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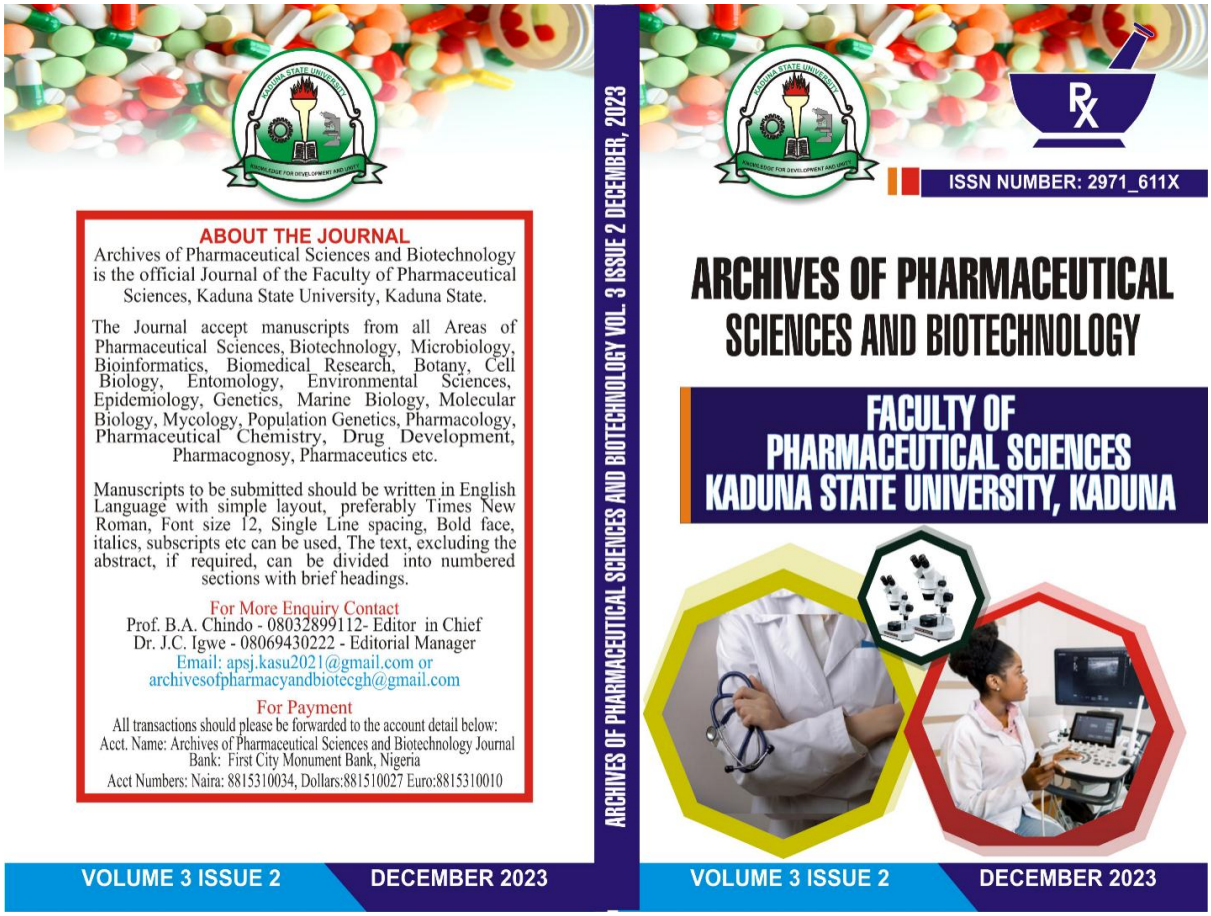

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PHYTOCHEMICAL EVALUATION AND IN VIVO ANTIDIARRHOEAIC EFFECTS OF TERMINALIA AVICENNIOIDES IN SWISS ALBINO RATS

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ABSTRACT

Aim: The study was undertaken to determine the *in vivo* antidiarrhoeaic effects of ethanol crude extract, aqueous, n-butanol and ethyl acetate fractions of *Terminalia avicennioides* leaf against clinical isolates of *Salmonella* Typhimurium isolated from diarrhoeaic patients attending some hospitals in Kaduna State.

Methods: The study was conducted in 2023. The phytochemical constituents of the leaf extract and its fractions were determined using standard analytical methods. The median Lethal Dose (LD_{50}) and the *in vivo* antidiarrhoeaic effects of the crude extract and fractions of the extract against clinical isolates of *Salmonella* Typhimurium were determined in Swiss albino rats using standard methods.

Results: The result of phytochemical screening revealed the presence of carbohydrates, cardiac glycosides, saponins, flavonoides, tannins, alkaloids, phenols and triterpenes. The LD_{50} of the crude extract and fractions of the crude extract were all found to be greater than 5000 mg/kg body weights of rats. None of the rats showed signs of abnormality in behaviour and mortality as in the control groups. The result of the *in vivo* antidiarrhoeal studies shows significant weight gain and reduction in the concentrations of *S.* Typhimurium in the faeces of some animal groups infected with 2.0 x 10^8 cfu/ml of *S.* Typhimurium cells during the eight (8) days treatment period with the 250 mg/kg body weight of crude extract and extract fractions. The effect was higher among the animal group treated with n-butanol fraction where fewer death were observed compared to other groups.

Conclusions: *Terminalia avicennioides* leaf has high *in vivo* antidiarrhoeal activities. The significant treatment effect, weight gain and reduction in concentrations of *S.* Typhimurium shed in the faeces of some infected groups of rats, may be attributed to the bioactive compounds present in the leaf extract of *T. avicennioides*.

Keywords: Antidirrhoeal effect, Terminalia avicennioides, n-Butanol fraction, *S.* Typhimurium, Swiss albino rats

1.0 INTRODUCTION

Worldwide, diarrhoeal diseases are reported as the leading cause of mortality among children aged five years and below where poor sanitation and insufficient potable water supply are key factors (1). *Salmonellae* are responsible for the second largest cause of bacterial food borne diseases. *Salmonella* species belong to predominant bacteria causing diarrhoeal illnesses in developing countries like Nigeria (2). *Salmonellae* are Gram negative, non-sporing, non -lactose fermenting, predominantly motile by means of peritrichous flagella, except for some serotypes. *Salmonella enterica serovar* Typhi and *serovar* Paratyphi cause diseases in only humans, whereas *Salmonella enterica serovar* Typhimurium can cause diseases in

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humans and animals (3). *Salmonella enterica* causes many diseases such as gastroenteritis, typhoid fever and salmonellosis. *S. enterica* are further classified into two sub-groups, namely, typhoidal or non-typhoidal. However, Salmonellosis is more frequent among people who consume foods from infected fresh foods and dairy products. Other enteric pathogenic bacteria associated with diarrhoea include *Vibrio cholerae*, *Vibrio mimicus, Yersinia enterocolitica, Citrobacter freundii,* and *Klebsiella pneumonia* (2). A study carried out by Ali (4) in 2023, on the prevalence of diarrhoea and the associated risk factors in Kano State reported that diarrhoea is a sign of infection in the intestinal tract caused by different bacteria, parasites and viruses. The pathogens cause increase in the mortality rate of infected patients due to their virulent nature as reported by Dairo (5) who determined the prevalence and determinants of diarrhoea among infants in Kaduna North.

Antimicrobial resistance makes *Salmonella* related cases difficult to treat resulting to ineffective response to medication and increased infection severity. Contaminated food, specifically pork meat, is responsible for the spread of multi-drug resistant *Salmonella*. However, *Salmonella* reaches the environment through faeces, thus making it harder to control their population. This could be due to temprature, nutrient availability, pH and the presence of other microorganisms in the environment which have signficant effect on the viability and proliferation of *Salmonella* (5). Antibiotics are widely used for effective treatment of bacterial diarrhoea. The indiscrimiate use of antibiotics contributed immensely to the rising prevalence of resistance among major human bacterial pathogens. This has prompted the use of plants as alternatives to antibiotics (2). Medicinal plants are effective against many diseases caused by microorganisms due to the secondary metabolites in plants (6).

The genus *Terminalia* belongs to the family Combretaceae. It is commonly called "Indian laurel" and locally referred to as "baushe" in Hausa, "Edo" in Igbo, and "Idiogon" in Yoruba. It comprises of approximately 200-250 species of medium to large flowering trees. *Terminalia* species are widely distributed in the savanna region of Africa and in the North -West of Nigeria, with the majority occurring in the Southern part of the continent. The ethanol leaf extract was reported to have some antibacterial effect against diarrhoeal pathogens like *Salmonella* Typhimurium and *Escherichia coli.* Traditionally, ointments of powdered leaves of *T. avicennioides* are used to treat rheumatic pains and other swollen joint conditions (6). Moreover, few studies have evaluated the anti diarrhoeal effect of *T. avicennioides.* Such studies include; the prevalence and determinants of diarrhoea among infants in selected primary Health Care Centers in Kaduna North Local Government Area, Nigeria and evaluation of phytochemicals and antibacterial properties of *Terminalia avicenioides* crude extract against selected bacteria from diarrhoeic patients. (5 and 6), .The present study was aimed to evaluate the phytochemical constituents, acute toxicity and *in vivo* antidiarrhoeal effects of *Terminalia avicennioides* Leaf extract and the extract fractions against some clinical isolates of *Salmonella* Typhimurium isolated from diarrhoeaic patients attending some hospitals in Kaduna State.

2.0 MATERIALS AND METHODS

2.1 Study Area/Design

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This study was carried out within Kaduna metropolis

2.2 Collection and Preparation of Plant Material

Fresh leaves of *Terminalia avicennioides* plant (Guil and Perr) were collected from Karaukarau village in Zaria Local Government area of Kaduna State, Nigeria in July 2022. The plant was identified and authenticated at the Biological Sciences Department, Kaduna State University, Nigeria and assighned a voucher number, 104. The leaves were thoroughly washed in a running tap, air dried under shade for two weeks and grounded to coarse powder using a mortar and pestle.

2.2.1 Extraction of Plant Material

Eight hundred grams (800g) of powdered leaf was in one (1) litre of 70% ethanol for 72hours. The filtrate collected in a crucible was concentrated in a water bath for two days at 40^0C to obtain dry crude extracts.

2.2.2 Fractionation of Crude Extract of *T. avicennioides* Leaf

Using separation funnel method (6), 120 g of the crude extract was dissolved gradually in 1.5 liters of sterile distilled water in a separating funnel. Two hundred and fifty milliliters (250 ml) of ethyl acetate was added to 250 ml of the extract solution (1:1v/v) the funnel was then shaken vigorously and allowed to stand for 15 minutes to allow for the separation of organic solvent from the mixture. The ethyl acetate was removed living the debris. The process was repeated twice to ensure complete separation. The same procedure was performed sequentially using n-butanol solvent. An aqueous layer (residue) which remained in the funnel was removed as the Residual Aqueous Fraction (RAF). Each fraction was collected in a beaker, labelled and evaporated to dryness at 40^0C using a rotary evaporator. Each extract fraction was tightly covered in a clean container and kept in a refrigerator at 4 ^{0}C for further use.

2.2.3 Phytochemical Screening of Crude Extracts and Extract Fractions of *Terminalia avicennioides* Leaf

The phytochemical screening of crude extracts and extract Fractions of *Terminalia avicennioides* Leaf was conducted in accordance with the methods of Evans (7) and Tiwari (8) to ascertain the presence or absence of carbohydrates, cardiac glycosides, saponins, flavonoides, Tannins, alkaloids anthraquinones, phenols, steroids, and triterpenes in the leaf extract and extract fractions of *Terminalia avicennioides.*

2.2.3.1 Detection of Carbohydrates

To 5ml of distilled water, 0.2 g of an extract was dissolved and filtered. The filtrate was used to test for the presence of carbohydrates as follows:

Molisch's Test: Filtrate was treated with drops of alcoholic a-naphthol solution in a test tube. The formation or non-formation of a violet ring at a junction indicated the presence or absence of carbohydrates.

Benedicts Tests: Filtrate was treated with Benedict's reagent and heated gently. Formation or non-formation of orange red precipitate indicated the presence or absence of reducing sugars.

Fehlings Test: Filtrate was hydrolyzed with diluted HCl, neutralized with alkali and heated with fehling's A and B solutions. Formation or non-formation of red precipitate indicated the presence or absence of reducing sugars.

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2.2.3.2 Detection of Cardiac glycosides

Keller-Kiliani Test: Five milliliters (5 ml) of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was mixed with 1ml of concentrated sulphuric acid. Formation or non-formation of brown ring at the interface, characteristic of cardenolides, indicated the presence or absence of a deoxysugar. A violet ring (may or may not appear below the brown ring, while in the acetic acid layer, a greenish ring may or may not form gradually), indicated the presence or absence of cardiac glycosides.

2.2.3.3 Detection of Saponins

Froth Test: To 1 g of extract 20 ml of distilled water was added and shaken in a graduated cylinder for 15 minutes. Formation or non-formation of 1cm layer of foam indicated the presence or absence of saponins.

Foam Test: Zero point five gram (0.5 g) of extract was shaken with 2 ml of water. Persistent production or non-production of foam for 10 minutes indicated the presence or absence of saponins.

2.2.3.4 Detection of Flavonoids

Alkaline Reagent Test: To 2 ml of water 0.2 g of extract was dissolved and treated with few drops of sodium hydroxide solution formation of intense yellow colour, which became colourless on addition of dilute acid or non-formation of yellow colour indicated the presence or absence of flavonoids.

Lead Acetate Test: To 2 ml of water 0.2 g of extract was dissolved and treated with 3 drops of lead acetate solution. Formation or non-formation of yellow colour precipitate indicated the presence or absence of flavonoids

2.2.3.5 Detection of Tannins

To 2 ml of water 0.2 g of extract was dissolved. About 1 ml of 1 % gelatin solution containing sodium chloride was added. Formation or non-formation of a white precipitate indicated the presence or absence of tannins

Ferric Chloride Test: To 2 ml of water 0.2 g of extract was dissolved and 1 % ferric chloride solution was added. Formation or non-formation of blue, green or brownish green colour indicated the presence or absence of tannins

2.2.3.6 Detection of Alkaloids

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium mercuric Iodide). Formation or non fomation of a yellow colour precipitate indicated the presence or absence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (iodine in potassium iodide). Formation or non-formation of a brown/reddish precipitate indicated the presence or absence of alkaloids.

Dragendoff's Test: Filtrates were treated with dragendroff's reagent (solution of potassium bismuth iodide). Formation or non-formation of a red precipitate indicated the presence or absence of alkaloids.

Harger's Tests: Filtrates were treated with Herger's reagent (Saturated picric acid solution). Formation or non-formation of a yellow colour precipitate indicated the presence or absence of alkaloids.

2.2.3.7 Detection of Anthraquinones

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Five grams (5 g) of each extract was stirred with 10 ml of aqueous sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of benzene. The benzene layer was then separated and 10% ammonia solution was added to half of its volume. Formation or non-formation of a pink red or violet colouration in the ammonia phase (lower layer) indicated the presence or absence of anthraquinone derviatives in the extract.

2.2.3.8 Detection of Phenols

Ferric Chloride Test: To 2 ml of water 0.2 g of extract was dissolved and treated with 3 drops of ferric chloride solution. Formation or non-formation of bluish black colour indicated the presence or absence of phenols.

2.2.3.9 Detection of Phytosterols and Triterpenes

Salkowski's Test: To 2 ml of water 0.2 g of extract was dissolved and treated with chloroform and filtered. The filtrate was treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance or non-appearance of golden yellow colour indicated the presence or absence of triterpenes.

Libermann Burchard's Test: To 2 ml of water 0.2 g of extract was dissolved and treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation or non formation of brown ring at the junction indicated the presence or absence of phytosterols.

2.2.4 Acute Oral Toxicity Studies or the median Lethal Dose (LD_{50}) of *Terminalia avicenniodes* Leaf Extracts and Extract Fractions

The LD_{50} of ethanol crude extracts, aqueous, n-butanol and ethyl acetate fractions of *T. avicennioides* leaf were carried out in accordance with the methods described by Lorke (9).

2.2.4.1 Experimental Animals

Swiss Albino rats (Wister strains) of same sex and matched weights were obtained from the animal house of the Department of Pharmacology and Clinical Pharmacy, ABU, Zaria. The animals were allowed access to pelleted feeds made from chick grower mash, purchased from Zaria. Kaduna, Nigeria. The animals were also allowed access to potable water in glass bottles ad libitum. They were also allowed to acclimatize to laboratory conditions for seven days prior to initiation of treatment with *T. avicennioides* extracts. Other care and handling of animals complied with the International Regulations for Animal Research (9).

2.2.4.2 Study Design

The study was conducted, in two phases using 16 rats for every test extract. Rats were fasted for two hours prior to treatment with each extract. In the first phase, 9 rats were divided into three groups of 3 animals each and were orally administered with an extract at dose levels of 10, 100 and 1000 mg/kg body weights (BW) of animals in the dose volume of 10mg/kg using a garvage needle. Another group (group four) was set as a control and the animals were given water and food only. The animals were observed for changes in general behaviour, toxic symptoms and mortality for a period of 2 hours and then 24 hours after treatment. The result from the first phase was used to determine the specific extract doses that were given separately to 4 rats (a dose per rat) in the second phase. The animals were also

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observed for changes in general behavior, toxic symptoms and mortality for a period of 24 hours. The median lethal dose (LD_{50}) after each treatment was calculated using the following formula;

$$LD_{50} = \sqrt{\text{Minimum Toxic dose} \times \text{Maximum Tolerated dose}}$$

2.2.5 Collection of Clinical Bacterial Isolates

A total of 31 Clinical isolates of *S.* Typhimurium isolated from patients suffering from diarrhoea were obtained from Yusuf Dantsoho Memorial Hospital (YDH) Tudun Wada, Gwamna Awan General Hospital (GAH) Kakuri and Barau Dikko University Teaching Hospital (BDUTH) Kaduna. Of the 31 isolates of *S.* Typhimurium collected, 15 (48.4 %) were from YDH, 5 (16 %) from GAH and 11 (35.5 %) from BDUTH. All isolates were inoculated in nutrient agar slants, labelled, placed in ice-pack and transported to the laboratory of the Department of Microbiology, Kaduna State University. Isolates were confirmed using standard microbiological methods () and stored in a refrigerator operating at 4^0C, till required.

2.2.6 Determination of Infective Dose of Bacteria

The infective dose of *S.* Typhymurium was determined in accordance with the method described by 10. Two (2) different sets of sterile test tubes (ten per set) were serially arranged and dispensed with 9 ml of sterile distilled water. One ml from a suspension containing *S.* Typhymurium cells was introduced into the first tube of one set of tubes making 1:10 dilution. This procedure was repeated for the remaining tubes. The remaining tubes were serially diluted. One ml from the dilutions containing *S.* Typhimurium cells w*as* pour plated on Salmonella, Shigella agar. All plates were incubated at 37 ^{0}C for 24 hours. Visible colonies were counted and estimated according to the dilution factors. Ten (10) groups of 3 rats each were challenged orally with 1ml of the different dilutions containing the test bacteria. The rats were observed for a period of three days for any clinical symptom of infection. The dilution that established infection in the rats and not high enough to cause rapid mortality and were able to produce clinical effects, such as unformed stool or diarrhoea, weakness and loss of appetite on the animals was used as the infective dose of the bacteria

2.2.7 *In vivo* Antidiarrhoeal Activities of *Terminalia avicenniodes* Leaf Extract and Extract Fractions

The *in vivo* antidiarrhoeal activities of ethanol crude extract, aqueous, n-butanol and ethyl acetate fractions of *T. avicennioides* leaf in Swiss albino rats were determined in accordance with the methods described by Momoh (11) and Bulus (12).

2.2.7.1 Infection of Albino Rats with *S.* Typhymurium and Treatment with *T. avicennioides* Leaf Extracts and Metronidazole

Two (2) months old albino rats of same sex and known weights were obtained from the animal house of the Department of Pharmacology and clinical Pharmacy Ahmadu Bello University Zaria. Animals were divided in to four groups of five animals (A, B, C and D) in separate cages including

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the controls, and maintained under 12/12 hours of light-dark cycle. They were allowed to acclimatize for 2 weeks during which they were fed with pelleted feeds and deionized water. After two weeks feaces of rats were examined using standard biochemical tests to ensure that they were negative for *S* Typhimurium before inoculation with 2.0 x 10^8 cfu/ml viable cells of *S* Typhimurium Group A (neutral control) was neither challenged orally with bacteria nor treated with extract, group B (negative control) was challenged with bacteria but not treated, group C was infected and treated with 50 mg/kgbody weights(kgbws)of metronidazole (positive control) and group D was infected and treated with 250 mg/kgbws of ethyl acetate fraction of *T. avicennioides* leaf fraction respectively. The frequency of diarrhoea was noted and scored based on consistency and number of defecation (greater than 2 times), starting from day 3 when infection was set in to the end of the treatment period (day 8).The mean weights and clinical symptoms observed in the experimental rats as well as the number of viable *S* Typhimurium cells per gram of faeces of rats were determined (12)

3.0 RESULTS

The yield of ethanol crude extract of *Terminalia avicenniodes* leaf after extraction is 18.8 %. The yields of fractions of the crude extract after fractionation of the crude extract are 28.1%, 13.2% and 5.7% respectively. The results of phytochemical screening of ethanol leaf extract and extract fractions of *T. avicennioides* revealed the presence of alkaloids, flavonoides, tannins, saponins, phenols, triterpenes, cardiac glycosides and steroids (Table 1). Steroids were only found in ethyl acetate fraction. Alkaloid was absent in ethyl acetate fraction but present in aqueous and n- butanol fractions.

Out of 31 (100%) clinical isolates of *S.* Typhimurium obtained from diarrhoeic patients attending the selected hospitals in Kaduna State (Table 2), 21 (67.7%) isolates were confirmed using standard microbiological methods.

The result of acute oral toxicity test or Lethal Dose (LD_{50}) of ethanol crude extract of *T. avicennioides* and extract fractions in Swiss albino rats is presented in Table 3. All the rats treated with the crude leaf extract and fractions of the extract showed no signs of abnormality in behaviour and death when compared with the control groups. $LD_{50.}$ of each extract was greater than 5000 mg/kg body weight of animals.

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Table 1: Phytochemical Constituents of Ethanol Leaf Extract and Extract Fractions of *T. avicennioides*

Constituents	Leaf Extract Ethanol	Leaf Fractions		
		Aqueous	N – butanol	Ethyl acetate
Carbohydrates	+	+	+	+
Anthraquinones	-	-	-	-
Steriods	-	-	-	+
Triterpenes	+	+	+	+
Cardiac glycosides	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Flavonoides	+	+	+	+
Alkaloids	+	+	+	-
Phenols	+	+	+	+

Table 2: Proportions of Clinical isolates of *S.* Typhimurium Confirmed from Various Hospitals

Hospitals	No of Isolates Collected (%)	No of Isolates Confirmed (%)
YDH	15 (48.4)	9 (42.9)
GAH	5 (16)	2 (9.5)
BDUTH	11(35.5)	10 (47.6)
Total	**31(100)**	**21 (67.7)**

Keys: YDH – Yusuf Dantsoho General Hospital, GAH – Gwamna Awan General Hospital, and BDUTH – Barau Dikko University Teaching Hospital, Kaduna

Table 3: Acute Oral Toxic Effects (LD_{50}) of Ethanol Crude Extract and Extract Fractions of *T. avicennioides in* Albino rats

Experiment	Extract Dose (mg/kg body weight)	ECE	AQF	BLF	EAF
		(Mortality after 24 hours of each phase)			
Phase 1	10	0/3	0/3	0/3	0/3
	100	0/3	0/3	0/3	0/3
	1000	0/3	0/3	0/3	0/3
Control	0	0/3	0/3	0/3	0/3
Phase 2	1600	0/1	0/1	0/1	0/1
	2900	0/1	0/1	0/1	0/1
	5000	0/1	0/1	0/1	0/1
	0	0/1	0/1	0/1	0/1

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Keys: ECE = Ethanol crude extract, AQF = Aqueous fraction, BLF = n- butanol fraction, EAF= Ethylactate fraction.

The result of *in-vivo a*ntidiarrhoeal effects of crude extract and extract fractions on the mean weights of rats infected with *S.* Typhimurium is presented in Table 4. The rats in group A (not infected and not treated) constantly gained weight while there was a decrease in the mean weights of all the infected groups of rats within 72 hours immediately infection was established followed by significant ($P < 0.05$) weight gain on day 5 among the control group treated with 50 mg/kg body weight (bw) of metronidazole and the groups treated with 250 mg/kgbw each of n- butanol and ethyl acetate fractions. Similar weight gain was observed on day 6 and 7 among the rat groups treated with similar doses of crude extracts of *T. avicennioides* Leaf and aqueous fractions of the leaf extract respectively.

Table 4: Effect of *T. avicennioides* Extracts and Fractions on the Mean Weights of Albino rats Infected with *S.* Typhimurium

Extacts	Duration of Treatment (days)/Weight of Albino rats Infected with *S.* Typhimurium (g)							
	0	**2**	**3**	**4**	**5**	**6**	**7**	**8**
A	166±1.69c	166±0.51c	167.00±1.82d	167.60±1.69d	167.80±1.71d	168.20±1.66d	168.40±1.69d	169.00±1.58d
B	160±0.71b	157.20±0.37a	157.80±0.73b	156.00±0.63ab	155.20±0.58ab	154.00±0.00ab	152.50±0.50a	152.00±1.00a
C	157.40±0.24a	158.00±0.00b	156.80±0.49b	156.80±0.49b	157.40±0.68b	157.80±0.49b	157.40±0.60b	158.80±0.58bc
D	155.80±0.37a	158.00±0.00b	154.80±0.37a	154.40±0.51a	153.80±1.02a	155.50±0.50b	155.50±0.50ab	156.75±0.49b
E	159.40±0.51ab	158.00±0.00b	157.80±0.86b	157.00±1.05b	156.20±1.24ab	152.60±0.51a	158.00±4.00b	158.50±3.50bc
F	160.80±0.37b	160.80±0.37c	158.00±0.00b	158.00±0.00b	161.20±0.37c	161.40±0.40c	162.60±0.40c	162.60±0.40c
G	167.80±0.37c	167.80±0.37d	158.00±0.00b	158.00±0.00b	166.80±0.73d	167.20±0.49d	168.25±0.75d	168.25±0.75d
p v	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*

Values are Means ± Standard Deviation of 5 determinants. Values with different letters in superscripts in a column are significantly different at $P< 0.05$

Keys: A= Not infected and not treated (neutral control), B= Infected but not treated (negative control), C= Infected and treated with metronidazol (positive control), D = Infected and treated with ethanol leaf extract, E= Infected and treated with aqueous fraction, F= Infected and treated with n- butanol fraction, G= Infected and treated with ethyl acetate fraction, pv = p value

About 60% of all infected rats in each dosed group manifested the symptoms of diarrhoea 72 hours after inoculation with the infective dose of selected viable *S.* Typhimurium cells (Table 5). Other symptoms observed were loss of appetite, general body weakness and deaths of some rats (Table 5)

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Table 5: Observed Mortality Rate /Clinical Manifestation in Infected Rats

Symptoms	Number of rats affected / total number of rats in each treatment group						
	A	B	C	D	E	F	G
Mortality rate (%)	0/5(0)	2/5(40)	0/5(0)	1/5(20)	2/5(40)	0/5(0)	2/5(40)
Watery diarrhea (%)	0/5(0)	3/5(60)	3/5(60)	3/5(60)	3/5(60)	3/5(60)	3/5(60)
Loss of appetite (%)	0/5(0)	2/5(40)	2/5(40)	1/5(20)	2/5(40)	1/5(20)	3/5(60)
Loss of weight (%)	0/5(0)	3/5(60)	2/5(40)	3/5(60)	2/5(40)	2/5(40)	3/5(60)
Body weakness (%)	0/5(0)	3/5(60)	2/5(40)	1/5(20)	2/5(40)	1/5(20)	2/5(40)

Keys: A= Not infected and not treated (neutral control), B= Infected but not treated (negative control), C= Infected and treated with metronidazol (positive control), D = Infected and treated with ethanol leaf extract, E= Infected and treated with aqueous fraction, F= Infected and treated with n- butanol fraction, G= Infected and treated with ethyl acetate fraction.

The result on faecal shedding of *S.* Typhimurium in the faeces of infected groups of rats is presented in Table 6. When comparing the activities of extracts with the controls, n-butanol fraction compared favourably with metronidazole as it was the first to eliminate *S.* Typhimurium from the faeces of rats

Table 6: Effect of Extracts of *T. avicennioides* Leaf on the Load of *S.* Typhimurium Shed in the Faeces of Infected Albino rats

Extract	Duration of Infection(days) and Effect of Treatment on the Load of *S.* Typhimurium (cfu/g)							
	1	2	3	4	5	6	7	8
A	ND	ND	ND	ND	ND	ND	ND	ND
B	ND	ND	$2.7x10^{2}b$	$3.6x10^{2}c$	$6.6\ x10^{4}c$ 2	$2.0\ x10^{3}b$	$1.5x10^{2}a$	$4.7x10^{1}a$
C	ND	ND	$2.7\ x10^{3}b$	$2.4±x10^{3}a$	$7.1\ x10^{2}d$	$3.6\ x10^{1}c$	ND	ND
D	ND	ND	$2.6x10^{2}a$	$2.6x10^{2}a$	$6.2\ x10^{2}c$ 1	$3.4\ x10^{2}b$	ND	ND
E	ND	ND	$4.0\ x10^{2}d$	$4.0x10^{3}d$	$1.5x10^{5}a$	$3.1\ x10^{5}c$	$2.5x10^{5}b$ 2	$2.5\ x10^{5}b$
F	ND	ND	$3.1\ x10^{3}c$	$3.2x10^{2}b$	$3.2\ x10^{1}b$	ND	ND	ND
G	ND	ND	$2.9x10^{2}b$	$3.1\ x10^{2}b$	$3.0\ x10^{3}b$ 2	$1.5\ x10^{2}a$	ND	ND
P value			0.02*	0.0088**	0.0001**	0.0002**	0.0001**	0.0001**

Values are Means ± Standard Deviation of 5 determinants. Values with different letters in superscripts in a column are significantly different at P< 0.05
Keys: A= Not infected and not treated (neutral control), B= Infected but not treated (negative control), C= Infected and treated with metronidazol (positive control), D = Infected and treated with ethanol leaf extract, E= Infected and treated with aqueous fraction, F= Infected and treated with n- butanol fraction, G= Infected and treated with ethyl acetate fraction, = Dead rats.

The result on mean haematological parameters of *S.* Typhymurium infected albino rats is presented in Table 7. The decrease in the average Packed Cell Volume (PCV) and Red Blood Cells (RBC)

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counts observed in all the infected groups of rats were found to be significant ($P < 0.05$) when compared with the control groups. The variations observed in the average counts of White Blood Cells (WBC), Neutrophils (N) and Lymphocytes of the infected rats were also found to be significant ($P < 0.05$). The infected non treated rats and the ethyl acetate treated rats had the least RBCs and Hb counts and the highest WBC and neutrophyl counts.

Table 7: Mean Haematological Parameters of Infected Albino Rats

Extract	Haematological Parameters of Infected Albino Rats.					
	PCV%	RBC(10^6/mm^3)	H b(g/dL)	WBC(10^6/mm^3)	N(10^3/mm^3)	L(10^3/mm^3)
A	39.00±1.39d	7.63±0.24b	12.83±0.35c	9.23±0.58b	2.43±0.15b	4.83±0.08a
B	32.00±1.39b	5.93±0.07a	9.67±0.35a	13.00±0.58c	3.83±0.15c	4.70±0.08a
C	39.33±1.39d	7.40±0.52b	12.20±0.35c	10.53±0.58b	2.70±0.15b	4.97±0.08b
D	37.33±1.39c	6.87±0.06ab	11.40±0.35b	8.00±0.58ab	1.87±0.15a	5.00±0.08c
E	28.33±1.39a	5.10±0.56a	9.67±0.35a	12.37±0.58c	3.63±0.15c	4.80±0.08a
F	37.67±1.39c	6.87±0.07ab	11.80±0.35b	8.83±0.58ab	2.23±0.15b	4.93±0.08b
G	37.00±1.39c	6.80±0.00ab	11.87±0.35b	7.23±0.58a	2.17±0.15b	5.10±0.08c
P value	0.0005*	0.0001*	0.0001*	0.0001*	0.0001*	0.069

Values are mean ± Standard deviation of 5 determinations. Data in the same column with different superscript letters are significantly different ($P < 0.05$)
Key: PCV= Parked Cell Volume, Hb = Hemoglobin, WBC = White Blood Cell, N = Neutrophil, L = Lymphocytes

4.0: DISCUSSION

Related findings on phytochemical studies of plant parts obtained from Bida, Niger State was reported by Itelima and Agina (13) where there was the absence of saponins in the leaf extract. Several factors like geographical source, soil condition, harvest processing and post-harvest processing time, moisture content, drying method and storage condition could be attributed to the variation in the composition of secondary metabolites extracted from plant tissues (5). Similarly, Mann (14) reported that the high temperature generated during tissue grinding can denature chemical constituents and this may invariably affect the composition of compounds and the level of biological activity of a plant material. Phytocompounds are known to be biologically active and can aid the antibacterial activities of *T. avicenniodes* and reports have shown that several compounds belonging to the investigated classes of metabolites showed antimicrobial activities (13, 5, 15). For example one of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms which have been widely studied for their potential use in the elimination and reduction compared to other active plant compounds (16). Carbohydrates being the main components of cell wall and protoplasm play important roles in diverse biological processes, including viral and bacterial infections, cell growth and proliferation, cell-cell communication, as well as immune response (17).

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The result of acute toxicity test conformed with the study conducted by Lorke (9) who provided the range of doses of plant extracts and LD_{50} values. This study also agree with the study with the conducted by Bulus (12), who evaluated the toxic effect of aqueous stem extract of *Terminalia avicennioides* in rats. The lack of mortality among rats that were orally treated could be due to first pass effect of the liver. Orally administered substances usually undergo three events; release from dosage form, mucosal barrier and passage to the liver for detoxification. Each of these stages has the potential to decrease the amount of any substance reaching the blood circulation (18). Fewer animals were used during the second phase of the toxicity test in order to obtain a realistic result and to minimize the number of rats used (9). This is in line with International Animal Research Regulation (19), who suggested using fewer animals in order to achieve significant results with minor pain and distress.The $LD_{50.}$ of each extract that was found to be greater than 5000 mg/kg body weight is an indication that the crude extract of *T. avicennioides* and extract fractions administered to the Swiss albino rats were safe.

The establishment of infection within 72 hours observed in infected rats during the *in vivo* studies could be due to the virulent properties of the test bacteria. The mortality recorded could be due to severity of diarrhoea caused by bacteria. The variation in mortality rates observed in the different groups may be attributed to individual host immune status (20).

The load of *S.* Typhimurium in the faeces of individual rats inoculated with viable cells of *S.* Typhimurium was found to be consistent with the short time intervention study conducted by 21 on faecal shedding of *S.* Typhimurium infected cattle, where bacteria were reduced to undetectable concentrations compared to non-antibiotic treated cattle within 7 days of treatment.The significant treatment and time effects demonstrated by the plant extracts in reducing the concentrations of *S.* Typhimurium in the infected rats, may be attributed to the type and quantity of bioactive ingredients present in the extracts of *T. avicennioides* (5).

The significant decrease in the average Packed Cell Volume (PCV) and Red Blood Cells (RBC) counts observed in all the infected groups of rats as well as the high neutrophil levels could be due to the severity of infection. The result corresponds with the report of Al-Hadithy (22) who reported similar findings on blood parameters of lactating ewes infected with mastitis

5.0 CONCLUSION

In conclusion, the phytochemical constituents detected in the *T. avicenioides* extract are carbohydrates, alkaloids, flavonoides, tannins, saponins, phenols, triterpenes, cardiac glycosides and steroids. The ethanol crude extract, aqueous, n-butanol and ethyl acetate fractions of *Terminalia avicennioides* leaf were each found to be safe in Swiss albino rats with LD_{50} greater than 5000 mg/kg body weights of rats. The significant treatment effects, weight gain and reduction in concentrations of *S.* Typhimurium shed in the faeces of some infected groups of rats, may be attributed to the bioactive compounds present in the leaf extracts of *T. avicennioides*. N-butanol fraction exhibited highest *in vivo* antidiarrhoeal activity compared to other extracts. N-butanol fraction can therefore be used to treat diarrhoea caused by *S.* Typhimurium. Further research should focus on isolating the active ingredients responsible

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for the observed *in vivo* antidiarrhoeal activities.

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Authors Contributions

Musa, F.M designed the entire aspect of the work. Muhammad-Idris Z.K. wrote the first draft of the manuscript with literature search. Wartu, J.R. wrote the second draft of the manuscript. All authors read and approved the final manuscript.

REFERENCES

1. Centres for Disease Control and Prevention.(2020) Water, Sanitation, and Environmentally-Related Hygiene https://www.cdc.gov/healthywater/hygiene/disease/chronic_diarrhea.html , Accessed 30th August 2023
2. Sule, E.I., Aliyu, A.M and Abdulaziz, B.M. (2011). Isolation of diarrheagenic bacteria in children attending some selected hospitals within Kaduna metropolis. *Continental Journal of Applied Science*. 6(1): 1 – 6.
3. Kato, K. and Ishiwa, A. (2015). The Role of Carbohydrates in Infection Strategies of Enteric Pathogens', *Tropical Medicine and Health*. 43 (1): 41-52
4. Ali, M., Ahmed, I., Yusha,u, M. and Shehu, A.A. (2023): Prevalence of Diarrhoea and Risk Associated Factors Among Children Under 5 in Kano, North Western Nigeria. *Archives of Pharmacy and Pharmacology Research* 3 (3): 1-7
5. Dairo, M.D., Ibrahim, T.F. and Salawu, A. T. (2017): Prevalence and Determinants of Diarrhoea Among Infants in Selected Primary Health Care Centers in Kaduna North Local Government Area, Nigeria. *Pan African Medical Journal*. 28: 109. 8159
6. Musa, F. M., Ameh, J. B., Ado, S. A. and Olonitola, O. S. (2016): Evaluation of phytochemical and antibacterial properties of *Terminalia avicenioides* crude extract against selected bacteria from diarrhoeic patients. *Bayero Journal of Pure and Applied Science*, 9(1): 129: 137.
7. Evans, W.S.(2009).*Trease and Evans Pharmacognosy*, 16th edition W.B. Saunders, Toronto. Harcourt Pub Ltd. Pp1-40,516-525
8. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kau, H. (2011). Phytochemical Screening and Extraction. AReview. International Pharmaceutica Sciencia, 1(1)
9. L orke, D. (1984). *A New approach to practical acute toxicity testng*. *Archives of Toxicology*, 54: 275-287.
10. Onanuga, A., Oluwatoyin, I., Adebayo, L. (2014). A study of the

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prevalence of diarrhoeagenic *Escherichia coli* in children from Gwagwalada, Federal Capital Territory, Nigeria. *The Pan African Medical Journal.* 17: 146.

11. Momoh, A.O., Adebolu, T.T and Ogundare, A.O. (2013). Evaluation of Beniseed extract and fermented liquor, in treatment of diarrhoea in albino rats infected with *Salmonella typhimurium*. *European Journal of Biology and Medical Science Research*, 1 (2) 16 – 23
12. Bulus, T., S.E. Atawodi and Mamman, M. (2011). Acute toxicity effect of the aqueous extract of *Terminalia avicennioides* on white albino rats. *Science World Journal,* 6 (2): 1 - 4
13. Itelima, J.U., Agina, S.E. (2014). *In vivo* Antimicrobial Activity of Plant Species on *Escherichia coli* O157:H7 inoculated into albino rats. *World Journal of Microbiology*. 1(1) 002-009.
14. Mann, A., Kuta, Y.A. (2014). Antibacterial activity of methanolic extracts of *Terminalia avicennioides* against fish pathogenic bacteria. *American Journal of Research Communication.* 2(4): 133 – 146.
15. Wendakoon. C., Calderon. P., Gagnoon. D. (2012). Evaluation of selected medicinal plants extracted in different ethanol concentrations for antibacterial activity organist human pathogens. Journal of medicinally active plants (1) 2
16. Hassan M., Musa F.M., Adamu A. and Baba Gabi. (2022): Phytochemical Analysis and Antibacterial Activity of Fractions of *Sida acuta* against Some Reference Isolates of Bacteria. *Science World Journal.* 17 (1): 26 – 30.
17. Bushra, S., Farooq, A., Muhammad, A. (2009). Effect of Extraction solvent / Technique on the Antioxidant Activity of selected Medicinal Plant Extracts. Molecules, 14:2167-2180.
18. Suleiman, M. M., Romanus, I.O, Yusuf, S. (2007): Gastro protective effect of the crude methanol extract of *Terminalia avicennioides in rats*. *Vertrinary Archives* 77, 345-354.
19. International Animal Research Regulations. (2012). Impact Research. National Academies Press (US). Workshop Summary.
20. Oladunmoye, M. K. (2007): The Immunostimulatory Effect of Ethanolic Extract of *Cassia alata* on immune system of Albino Rats Dosed with *Staphylococcus aureus* (NCIB 8588). *Journal*
21. Elder, R.O., Keen, J.E., Wittum, T.E., Callaway, T.R., Endrinton, T.S., Anderson, R.C., Nisbet, D.J, (2002). Intervention to Reduce Faecal Shedding of Enterohaemorrhagic *Escherichia coli* O157:H7 In Naturally Infected Cattle Using Neomycin Sulfate. *Journal of Animal Science,* 80(1): 151
22. Al-Hadithy, H.A. and Suleiman, J.M. (2014). The hematological parameters in clinically normal lactating and ewes affected with mastitis. *Kaufa Journal for Vetenary Medical Sciences vol. 5 No. 5*.