



## Acute toxicity of the extracts of some medicinal plants in Nigerian ethno-botanicals using broiler chicken model

Joseph Olowo Arogbodo\*, Isaac Abayomi Adebayo, Innocent Bamidele Osho

Department of Animal Production and Health, School of Agriculture and Agricultural Technology, Federal University of Technology, P.M.B. 704, Akure, Nigeria



\*Corresponding author: arogbodojo@futa.edu.ng

(Accepted February 16, 2024)

**Abstract:** Therapeutic and nutraceutical application of medicinal plants in animal production are becoming very popular in the recent time. Many medicinal plants were reported to have shown adverse effects due to their toxicity, which underscores the necessity of their acute toxicity determination prior to the approval of their usage. This study examined the acute toxicity ( $LD_{50}$ ) of four medicinal plants (*Solanum elaeagnifolium* D. Don., *Petiveria alliacea* L., *Alternanthera brasiliana* (L.) Kuntze, and *Hoslundia opposita* Vahl) in Nigerian ethno-botanicals in broilers using standard procedure. Sixty-eight (68) three weeks old broilers were divided into two groups of forty eight (48) and twenty (20) for the first and second phase of the study respectively. Ethanolic extracts were administered at 10, 100, and 1,000 mg/kg during the first stage and 1,000, 1,600, 2,900, and 5,000 mg/kg at the second stage of the experiment. There were no observable hazardous or debilitating effects, injuries or deaths caused by the extracts, even at 5,000 mg/kg to the experimental birds. By deductive inference, the  $LD_{50}$  of the extracts was above 5000 mg/kg. It was concluded that the extracts were practically non-toxic by classification of  $LD_{50}$ , based on dose range. Further studies on sub-chronic and chronic toxicity effects of the test plants on other species of animals were recommended. Their histopathological effects can thenceforth be adequately assessed on selected vital organs.

**Keywords:** Acute toxicity, medicinal plants, leaf extracts, broiler chickens

## INTRODUCTION

Poultry infections from pathogenic bacteria like *Salmonella species*, *Escherichia coli*, *Pasteurella multocida*, etc., and Coccidiosis from protozoans are of major concern in poultry production in Nigeria. The potentials of medicinal plants in the management of bacterial and coccidial surge were reported in literature. Interests in the study and use of medicinal plants have unarguably grown and still growing amongst scientists worldwide as more and more of their importance are being discovered in various fields like; Veterinary Medicine, Agriculture, Pharmacognosy, Ethnobotany, etc. Modern Pharmaceutical industries have also discovered that traditional medicine could be a veritable source of bio-active compounds needed in the preparation of synthetic drugs (Odugbemi, 2008). Plants have been a source of inspiration for novel drug compounds necessary for the treatment of many diseases in veterinary and human medicine (Iwu *et al.*, 1999).

It was reported that the cold decoction of the leaf and seed of *Phyllanthus amarus* are potent immune booster, analgesic, antidiabetic, hepatoprotective, anti-inflammatory and antimicrobial (Odugbemi, 2008). According to Ilori *et al.* (1996), *Ocimum gratissimum* was used in the treatment of the disease of upper respiratory infections, headaches, fever, and pneumonia in humans. The use of medicinal plants in the prevention and treatment of animal's ailments and incorporation into their diets are becoming very popular in recent times. The report of Arogbodo *et al.* (2021) showed the antibacterial efficacy of *Chrysophyllum albidum* (G. Don) leaf extract against some clinical pathogenic bacteria. Likewise, the administration of aqueous ginger extract was reported to inhibit the growth of *Salmonella spp* in the crop of broiler chickens (Okolo *et al.*, 2022). Anticoccidial evaluation of aqueous extracts of *Prosopis africana* and Aloe vera leaf was carried out and found effective in the reduction of coccidial oocysts infected West African Dwarf goats and broiler chickens respectively. The leaves were recommended as natural ant coccidiosis and with further advantage of reducing the residual effects of synthetic drug (Mohammed *et al.*, 2022; Idowu *et al.*, 2022).

Anthelmintic efficacy of methanol stem-bark extract of *Anogeissus leiocarpus* was demonstrated against *Ascaridia galli* with reasonable larvicidal activity (Yahaya *et al.*, 2022). According to Suleiman (2022), farmers can add sweet potato (*Ipomea batatas*) meal to layers feed at the level of 10% to 15% level for better performance and without any damaging outcome. The leaf of *Solanum erianthum* (solanaceae) is used in trado-medicine for the treatment of piles, headache, constipation and wound (Burkil, 2000). According to the report of Battiwala *et al.* (2014), the root bark and leaf of *S. erianthum* possess antipyretic, anti-inflammatory, anti-arthritis and analgelsic properties. *Petiveria alliacea* (Phytolaccaceae) is called by various names like 'guine' in Latin America, 'Guinea Hen Weed', 'anamu' in South America, 'awogba arun' in Yoruba etc (2004). It has antibacterial (broad spectrum), anticancer, anti-inflammatory, antispasmodic, antehelmintic properties etc (Lima *et al.*, 1991).

*Alternanthera brasiliiana* (Amaranthaceae) was reported to have originated from Brazil. The abundance of this plant in Brazil earned it the nick name "Brazilian joy-weed" (Barua *et al.*, 2012). Though every part of the plant is useful in traditional medicine, the leaf is frequently used (Saawan *et al.*, 2011). It is very useful in the treatment of many ailments like bronchitis, asthma, wound infection, etc (Barua *et al.*, 2009). *Hoslundia opposita* is an herbaceous plant from the family Lamiaceae and usually located in Africa's tropics and subtropics. The plant is called 'Efinrin odan/Efinrin Oso' among the Yorubas and 'Oke Ota' by the Igbos in Nigeria (Onwuka *et al.*, 2016). Traditionally, various medicinal usefulness of the plant had been well documented in literature some of which are; antibacterial, antimalarial, wound healing, anti-inflammation potentials among others (Anchebach *et al.*, 1992; Sadri, 2017). The toxicity effects of some medicinal plants were recently

reported, indicating that there may be potential adverse effects following chronic administration of such plants. Hence, examination of the toxicological impact of any medicinal plants due for clinical or preclinical use is an indispensable aspect of its evaluation (Mayur *et al.*, 2017). After keen observations from literature, large percentages of experiments carried out on animals were without the determination of the toxicity (acute, sub-acute, and chronic) profile of the experimented plant or plants before the actual administration to animals. The limited volumes accessed are most of the time on rats, mice, and guinea pigs, but very few reports on broiler chickens. The reason might be due to higher volume of extract needed for broiler chickens' experiment *vis a vis* the cost of extraction. This study therefore set out to assess the toxicological effects of *S. erianthum*, *P. alliacea*, *A. brasiliensis* and *H. opposita* prior to administration in further broiler chickens' experiments. These plants were chosen for this study also because of their scarce use in animal production in Nigeria.

## MATERIALS AND METHODS

*Plants collection, identification and preparation of extracts:* The plants evaluated in this study were harvested fresh from Akure and taken to the Medicinal Faculty of Pharmacy, Department of Pharmacognosy, Obafemi Awolowo University Ile-Ife Nigeria where they were identified and authenticated by an experienced botanist. Each of the plants was assigned Herbarium Number viz; *Petiveria alliacea* L.; FPI 2269, *Solanum erianthum* D. Don.; FPI 2270, *Alternanthera brasiliensis* (L.) Kuntze; FPI 2271, and *Hoslundia opposita* Vahl.; FPI 2272. Their pictures are shown in Plates 1 – 4. They were extracted in 99.70 - 100 % (absolute) ethanol sourced from AnalaR® BDH Limited Poole England for 72 hours with intermittent stirring using glass rods as described in our earlier experiment on the same plants (Arogbodo *et al.*, 2023). The percentage yield of the extract from each of the samples is as presented in Table 1.

*Experimental site:* this experiment was conducted at the Phytomedicine and Toxicology Unit of the Department of Animal Production and Health, Federal University of Technology Akure. The experiment was carried out according to the Ethical Code of the University on Animal Use, Guidelines of the Organization of Economic Cooperation Development (OECD, 2008) and World Health Organization (WHO, 2000).

*Experimental broiler chickens:* one hundred (100) unsexed Arbor acres breed of broiler chicken were sourced from a commercial hatchery having a track record of good quality day-old broiler production and used for this experiment. The brooding of the birds was done in a serene environment where there was a strict compliance to bio-security measures. They were given water and feed (commercial feed) *ad libitum* while necessary medications and vaccinations were appropriately administered.

*Reconstitution of the extracts:* exactly 60 g each of the extracts was reconstituted with 5 % Di methyl sulfoxide (DMSO) and distilled water (500 mL) at the ratio of 1:10 (v/v) to give the working concentration of 120 mg/mL.

*Acute oral toxicity (LD<sub>50</sub>):* acute toxicity according to Enevide *et al.* (2013) is the unwanted effect(s) that emanated either immediately or at a short time interval after a single or multiple administration of such substance within short hours of 24. It was also defined as the detrimental and manifested effects observable after the administration of a single dose of a test substance within 24 hours by a known route (Saganuwan, 2016). The acute toxicity in this study was determined in two stages as

described by Lorke (1983) with slight modifications. The first stage involved the usage of forty-eight (48) broiler chickens (male and female) of three weeks old (3 weeks) weighing 608.20 – 730.30 g.



Plate 1 *Petiveria alliacea*



Plate 2 *Hoslundia opposita*

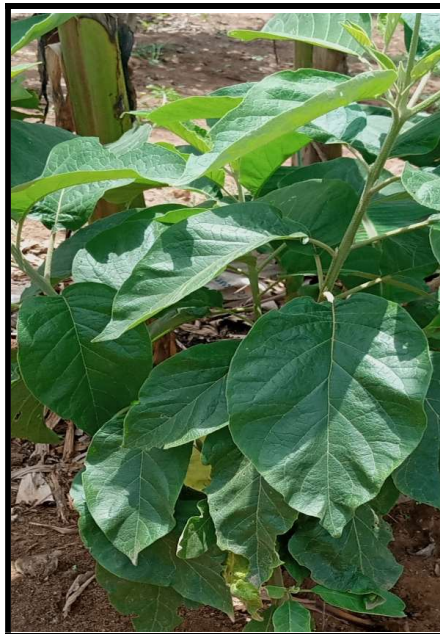


Plate 3: *Solanum erianthum*



Plate 4: *Alternanthera brasiliana*

Nine (9) broilers were used per plant sample divided into four groups of dosage administration with three broilers per group per plant. The extracts were orally administered in mg/kg body weight basis. The control birds were given the solution used for reconstituting the extract (DMSO + distilled water) only as a placebo. The birds were fasted overnight prior to the extracts' administration. The birds were observed for twenty-four (24) hours for toxicity signs. The follow up stage of the acute toxicity study entails the use of twenty (20) birds distributed into five (5) groups of 0, 1000, 1600, 2900, and 5000 mg/kg at the rate of ten (5) birds per group in which one (1) bird was housed per treatment (i.e. *S. erianthum* (SER), *P. alliacea* (PAL), *A. brasiliiana* (ABR), and *H. opposita* (HOP)). The birds were observed for twenty-four (24) hours for morbidity, mortality and acute toxicity signs.

## RESULTS

The results of the yield of the extracts expressed as percentage are shown in Table 1. The calculated yield gave important information regards the expected volume of extract from known powdered sample of individual plant. Highest yield was obtained from *S. erianthum*, followed by *H. opposita*. Similar yield was recorded in *A. brasiliiana* and *P. alliacea*. The noticeable signs in the birds within 24 hours after extracts' administration are tabulated in Table 2 while the mortality records of the experimental broiler chickens administered varying doses of the extracts are presented in Tables 3 and 4. Observed behavioural signs in the treated groups include sneezing, neck stretching, and prostration. The control groups did show normal behaviour while the behavioural signs in the other groups disappeared after twenty-four hours (24 hours) of the extracts' administration. There was no record of mortality (Table 3 and 4) in all the groups (treated and untreated) at the two stages of the experiment.

Table 1: Percentage yield of the ethanolic extracts from the tested plants

S/N	Medicinal plants	Soaked weight (g)	Volume of solvent (mL)	Ratio (w/v)	Yield (g)	% Yield
1	<i>S. erianthum</i>	400	4000	1:10	47.30	11.83
2	<i>P. alliacea</i>	400	1600	1:4	18.80	4.70
3	<i>A. brasiliiana</i>	400	1600	1:4	18.70	4.68
4	<i>H. opposita</i>	400	1600	1:4	25.19	6.30

## DISCUSSION

The percentage yield of the ethanolic extracts (Table 1) was highest in SER, higher in PAL and ABR, but lowest in HOP. The expressed ratio among them depicts SER: PAL (2.52:1), SER: ABR (2.51:1) and SER: HOP (1.87:1). The highest value of SER may be attributed to the more solvent consumed by the plant sample during the extraction process as equal volume of solvent did not get the SER soaked and well covered. This is a remarkable discovery showing plainly that the volume of extractive solvent as well as the concomitant yield differ or vary from plant to plant. The behavioural signs observed (Table 2) after the oral administration of the extracts in all the groups within four hours (except the control that was normal all through) include, sneezing, neck stretching, gasping and prostration. The exhibited sign of prostration may be due to handling stress during the course of the extracts' administration. As the level of the dose increases, more of the characteristics listed above became

more evident. Sneezing and gasping were peculiar to birds on *S. erianthum*, which is suggestive of some level of irritants in the archive of its chemical constituents.

Table 2: Characteristic behaviour manifested by birds during acute toxicity test

Groups	Doses (mg/kg)	Plant samples				Control
		<i>Solanum erianthum</i>	<i>Petiveria alliacea</i>	<i>Alternanthera brasiliana</i>	<i>Hoslundia opposita</i>	
First stage						
1	10	Sneezing and neck stretching	Normal	Normal	Normal	Normal
2	100	Sneezing and neck stretching	Normal	Normal	Normal	Normal
3	1000	Sneezing and neck stretching	Neck stretching	Neck stretching	Neck stretching	Normal
Second stage						
1	1000	Sneezing and neck stretching	Neck stretching	Neck stretching	Neck stretching	Normal
2	1600	Sneezing and neck stretching	Neck stretching	Neck stretching and prostration	Neck stretching and prostration	Normal
3	2900	Sneezing, neck stretching, gasping and prostration	Neck stretching and prostration	Neck stretching and prostration	Neck stretching and prostration	Normal
4	5000	Sneezing, neck stretching, gasping and prostration	Neck stretching and prostration	Neck stretching and prostration	Neck stretching and prostration	Normal

Table 3: Mortality record of broiler chickens at the first stage of LD<sub>50</sub> determination

Groups	Doses (mg/kg)	Plant samples			
		<i>Solanum erianthum</i>	<i>Petiveria alliacea</i>	<i>Alternanthera brasiliana</i>	<i>Hoslundia opposita</i>
1	Control	0/3	0/3	0/3	0/3
2	10	0/3	0/3	0/3	0/3
3	100	0/3	0/3	0/3	0/3
4	1000	0/3	0/3	0/3	0/3

0= number of deaths, 3= number of broilers used for the trial

Table 4: Mortality record of broiler chickens at the second stage of LD<sub>50</sub> determination

Groups	Doses (mg/kg)	Plant samples			
		<i>Solanum erianthum</i>	<i>Petiveria alliacea</i>	<i>Alternanthera brasiliana</i>	<i>Hoslundia opposita</i>
1	Control	0/1	0/1	0/1	0/1
2	1000	0/1	0/1	0/1	0/1
3	1600	0/1	0/1	0/1	0/1
4	2900	0/1	0/1	0/1	0/1
5	5000	0/1	0/1	0/1	0/1

0= number of deaths, 1= number of broilers used for the trial

The mortality record at the first stage of the acute toxicity study (Table 3) was zero in all the groups. This observation may be pointing to the fact that these extracts were safe at this stage and at all administered doses. The above observation was equally witnessed at the second stage (Table 4) of the acute toxicity determination study even with higher doses of 1000, 6000, 2900, and 5000 mg/kg. This indicates that the LD<sub>50</sub> of tested medicinal plants was above 5000 mg/kg and can therefore be labelled as safe among the categories of medicinal plants (Lorke, 1983). According to Loomis and Hayes (1996) classification, LD<sub>50</sub> dose range < 5 mg/kg is extremely toxic; 5 – 50 mg/kg is highly toxic; 50 – 500 mg/kg is moderately toxic; 500 – 5,000 mg/kg is slightly toxic; 5,000 – 15,000 mg/kg is practically non-toxic and > 15,000 mg/kg is relatively harmless. LD<sub>50</sub> for all the plants investigated in this study was higher than 5,000 mg/kg body weight. This value falls within the range of 5,000 – 15,000 mg/kg of Loomis and Hayes (1996) classification, indicating that the four plants were practically non-toxic to the experimental birds. The report of Hashemi *et al.* (2008) on acute toxicity of selected medicinal plants (*Solanum torvum*, *Euphorbia hirta*, *Zingiber officinale*, *Curcuma longa* and *Zingiber zerumbet*) orally dosed at 2000 mg/kg body weight also showed non-toxic effects on experimental broilers. One of the species of *Solanum* (used in this study) known as *Solanum torvum* was investigated by them, though the fruits were evaluated as against the leaves assessed in this study. The LD<sub>50</sub> of above 5,000 mg/kg obtained in this study agrees with that of Hauwa *et al.* (2015) and Ibrahim *et al.*, (2021) with similar results on Wistar rats. It is noteworthy that the acute toxicity determination of the selected plants in this study was not accessed in literature despite the volume of their usage in traditional medicine. This in itself has probably confirmed and lent credence to the essentiality of this research.

## CONCLUSION

The LD<sub>50</sub> of the considered medicinal plants was above 5000 mg/kg body weight. This is an indication of their safety at the level of acute toxicity. Recommended further studies on the plants include the evaluation of their sub-chronic and chronic toxicity on other species of animals, antibacterial evaluation of the extracts, and GC-MS analysis of the bio-active elements. This may form the bedrock and focus of the next research. Their analytical and experimental results may be useful in the formulation of relevant drugs for poultry birds and other livestock. Eventually, over-dependency syndrome on synthetic drugs for the management of animals in Nigeria could be significantly extenuated.

## DECLARATION OF CONFLICT OF INTEREST

No conflict of interest to declare.

## DECLARATION OF HONOUR

We declare on our honour that our results are not fake and made up.

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