and 57.1 % respectively. This indicates a significant ($p < 0.05$) antidiarrheal activities as compared to the standard. The higher dose of the extract (900 mg/kg) in group D produced a better effect as compared with the group B (300 mg/kg) and C (600 mg/kg) as shown in Table 3.

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C	Extract (600 mg/kg)	98.4±13.2	51.9±7.2 [b]	52.7	47.1[b]
D	Extract (900 mg/kg)	120.5±23.3	50.8±4.0[b]	42.2	49.4[b]
E	Atropine sulphate (3 mg/kg)	115.4±1.17	33.0±1.3 [b]	28.6	71.3[b]

Within column, mean with the same superscript are statistically significant p>0.05 when compared with control group.

DISCUSSION

Plant based medicinal products of natural origin have been used traditionally and were considered relatively safe to the body compared to synthetic drugs (Kangwan *et al.,* 2014). Several phytochemicals constituents have been well described for their anti-diarrheal properties in plants such as *Lantana camara, Plumbago indica,* and *Achillea millefolium* among others (Babaeli, *et al.,* 2007; Baggio, *et al.,* 2009).

Cardiac glycosides, terpenoids and saponins present in the aqueous extract of *H. auriculata* are reported to have antidiarrheal activities (Ataka *et al*., 2005; Cowan, 1999).

From the result of acute toxicity, a conclusion can be made on the toxicity status of *H. auriculata* as slightly toxic. This finding agrees with the report of Loomis and Hayes, (1996) and Erhirhie, *et al.* (2018), that a test substance with LD_{50} between 500 – 5000 mg/kg is classified as slightly toxic. The standard drug used, atropine sulphate is known to inhibit intestinal transit possibly due to its anticholinergic effect (Mascolo, *et al.,* 1993). According to Akah (1996), drugs which inhibit intestinal motility can also possess anti-diarrheal activity. The extract *H. auriculata* dose dependently reduced the intestinal propulsive movement of the charcoal meal in the treatment groups and also increased in percentage inhibition of the distance travelled by charcoal meal in the intestine of the rats. Thus, the extract showed activity similar to that of atropine sulphate. Previous studies show that activated charcoal lowered the absorption of ingested gastric contents (Robert *et al.,* 1984).

Castor oil or its active components ricin oleic acid causes permeability changes in mucosal fluid and electrolyte transport that result in a hypersecretory response and diarrhea (McQuaid, 2012). Ricin oleic acid increase the prostaglandin E_2 (PGE_2) content in portal venous and diarrhea causes an increase in secretion of water and electrolytes into the small intestine (Beubler and Juan, 1979). Ricin oleic acid also produces irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins which stimulate motility and secretion (Mascolo, *et al*., 1993). The percentage reduction of the intestinal contents of the rats administered the highest dose of the extract in group D (900 mg/kg) was closer to the percentage reduction produced by the standard drug (Atropine sulphate 3mg/kg) in group E which implies that the plant extract inhibits diarrhea more effectively at relatively higher doses (Chaddha, *et al*., 2013). Based on these observations, it is suggestive that the antidiarrheal effect of the aqueous extract of *H. auriculata* may be due to the presence of saponin and terpenoid which are associated with increase in the reabsorption of water and NaCl by inhibiting intestinal motility or reduced mucosal secretion and also prevent prostaglandin release from intestinal mucosa (Orooba, and Muhammad, 2016). These phytochemical constituents may probably be responsible for the antidiarrheal effect of this plant extract. Most antidiarrheal agents act by decreasing secretion and/or reducing the propulsive movement of gastrointestinal smooth muscles (Matias *et al*., 1978). However, the specific phytochemical constituents responsible are yet to be identified since our current experimental data is insufficient to directly ascribe the observed anti-diarrheal activity to any of them.

Conclusion

The results of this study revealed that the aqueous extract of the aerial part of *H. auriculata* has anti-diarrheal activity. The effect of the extract in the reductions of the total fecal output and gastrointestinal motility supports its antidiarrheal activity. Some of the phytochemicals identified in this study may also be responsible for the antidiarrheal activity of the extract through various possible mechanisms of action proposed. These findings provide a scientific support for a traditional use of the aerial part of *H. auriculata* as an anti-diarrheal agent. However, there is need for further research to elucidate the specific mechanism (s) of action and isolate the bioactive constituents responsible for the anti-diarrheal activity.

Author Contribution

Salihu, S.I designed and carried out the anti-diarrhea study. Chiroma, M conducted the extraction and phytochemical study. Telta, A did the data analysis and wrote the draft manuscript. Daniel, N and Yakubu, C participated in the study design and manuscript write up. Mr. Ibrahim Wiam sourced the experimental animals and provided technical support for the study.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

REFERENCE

Akah, P.A. (1996). Anti-diarrheal activity of *Kigelia afriana* in experimental animals. J Herbs, Spices and Med Plants 4:31-8.

Akah, P.A. and Nwafor, S.V. (1999). Studies on anti – ulcer properties of *Cassampelos mucronata* leaf extract. Indian J. Exp. Biol. 37: 936 – 938.

Alam, S. and Bhatnagar, S. (2006). Current status of anti-diarrheal and anti-secretory drugs in the management of acute childhood diarrhea. Ind. J. Pediatr., 73(8): 693– 696.

Ataka, K., Ito, M. and Shibata, T. (2005). New views of antidiarrheal effect of wood creosote: Is wood creosote really a gastrointestinal antiseptic? Yakugaku Zasshi: 125:137-950.

Babaeli, M., Abarghoei M.E., Akhavan M.M., Ansari, R., Vafaei, A.A., Taherian, A.A., Mousavi S. & Toussy J. (2007). Antimotility effect of hydroalcoholic extract of yarrow (Achillea millefolium) on the guinea-pig ileum. Pak. J. Biol. Sci.. 10: 3673-3677.

Baggio, C.H., Freitas, C.S., Mayer, B., Dos Santos, A.C., Twardowschy A., Potrich F. B., Cipriani T. R., De Souza L. M., Sassaki G. L., Iacomini M., Marques M. C. & Mesia-Vela S. (2009). Muscarinic-dependent inhibition of gastric emptying and intestinal motility by fractions of *Maytenus ilicifolia* Mart ex. Reissek. J. Ethnopharmacol. 123: 385-391.

Beubler, E. and Juan, H. (1979). Effect of ricin oleic acid and other laxatives on net water flux and prostaglandin E released by the rat colon. J. Pharm. Pharmacol. *31:681-685*.

Chaddha, V., Kushwah, A.S. & Shrivastava, V. (2013). An importance of herbal drugs as antidiarrheal: a review. Intl. J. Res. Appl, Nat. Soc. Sci..1 (7): 25–28.

Cioms and Iclas (2012). Council for International Organizations of Medical Sciences and the International Council for Laboratory Animal Science. International Guiding Principles for Biomedical Research Involving Animal, December 2012.

Cowan, M. M. (1999). Plant products as antimicrobial agents. Clin. Microbiol. 12:564-582

Dixon WJ (1991). Staircase Bioassay: The up and down method. Neurosci. Biobehav. R. 15 (1), 47-50.

Erhirhie, O., Ihekwereme, C.P and Ilodigwe E.E. (2018). Review: Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance Earnest. Interdiscip Toxicol. 11(1): 5–12.

Gascon, J., Vargas, M. and Schellenberg, D. (2000). Diarrhea in Children under 5 Years of Age from Ifakara, Tanzania: a Case-Control Study. J Clin Microbiol. 38: 4459–4462.

Gnanavel, V., Veluchamy, P. & Anand, P.R. (2018). Phytochemical and Pharmacological Importance of Plant Secondary Metabolites in Modern Medicine. *In book: Bioorganic Phase in Natural Food:* An Overview.

Grooms, D.L. (2004). Reproductive consequences of infection with bovine viral diarrhea virus. The Veterinary Clinics of North America. Food Anim. Pract. 20(1):5-19.

House, J.A. (1978). Economic impact of rotavirus and other neonatal disease agents of animals. J. Am. Vet. Med. Assoc. 173: 573-576.

http://www.efloras.org/florataxon.aspx? *Hygrophila auriculata* in *Flora of Pakistan*, at Efloras.org at. Accessed 9th may 2020

https://en.wikipedia.org/wiki/Hygrophila_auriculata. Accessed 9th may 2020

Jones, P. and Kinghorn D. (2006). Extraction of plant secondary metabolites. In: Sarker D, Latif Z, Gray A, editors. Methods in Biotechnology Natural Products Isolation. 2nd ed. Totowa, New Jersey: Human Press pp. 269–273.

Kangwan, N., Park, J.M., Kim, E.H. & Hahm, K.B. (2014). Quality of healing of gastric ulcers: Natural products beyond acid suppression. World J Gastrointest Pathophysiol. 5(1):40-7.

Loomis, T.A. and Hayes, A.W. (1996). Loomis's essentials of toxicology. 4. California: Academic press; . 208–245.

Mascolo, N., Izzo, A.A., Barbato, F. & Capasso, F. (1993). Inhibitors of nitric oxide synthetase prevent castor-oil-induced diarrhoea in the rat. Br J Pharmacol. 108 (4) 861–864.

Matias, J. R., Martin, J.L. & Burns, T.W. (1978). Ricinoleic acid effect on the electrical activity of the small intestine in rabbits. J Clin Invest. 61:640–644.

McQuaid, K.R. (2012). Drugs used in the treatment of gastrointestinal diseases. In *Basic and Clinical Pharmacology*, B. G. Katzung, S. B. Masters, and A. J. Trevor, Eds., pp. 1081–1115, McGraw-Hill, New York, NY, USA.

Medscape.(2020). How are acute and chronic diarrhea defined? https://www.medscape.com/answers/928598-25392/how-are-acute-and-chronic-diarrhea-defined.Accessed 9th- march- 2020

OECD, (2000). Guidance Document on Acute Oral Toxicity. Environmental Health and Safety monograph Series on Testing and Assessment No 24.

Orooba, M.S.I. and Muhammad, M.S. (2016). Evaluation of *Punica granatum* Peels Extracts and its Phenolic, Alkaloid and Terpenoid Constituents Against Chemically Induced Diarrhoea in Rats. Adv. Anim. Vet. Sci, 4 (3):161.168

Park, K. (2000). Park's. Textbook of Preventive and Social Medicine. Jabalpur, India: M/S Banarsidas Bharat Publishers; pp. 172–175.

Reda, A., Seyoum, B. and Yimam, J. (2011). Antibiotic susceptibility patterns of *Salmonella* and *Shigella* i solates in Harar, Eastern Ethiopia. J Infect Dis Immun. 3:134–139.

Robert, A. C., Joseph, G. B. & Nicola, G. (1984). Efficacy of Ipecac and Activated Charcoal/Cathartic Prevention of Salicylate Absorption in a Simulated Overdose. Arch *Intern* Med . *144(1):48-52.*

Sarvananda, L. and Premarathna, A.D. (2018). Ethnopharm acolological potential and medicinal uses of *Hygrophila auriculata*. J Ayu Herb Med. 4(4):185-188.

Sodipo, O. A and Wannang, N. N. (2015). Ethnopharmacolo gical Survey of Plants Used by Trado-Medical Practitioners (TMPs) in the Treatment of Typhoid Fever in Gomari Airport Ward, Jere Local Government Area, Borno State, Nigeria. American J. Ethnomed., 2 (4) 184-218.

Sorouri, M., Pouhoseinli, M. A., Vehedi, M., and Moghimi-Dehkordi, B. (2010). Functional bowel disorders in Iranian population using Rome III criteria. Saudi J. Gastroenterol 16:154-160.

Snyder, J.D. & Merson, M.H. (1982). The magnitude of the global problem of acute diarrhoeal disease: a review of active surveil- lance data, Bulletin of the WHO. 60(4): 605–613,

Tagne, F.M.A., Rekabi, Y., Noubissi, P.A., Fankem, G.O., Akaou, H., Wambe, H. and Kamgang, R. (2019). Evaluation of Antidiarrheal Activity of Aqueous Leaf Extract of Anogeissus leiocarpus on Castor Oil Induced Diarrhea in Rats. Am J Biomed Sci & Res. 3(1): 27-34. AJBSR.MS.ID.000629.

Than, A., Kulkarni, H. J., Hmone, W. and Tha, S.J. (1989). Anti-diarrhoeal efficacy of some Burmese indigenous drug formulations in experimental diarrhoeal test models. Int. J. Crude Drug Res. 27:195–200.

Thapar, N. and Sanderson, I.R. (2004). Review on diarrhea in children: an interface between developing and developed countries. Lancet. 363:641–653.

Trease, G.E. and Evans, W.C. (2002). Pharmacognosy. 15th ed. London: WB Saunders; pp. 33–35.

Tsai, H.H., Lin, H.W., Pickard, S.A., Tsai, H.Y. & Mahady, G.B.(2012). Evaluation of documented drug interactions and contraindications associated with herbs and dietary supplements: a systematic literature review. Int J Clin Pract. 66(11):1056-78.

Umeh, E.U., Oluma, H.O.A. and Igoli, J.O. (2005). Antibacterial screening of four local plants using an indicator base microdilusion technique. Afri. J. Trad. 2(3): 238-243.

Wendy, B.M.D. & Andrew S.M.D. (2014). Acute Diarrhea in Adults. Am Fam Physician. 89(3):180-189.