



Short Communication

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Acute Toxicity and Anti-diarrhoeal Activity of Aqueous Extract of Aerial Parts of *Hygrophila Auriculata* in Albino Rats

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ABSTRACT

Hygrophila auriculata (*H. auriculata*) plant extract was studied for its phytochemical constituents, acute toxicity and its anti-diarrhoeal 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 diarrhoea 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 phytochemicals in the plant extract. The results in this study confirmed the anti-diarrhoeal 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 diarrhoea.

Keywords: Anti-diarrhoeal activity; Castor oil; Gastrointestinal motility; *H. auriculata*; Acute toxicity

INTRODUCTION

Diarrhoea 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 diarrhoea is caused by bacteria e.g. *Campylobacter*, *Salmonella*, *Shigella*, and *Escherichia coli* (Gascon et al., 2000; Reda et al., 2011) and viruses e.g. rotavirus and bovine viral diarrhoea in cattle (Grooms, 2004). Acute diarrhoea is also caused by drug medications like antibiotics, anticancer and antacids containing magnesium (Wendy, and Andrew, 2014). Chronic diarrhoea 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 diarrhoea (Thapar and Sanderson, 2004).

Infectious diarrhoea of neonatal animals is one of the most common and economically devastating conditions encountered in the livestock industry (House, 1978).

Diarrhoea 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 diarrhoea in developing countries coupled with limitations of currently available anti-diarrhoeal drugs and poor healthcare treatment may make traditional medicines good alternative agents for the management of diarrhoea. The WHO has encouraged studies into the treatment and prevention of diarrhoea using traditional medical practices (Snyder and Merson, 1982). Currently, most available drugs are linked with adverse effects and contra-indications (Tsai, et al., 2012). Antibiotic resistance is a major problem in the treatment of diarrhoea (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 grows 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 *gokulakanta* (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, anaemia, 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 diarrhoea 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 (LD₅₀) and to evaluate the anti-diarrhoea 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 ase 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 (LD₅₀)

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 provided 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.

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±2 °C) for two weeks.

Plant Extraction

The dried plant material of *H. auriculata* was grounded manually using pestle and mortar 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;

$$\text{Extract yield} = \frac{W1 \times 100}{W2}$$

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 Tre

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).

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

$$\text{Percentage inhibition} = \frac{Dc-Dt}{Dc} \times 100$$

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 anti-diarrhoea 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). Diarrhoea 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 °C for 2 hours, weighed and then recorded. The percentage reduction in intestinal content was calculated using the formula;

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

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 \pm 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 (LD₅₀)

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 LD₅₀ 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 Saline 5ml/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 anti-diarrhoea l activity of *H. auriculata* on castor oil induced diarrhoea in albino rats were expressed as percentage reduction in faecal content of the intestine as shown in table 3. The extract had significantly ($p < 0.05$) decreased the faecal 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 ($p < 0.05$) anti-diarrhoea l 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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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-diarrhoea l 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 anti-diarrhoea l 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 LD₅₀ 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-diarrhoeal 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 diarrhoea (McQuaid, 2012). Ricin oleic acid increase the prostaglandin E₂ (PGE₂) content in portal venous and diarrhoea 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 diarrhoea more effectively at relatively higher doses (Chaddha, et al., 2013). Based on these observations, it is suggestive that the anti-diarrhoea l 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 anti-diarrhoea l effect of this plant extract. Most anti-diarrhoea l 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-diarrhoea l 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-diarrhoea l activity.

The effect of the extract in the reductions of the total faecal output and gastrointestinal motility supports its anti-diarrhoea l activity. Some of the phytochemicals identified in this study may also be responsible for the anti-diarrhoea l 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-diarrhoea l 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-diarrhoea l activity.

Author Contribution

Salihu, S.I designed and carried out the anti-diarrhoea 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.

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