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Evaluation of the Antimicrobial Activity and Toxicity of Local and Foreign Seeds of *Azanza garckeana* (Goron Tula) Extracts

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Abstract

Drug resistance challenges antimicrobial treatment options, necessitating the continuous search for plant extracts with therapeutic potential. The study determines the antimicrobial activities and toxicity effects of local and foreign seeds of Azanza garckeana. Constituents of the seeds of A. garckeana were extracted and identified using standard phytochemical procedures. Clinical isolates from patients diagnosed with Urinary Tract Infections (UTIs) were confirmed using standard microbiological procedures. Disk diffusion techniques were used to assess the antimicrobial properties of the plant extract, and Mass Spectrometry and Gas Chromatography were used to identify the bioactive components. The toxicity of plant extract was assessed using acute toxicity tests and histopathological and hematological studies. The findings indicated the existence of alkaloids, carbohydrates, saponins, tannins, and flavonoids in both local and foreign seeds of A. garckeana. Five (5) organisms were identified from patients with UTIs. Antimicrobial activities showed that the Petroleum ether extract of foreign A. garckeana exhibited higher antibacterial activity against Staphylococcus aureus (15mm), Pseudomonas aeruginosa (13mm) than Petroleum ether extract of local A. garckeana at a concentration of 200µg/ml respectively. Similarly, A. garckeana foreign aqueous extracts showed higher activity against S. aureus (13mm) and P. aeruginosa (12mm) than local Aqueous A. garckeana at 200µg/ml concentrations, respectively. The GCMS analysis showed the existence of Dodecanoic acid, ethyl ester, Octadecenoic acid, Undecanoic acid, and methyl ether. The local and foreign seeds of A. garckeana were non-toxic at ≤ 600 mg/kg. Histopathological and hematological studies showed a heart with normal features, a kidney with slight hyperplasia of inflammatory cells, and a liver with slight hepatic necrosis at 1000 mg/kg. The study demonstrated that the local and foreign seeds of Azanza garckeana had antimicrobial therapeutic potential, but its usage should be dose-dependent, not exceeding ≤ 600 mg/kg.

Keywords: Azanza garckeana, Antimicrobial activity, Toxicity, Histopathology, Hematology

INTRODUCTION

Drug resistance continues to pose a challenge to antimicrobial treatment options around the globe, necessitating the continuous search for plant extracts with antimicrobial therapeutic potential. Vennaposa *et al.* (2013) noted that demand for medications, health products, food supplements, cosmetics, and other plant-based items is rising. In an earlier study, Parekh and Chanda (2007) noted that the growing prevalence of antibiotic side effects and antibiotic resistance in harmful microorganisms necessitate the development of different antimicrobial medications with unique modes of action and a range of chemical structures for treating infectious diseases.

Azanza garckeana is a valuable edible indigenous fruit tree and can be found in Nigeria at Kankiya, Daggish, Northwest Katsina State, Daggish in the middle belt and mile north of River Benue and can also be found in the majority of Northeastern markets, particularly in rural regions, and in the Tula area of Kaltungo local government area of Gombe State, as well as the Kali hills of Zah district of Michika local government area of Adamawa State (Yusuf, 2020). Lako *et al.* (2007) opined that being one of the few plant species, *A. garckeana* benefits the nutritional, medicinal, and economic security of local communities in sub-Saharan Africa and should be included in the domestication process in farming systems. Van Wyk (2011) asserts that *A. garckeana* fruits have promise to create novel food and beverage products.

Examining medicinal plants that are readily available in an area and searching the biologically active chemicals from the extracts of the plant species for utilization in traditional and herbal medicine for potential antibacterial

qualities becomes one of the important methods. The choice of *A. garckeana* in this study was connected to the extensive historical use of the plant, particularly the seed, in treating various ailments in different parts of Nigeria, especially the Northern and Southern parts. Additionally, documented works reported that various plant sections had different pharmacological activities and are used for various ailments (Yusuf *et al*., 2020; Ahmed *et al*., 2016); Mshelia *et al*., 2016). However, reviewed literature indicated that there was inadequate knowledge describing the plant's toxicity, especially regarding histopathological studies as well as the effect of the plant on kidney and liver function. The current investigation seeks to demonstrate the antibacterial efficacy of both foreign and local seeds of *A. garckeana* and evaluates its toxicity and effect on kidney and liver function.

MATERIALS AND METHODS

Fruits of *A. garckeana* were purchased from a local market in Tula Kaltungo, Gombe State, Nigeria, while the foreign seed was purchased from Islamic Chemist Shop at Kasuwar Rimi Market, Kano, Nigeria. Identification of the plant materials was done according to the methods of Demotrio *et al*. (2015) at Bayero University, Kano, Department of Plant Biology.

Drying and Processing of *A. garckeana* Seed

The seeds of *Azanza garckeana* were properly cleaned several times under running water and stored at room temperature in the shade to dry. After drying up, the seeds were processed into a powder and stored in an appropriately sealed labeled plastic bag, as stated by Tukur and Mukhtar (1999).

Extraction of the *A. garckeana*

The procedure for extracting plant material was done using the percolation method described by the Association of Official Analytical chemist AOAC (2012). One hundred grams (100g) of the dehydrated, powdered plant material of both local and foreign *Azanza garckeana* were soaked in water (250ml), petroleum ether (250ml), and methanol (250ml), respectively, in a volumetric flask for one week. Each solvent mixture was mixed and shaken for an entire night in a mechanical shaker. It was then filtered and concentrated in a water bath set at 560ºC and moved to a beaker. The filtrates were then evaporated, and phytochemical evaluation and bioassay were conducted using the residues.

Determination of Phytochemical Constituents of *Azanza garckeana* extracts

Phytochemical screening was carried out to detect the presence of some metabolites. The presence of alkaloids was detected according to the methods of Lalitha and Jayanthi (2012). Tannins were detected based on the procedure that Ciulci (1994) outlined. There was a noticeable green-black or blue tint, suggesting tannin's presence. Flavonoids were detected according to AOAC (2012), where a red or intense red coloration indicated the presence of flavonoids. The presence of Saponins was determined as demonstrated by Sofowora (1993), where continuous foam that persisted for roughly fifteen minutes indicates saponins' existence. Steroids were detected, as demonstrated by Soforowa (1993), and the presence of steroids was indicated by a violet tint in the supernatant layer and a reddish-brown ring at the interface of the two liquids. Glycosides were determined as demonstrated by Soforowa (1993), and the appearance of brick red precipitate indicates glycosides' existence. The presence of terpenoids was determined according to Ciulci (1994), and the interface's reddish-brown coloring suggests the presence of terpenoids.

Collection and confirmation of clinical isolates

The isolates for the study were obtained from patients with Urinary Tract Infections from the Microbiology Department of Aminu Kano Teaching Hospital, Kano (AKTH**).** The isolates included *Escherichia coli*, *Klebsiella sp*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. The isolates were confirmed using standard microscopic, cultural, and biochemical tests according to the method of Cheesbrough (2006). The pure cultures of the identified bacteria were streaked onto Nutrient Agar slants incubated at 24 hours and thereafter stored at 4ºC.

Standardization of the inoculum

The bacterial isolates were cultivated in nutrient broth for a duration of 18 to 24 hours, after which a loopful of it was diluted in normal saline (0.85% Nacl w/v) until its turbidity matched the standard turbidity of 1% (w/v) barium sulphate solution (Mukhtar and Tukur, 1999). The resulting turbidity was approximately 3.33×10^6 cfu/ml.

Preparation of Different Concentrations of the Extracts

Various concentrations of the extracts were prepared, as described by Taura and Oyeyi (2009). A stock solution of 100 mg/ml, one (1) gram of each plant extract was reconstituted in 10 ml of dimethyl sulphoxide (DMSO) for the methanol and petroleum ether extract and distilled water for the aqueous extract.

To get lower concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.25 mg/ml, and 1.125 mg/ml were obtained using the twofold dilution technique.

Antimicrobial Susceptibility Testing of *A. garckeana* Extract

The susceptibilities of the bacterial isolates to *A. garckeana* were determined using the agar well diffusion method according to Nester *et al.* (2004). Muller Hinton Agar was made according to the manufacturer's instructions, autoclaved, and aseptically poured into sterile Petri dishes and allowed to gel. Each agar plate was streaked evenly with a loopful of the standardized bacterial suspension, and a sterile cork borer was used to make wells (6 mm diameters) on each plate. Next, 0.1 milliliters of the extracts at different concentrations (50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.25 mg/ml, and 1.125 mg/ml, respectively) were added to the wells. 0.1ml of distilled water and 0.1ml 250 µg/ml of Ciprofloxacin were used as negative and positive controls, respectively. After pre-diffusion for 30 minutes on the table, the plates were incubated for 24 hours at 37^0C. After incubation, zones of inhibition created by the extracts against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Staphylococcus aureus* were measured and recorded in millimeters (mm). Cheesbrough, M. (2006)

The Minimum Inhibitory Concentration (MIC) was obtained using the broth dilution method in accordance with the methods of Ali *et al.* (2017). Sterilized distilled water was used to create a solution of two-fold dilutions with concentrations of 25, 12.5, 6.25, 3.125, and 1.56 mg/ml. Equal volumes of the aforementioned concentrations were mixed with nutrient broth in a 1:1 ratio, and each test tube was filled with 0.1 ml of the test organisms' standard suspension (3.33 × 106 cfu/ml). After that, the tubes were incubated aerobically for 24 hours at 37^0C. As positive controls, tubes with just broth and extract were used, while as negative controls, tubes with broth and inocula were used. The extract's highest dilution (least concentration) demonstrating no visible growth was considered the minimum inhibitory concentration.

Ali *et al.* (2017) used the technique to determine the Minimum Bactericidal Concentration (MBC). The tubes from MIC that showed no discernible growth were used. Nutrient agar plates were coated with precisely 0.1 ml of the bacterial culture from the MIC tubes, and these plates were then incubated at 37^0C for 24 hours. Plates that did not exhibit any visible bacterial growth were classified as MBC.

Identification of the Bioactive Compounds of the Crude Extracts Using Gas Chromatographic Mass Spectrometry (GC-MS) Analysis

Gas Chromatographic Mass Spectrometry (GC-MS) analysis was used to identify the bioactive substances in the petroleum ether and methanol seed extract of *A. garckeana* analysis according to manufactures instruction (GC-MS model, QP2010 PLUS, Shimadzu, Japan). The GC-MS analysis was predicated on comparing the bioactive compounds' retention indices and mass spectra fragmentation patterns with those kept in the machine's computer library (i.e., comparing the unknown component's spectrum with the known component's spectrum kept in the machine) and interpreting the mass spectrum. The National Institute for Standard Technology's mass spectrum database was used for the GC-MS analysis (NIST05.LIB). The compound's name was determined using the molecular weight, molecular formula, and Hits number from NIST05 and LIB library recorded.

Determination of Toxicity (LD_{50}) of the Crude Extracts of *Azanza garckeana*

The toxicity of the crude extracts of *A. garckeana* was determined according to the methods of Lorke (1983) using female albino rats. The choice of female rats was in accordance with the Organisation for Economic Cooperation and Development (OECD) (1998) established guidelines, which recommend that only female albino rats should be used in acute oral toxicity tests in the absence of an indication that males are more sensitive to the compound being tested. Recent studies by Levy *et al.* (2023) also indicated that female mice exhibit more stable exploratory behavior despite hormonal fluctuations than their male peers. Twelve (12) female adult albino mice weighing 17 and 22g body mass were obtained from Small Laboratory Animal House, Department of Pharmaceutical Science, Bayero University Kano, Nigeria. The animals were kept in standard settings with a 12-hour light/dark cycle, 25^0C temperature, and humidity in the experimental facility with standard feed and free access to water. Before the commencement of the research, the animals were given seven days to acclimate. The study was carried out following the Good Laboratory Practice (GLP) regulations recommended by WHO (1992).

Nine (9) mice were employed in the initial phase. The nine animals were split up into groups of three. Each group comprised three mice and was given extracts at doses of 10, 100, and 1000 mg/kg body weight, and they were watched for 24 hours to see if there were any behavioral changes or deaths. Four animals total—four groups of one animal each—were employed in the second part of the experiment, and they received doses of the extracts of 140 mg/kg, 225 mg/kg, 370 mg/kg, and 600 mg/kg body weight, respectively. Their behavior and mortality were monitored.

LD_{50} was calculated using the formula: $LD_{50} = \sqrt{(D_0 \times D_{100})}$
Where: D_0 = Highest dose that gave no mortality, D_{100} = Lowest dose that produce mortality

Assessment of the Haematological indices of the test animal

The test animals' hearts were punctured to get blood, which was stored in EDTA tubes. The collected blood was used to assess the haematological indices of the test animals and their liver and kidney functions.

An automated hematology system was used to analyze blood samples for red blood cell count (RBC), packed cell volume (PCV), hemoglobin (Hb) concentration, platelet count (PLT), erythrocyte indices, total white blood cell counts, and their differentials (Sysmex Hematology Systems, 2008).

Tests for kidney and liver function

To assess liver toxicity, four enzyme indicators of liver damage were measured. ALP (alkaline phosphatase) activity was measured using the technique outlined by Karmen (1955), Reitman and Frankel's (1957) techniques were used to measure the activities of aspartate transaminase (AST) and alanine transaminase (ALT), while Jendrassik and Grof's (1938) approach was used to measure the amount of bilirubin. Using the techniques outlined by Henry (1974), the levels of the kidney function indices—urea, creatinine, sodium, potassium, chloride, and bicarbonate—were measured to assess renal function.

Histopathological Studies

Cervical decapitation was used to sacrifice every animal. The liver, kidneys, heart, and spleen were removed, and the organs were then frozen, cleaned, and weighed on a digital scale (KERRO BL 200001, MxRady Lab Solutions Pvt. Ltd., Delhi, India). Following that, samples of each animal's kidney, liver, spleen, and heart were removed and processed using the methods of Drury *et al*. (1976). The tissue samples were embedded in paraffin wax, dried in alcohol, and fixed with a 10% neutral buffered formalin solution. Hematoxylin and eosin (H&E, Thermo Shandon, USA) were used to stain sections that were cut at thicknesses of 5 µm. (H&E, Thermo Shandon, USA).

RESULTS

All the extracts of both the local and foreign seeds of *A. garckeana* contain alkaloids, carbohydrates, flavonoids, saponins, and tannins, but only the three extracts of the local seeds contain resins and steroids (Table 1).

Organisms confirmed from urine samples of patients with UTI were *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureaus,* and *Pseudomonas aeruginosa*).

The antimicrobial activity of both foreign and local *A. garckeana* extracts on some selected Urinary tract infection isolates shows that both extracts exhibited some antimicrobial activity against some of the isolates, although all activities were lower in relation to the control (Table 2). At a concentration of 200µg/ml, the foreign and local *A. garckeana* petroleum ether extract exhibited the maximum activity of the 15mm and 13mm zones of inhibition against *S. aureus* (Table 2). Noticeably, both the local and foreign *A. garckeana* methanol extract recorded zones of inhibition against *E. coli* (7mm) and *K. pneumoniae* (11mm) at 200µg/ml (Table 2). More so, both the local and foreign aqueous seed extracts of *Azanza garckeana* recorded zones of inhibition of 12mm against *S. aureus* and 11mm against K. pneumoniae at a concentration of 200µg/ml, respectively (Table 2). Both foreign and local A. garckeana extracts showed a minimum inhibitory concentration of 6.25 mg/ml and a minimum bactericidal concentration of 25mg/ml against the test organisms (Table 3).

In the acute toxicity study of *A. garckeana,* as shown in phase one of the toxicity test, none of the animals died within 24 hours after the application of the extract at a concentration of 10mg/kg and 100mg/kg. However doses at 1000mg/kg caused death to the mice (Table 4). No death was recorded among all the groups throughout the second phase of the test, and the LD_{50} was greater than 600mg/kg (Table 4).

The outcome of receiving acute oral treatment with local and foreign *A. garckeana* extracts on the organ weight index shows that dose-dependent changes occurred in both the body and organ weight of all groups but were found to be insignificant as compared to the control group ($p>0.05$) (Table 5).

Liver function test revealed that animals that had foreign *Azanza garckeana* showed a higher level of alkaline phosphatase of 235.8±2.55 U/l to 323.5±3.00 U/l compared to those that had local aqueous *Azanza garckeana* (82.6±0.85 U/l to 100.165±3.92 U/l) and the control (100.165±3.62 U/l) (Table 6).

garckeana had higher levels of unconjugated bilirubin (0.05±0.05 µmol/l to1.1835±0.15 µmol/l) compared to local *azanza garckeana* (0.1±0.05 µmol/l to 0.574±0.1625 µmol/l) and the control (1.148±0.325 µmol/l). Samples of test animals that had foreign seeds of *Azanza garckeana* had a lower amount of Aspartate

transaminase (0.212±0.15 U/l to 37.065±0.515U/l) compared to Local *Azanza garckeana* (11.5±0.5 U/l to 42.334±2.35 U/l) and the control (54.00±0.67 U/l) (Table 6).

The study revealed that evaluation of the renal and liver functions of the test mice indicated either higher or lower values compared with the control. Test mice that had the extracts of both local and foreign seeds reported higher Urea values (30.45±1.78 mg/dl to 78.31 ± 1.55 mg/dl) compared with 25.72±0.83 mg/dl of the control and lower Creatinine levels of 62.5 ± 1.00 µmol/l to 93.163 ± 1.59 µmol/l compared to186.3±3.18 µmol/l of the control (Table 7). The Sodium (Na) levels were lower in the test Mice (85.215 ± 1.00 mEq/L to 122.47 ± 1.08 mEq/L) compared with the Control (219.5±2.31 mEq/L), the Potassium (K) levels were also lower with the test mice recording a value of 1.53±0.05 mEq/L to 2.93 ± 0.40 mEq/L and control mice recording a value of 5.86±0.80 mEq/L, and the Chloride levels were also lower in the test mice (37.855 ± 0.52 mEq/L to 39.45±0.17 mEq/L) in contrast to the control (75.71±1.04 mEq/L) (Table 7). The test mice recorded lower values of HCO of 12.165 ± 0.6mEq/L to 22.34±0.55 mEq/L compared with the control mice with 44.33±1.03 mEq/L (Table 7).

Histopathological studies showed a heart with normal features, a kidney with slight hyperplasia of inflammatory cells, and a liver with slight hepatic necrosis at 1000 mg/kg (Plate a-i) (Table 8).

With the exception of red blood cells and white blood cells, and lymphocyte counts that are within the normal range, all other haematological indices of the test mice were either higher or lower than the normal values (Table 9).

The results of the GCMS analysis showed the presence of several hydrocarbon molecules, including Dodecanoic acid, ethyl ester, Octadecenoic acid, Undecanoic acid, and methyl ether, with Dodecanoic acid having the highest percentage abundance of 88% (Figure 1).

Table 1: Phytochemical Characteristics of Seed extract of local and foreign *Azanza garckeana*

Phytochemicals		Extracts of seeds of *Azanza garckeana*		
	Plant type	Methanol	Aqueous	Petroleum Ether
Alkaloids	Local	+	+	+
	Foreign	+	+	+
Carbohydrates	Local	+	+	+
	Foreign	+	+	+
Flavonoids	Local	+	+	+
	Foreign	+	+	+
Resins	Local	+	+	+
	Foreign	-	-	-
Saponins	Local	+	+	+
	Foreign	+	+	+
Steroids	Local	+	+	+
	Foreign	-	-	-
Tannins	Local	+	+	+
	Foreign	+	+	+

Key: + = Detected, - = Not detected

Table 2: Antimicrobial activity of local and foreign *Azanza garckeana* seed extracts on some selected Urinary tract infection isolates

Isolate	Plant type	*Azanza garckeana* seed extracts concentration (µg/ml)/Zone of Inhibition (mm)												
		AGM				AGP				AGPE				Cipro
		200	100	50	25	200	100	50	25	200	100	50	25	200µg/ml
E. coli	Local	10	7	5	3	0	0	0	0	11	9	7	6	23
	Foreign	8	7	5	4	0	0	0	0	0	0	0	0	23
K. pneu	Local	11	10	8	7	0	0	0	0	11	8	7	6	22
	Foreign	11	9	8	7	0	0	0	0	0	0	0	0	22
P. aeru	Local	0	0	0	0	12	8	6	5	0	0	0	0	33
	Foreign	0	0	0	0	0	0	0	0	13	12	10	10	33
S. aureus	Local	0	0	0	0	12	11	10	9	13	12	10	9	27
	Foreign	0	0	0	0	12	11	10	9	15	13	9	8	27

Key: AGM = *Azanza garckeana* methanol, AGA = *Azanza garckeana* aqueous, AGPE = *Azanza garckeana* petroleum ether, E=*Escherichia*, K=*Klebsiella*, P=*Pseudomonas*, *aeru=aeruginosa* S=*Staphylococcus*, Pneu= *pneumonia*, Cipro=Ciprofloxacin

Table 3: Minimum Inhibitory and Minimum Bacterial Concentrations (mg/ml) of *Azanza garckeana* of seed extracts

Isolate	Extract Type	Concentration of *Azanza garckeana* Extracts (mg/ml)					
		Methanol		Aqueous		Petroleum ether	
		MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	Local	12.50	50	-	-	6.25	25
	Foreign	12.50	50	-	-	6.25	25
Klebsiella pneumoniae	Local	12.50	50	-	-	6.25	25
	Foreign	12.50	50	-	-	6.25	25
Pseudomonas aeruginosa	Local	-	-	6.25	25	-	-
	Foreign	-	-	6.25	25	6.25	25
Staphylococcus aureus	Local	-	-	6.25	25	6.25	25
	Foreign	-	-	6.25	25	6.25	25

Key: - = No Activity

Table 4: Phase I and II LD_{50} of the local and foreign seed extract of *Azanza garckeana*

Extract type	Phase	No. of Animals	Doses (mg/kg)	No. of Death
Aqueous Azanaza garckeana	**Phase I**	3	10	0
		3	100	0
		3	1000	3
	Phase II	1	600	0
		1	370	0
		1	225	0
		1	140	0
Petroleum ether *Azanza garckean*	**Phase I**	3	10	0
		3	100	0
		3	1000	3
	Phase II	1	600	0
		1	370	0
		1	225	0
		1	140	0
Methanol *Azanza garckeana*	**Phase I**	3	10	0
		3	100	0
	Phase II	3	1000	3
		1	600	0
		1	370	0
	Phase I	1	225	0
		1	140	0
	Phase II			
Control (Distilled water)		3	10	0
		3	100	0
		3	1000	0
		1	600	0
		1	370	0
		1	225	0
		1	140	0

Table 5: Effect of Oral Acute treatment with local and foreign *Azanza garckeana* extracts on the organ weight index

Extract type	Organs	Organ Weight Index 1000mg/kg (local)	Organ Weight Index 1000mg/kg (Foreign)	Organ Weight Index 1000mg/ml (Control)
Aqueous *Azanza garckeana*	Liver	0.0122±0.0004	0.0135±0.0006	0.147±0.0065
	Kidney	0.0012±0.0003	0.0014±0.0005	0.0016±0.0005
	Spleen	0.001785±0.0004	0.001885±0.0004	0.002085±0.0025
	Heart	0.0015±0.0005	0.0016±0.0005	0.0017±0.0002
Petroleum ether *Azanza garckeana*	Liver	0.0147±0.0007	0.0142±0.00065	0.147±0.0065
	Kidney	0.0016±0.0005	0.0015±0.0005	0.0016±0.0005
	Spleen	0.002085±0.00025	0.001985±0.00025	0.02085±0.0025
	Heart	0.0019±0.0003	0.0018±0.0002	0.0017±0.0002
Methanol *Azanza garckeana*	Liver	0.0197±0.00115	0.01895±0.0004	0.147±0.0065
	Kidney	0.0022±0.0001	0.0021±0.0001	0.0016±0.0005
	Spleen	0.0021±0.0001	0.002±0.0001	0.02085±0.0025
	Heart	0.002585±0.0003	0.0023±0.0002	0.0017±0.0002

Table 6: Effect of Oral administration of local and foreign Seed extracts of *Azanza garckeana* on liver enzymes of mice

Extract type	Plant type	ALP / (U / l)	AST / (U / l)	ALT / (U / l)	U. Bil (µmol/l)
Aqueous *Azanza garckeana*	Local	100.165±3.92	42.334±2.35	19.5±1.525	0.574±0.1625
	Foreign	323.5±3.00	0.212±0.15	25.25±0.75	0.265±0.035
Pet. ether *Azanza garckeana*	Local	85.5±0.5	11.5±0.5	20.5±0.5	0.42±0.045
	Foreign	287.5±8.43	37.065±0.515	26.5±0.285	1.1835±0.15
Methanol *Azanza garckeana*	Local	82.6±0.85	36.9±2.18	23.2±0.9	0.1±0.05
	Foreign	235.8±2.55	10.8±0.8	19.8±0.6	0.05±0.05
Control		100.165±3.62	54.00±0.67	15.00±1.02	1.148±0.325

Key: AST = Aspartate transaminase; ALT = Alanine transaminase; ALP = Alkaline phosphatase; U.Bil = bilirubin

Table 7: Effect of Oral administration of local and foreign Seed extract of *Azanza garckeana* on Urea, creatinine, and electrolytes in mice

Biochemical parameters	Aqueous *A. garckeana* (local)	Aqueous *A. garckeana* (Foreign)	Pet.ether *A. garckeana* (local)	Pet.ether *A. garckeana* (Foreign)	Methanol *A. garckeana* (local)	Methanol *A. garckeana* (Foreign)	Control
Urea (mg / dl)	78.31 ± 1.55	38.635 ± 0.645	40.87 ± 0.66	38.58 ± 1.25	34.32±1.17	30.45±1.78	25.72±0.83
Creatinine (µmol / l)	93.163 ± 1.59	79.305 ± 1.55	62.5 ± 1.00	72.5 ± 1.00	63.88±1.87	71.88±1.05	186.3±3.18
Na^+ (mEq / L)	109.74 ± 1.16	95.215 ± 1.66	121.8 ± 1.5	122.47 ± 1.08	85.5±1.00	107.8±1.00	219.5±2.31
K^+ (mEq / L)	2.93 ± 0.40	1.865 ± 0.075	1.77 ± 0.05	2.59 ± 1.05	2.72±0.34	1.53±0.05	5.86±0.80
Cl^- (mEq / L)	37.855 ± 0.52	44.185 ± 0.76	40.39 ± 0.5	44.15 ± 0.32	43.24±0.98	39.45±0.17	75.71±1.04
HCO_3 (mEq / L)	22.165 ± 0.5	12.165 ± 0.6	21.00 ± 1.00	21.00 ± 0.5	22.34±0.55	22.34±0.55	44.33±1.03

Key; Na^+ = Sodium ion, K^+ = Potassium ion, CL^- = Chlorine ion, HCO_3 = Hydrogen trioxocarbonate (V) acid

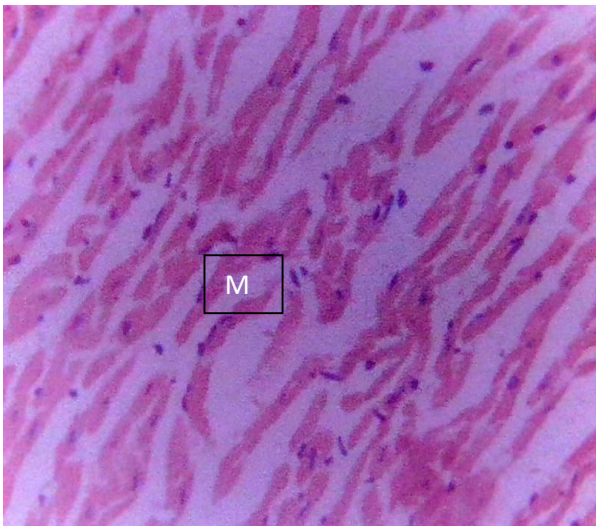


Plate a: Heart shows normal myocardium (M) hormone

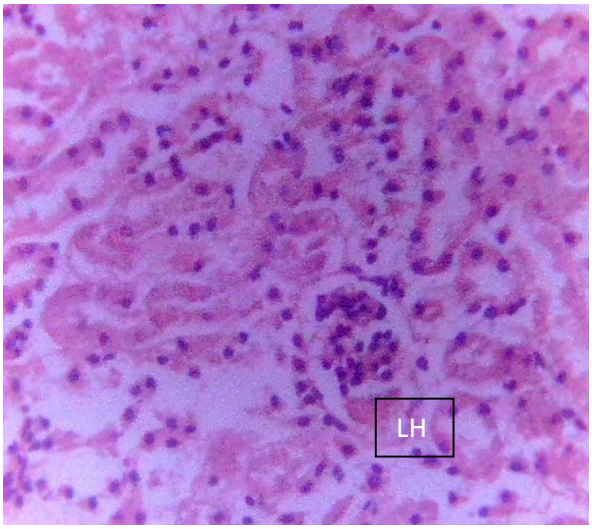


Plate b) Kidney shows moderate (Luteinizing (LH)

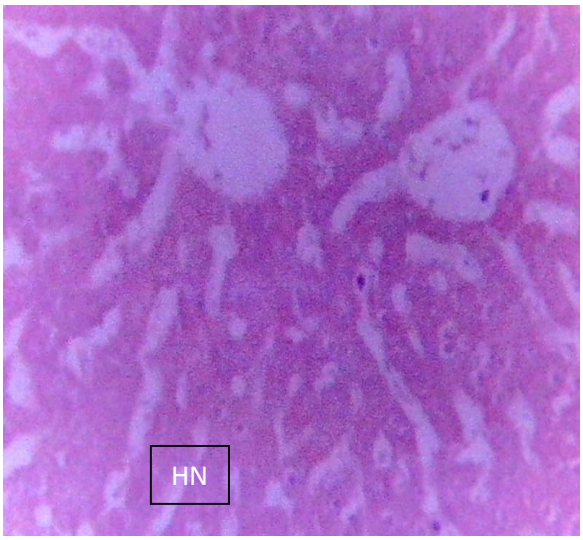


Plate c) Liver Show Moderate Liver Hepatocyte nuclear factor 4-alpha (HNF4-α) (HN)

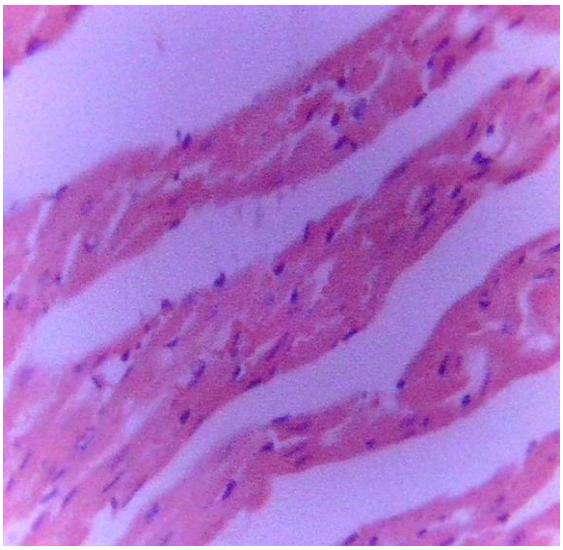


Plate d) Heart show normal features slight Proton (H)

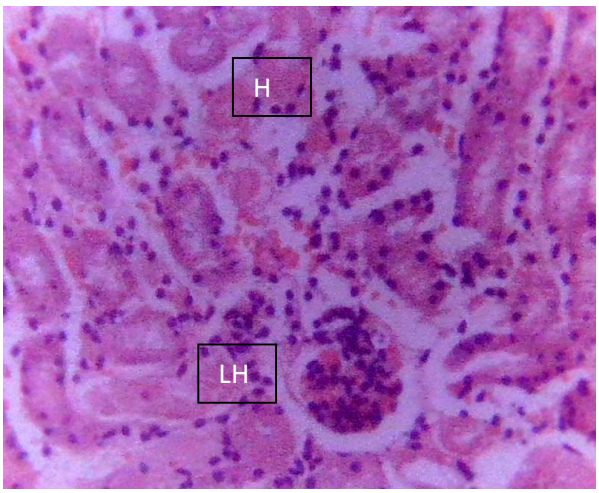


Plate e) Kidney show moderate Luteinizing hormone

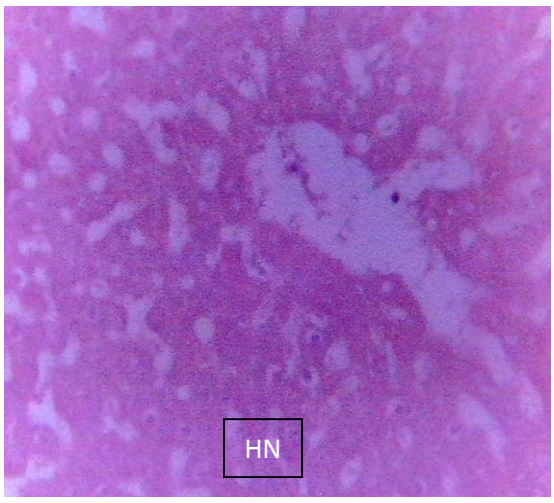


Plate f) Liver shows slight HN (LH) with

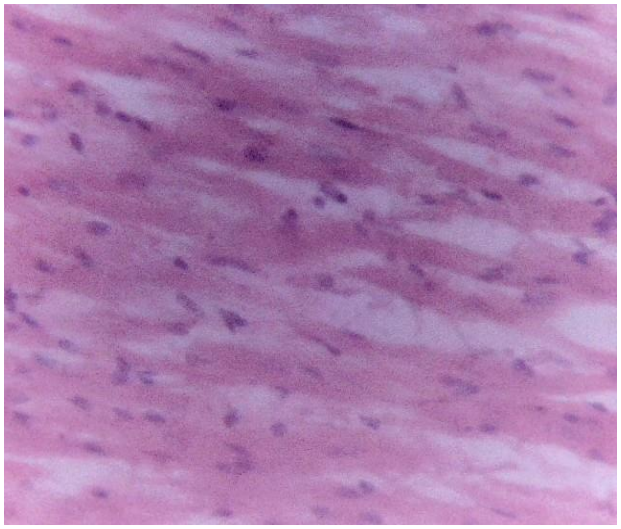


Plate g) Heart show normal features

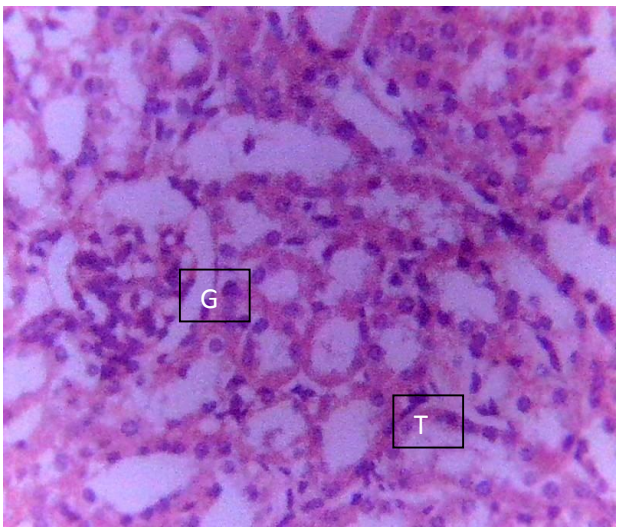


Plate h) Kidney show normal Glomerulus (G) and Tubules T

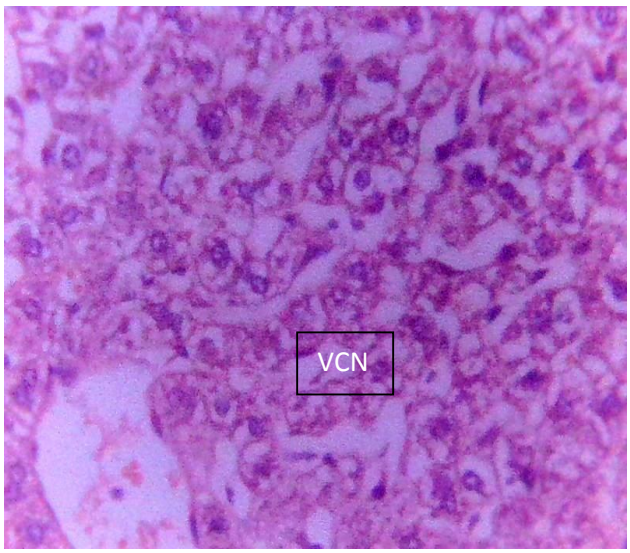


Plate i) Liver shows moderate Virtual non-contrast (VCN)

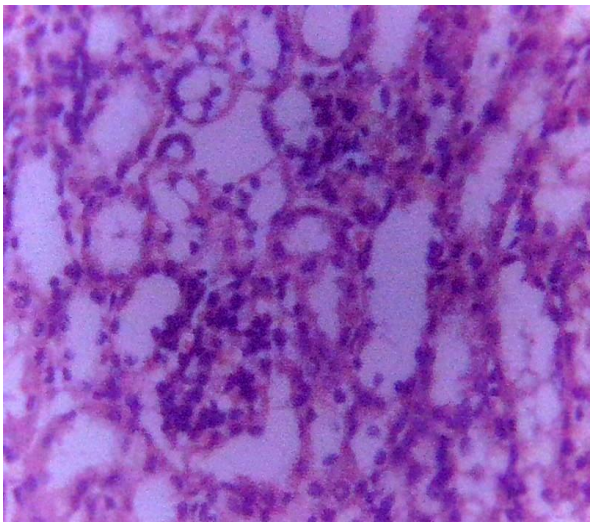


Plate j) Heart Show Normal Features

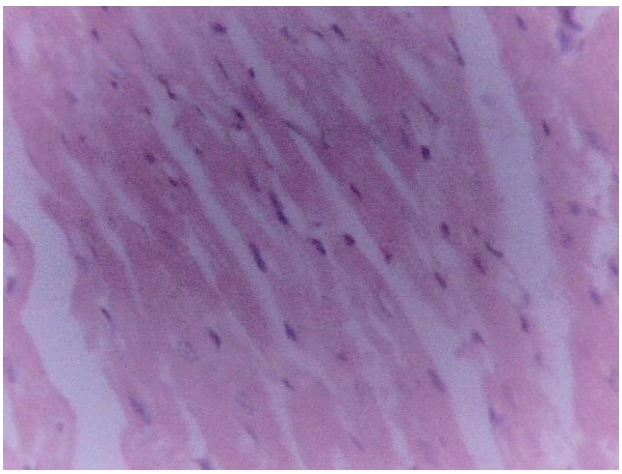


Plate k) Kidney Show Slight Luteinizing hormone (LH)

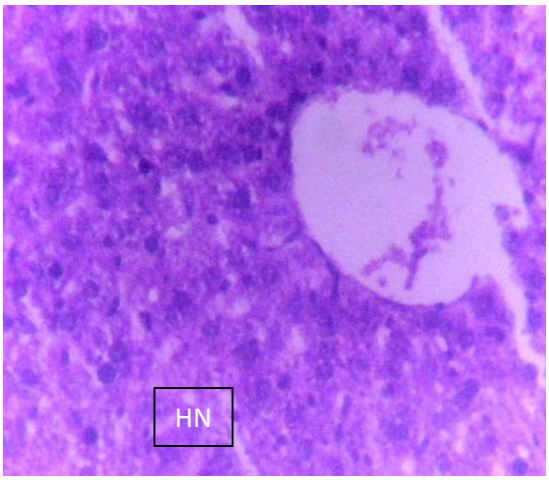


Plate l) Liver show slight Liver Hepatocyte nuclear factor 4-alpha (HNF4-α) (HN)

Key: Liver HN=Liver Hepatocyte nuclear factor 4-alpha (HNF4-α), Kidney LH= Luteinizing hormone, Kidney H=Proton (H^+) identified in the kidney tissue, Liver VCN= Virtual non-contrast, Kidney T =Tissue

Table 8: Acronyms, meaning, and description of some of the histopathological sections of the studied Mice tissues administered with extracts of *Azanza garckeana*

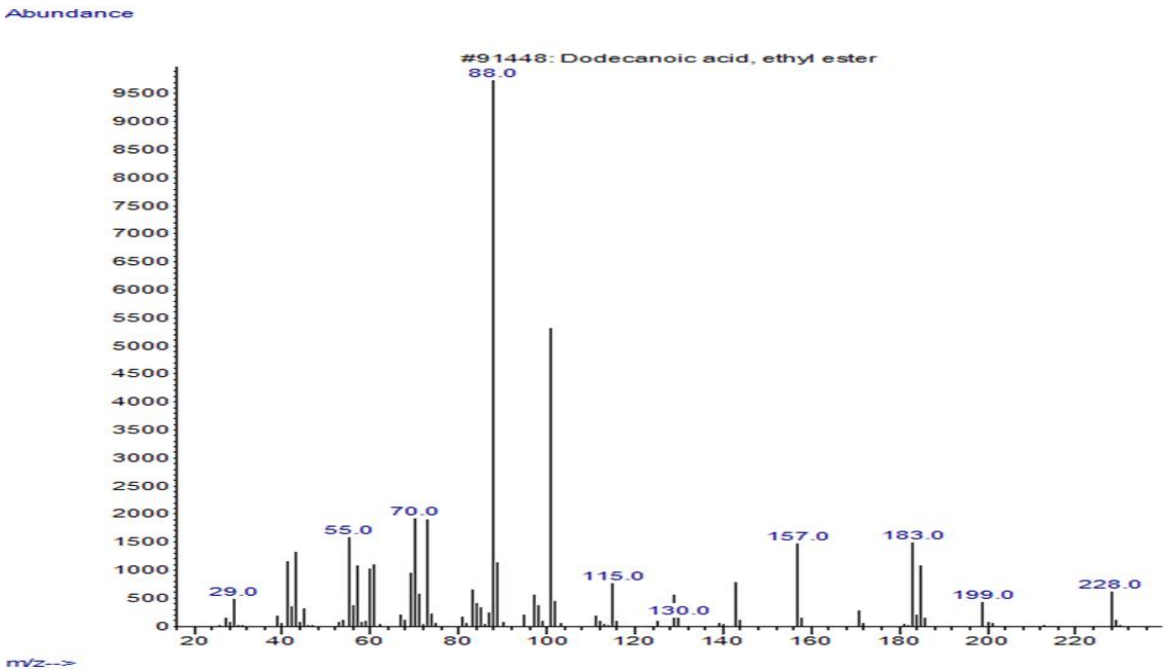
SN	Acronyms	Meaning	Description
1	Heart M	Myocardium	The Cardiac muscle or myocardium makes up the thick middle layer of the heart.
2	Liver HN	Liver Hepatocyte nuclear factor 4-alpha (HNF4-α)	This nuclear receptor regulates metabolism, cell junctions, differentiation, and proliferation in liver and intestinal epithelial cells.
3	Kidney LH	Luteinizing hormone	Revealing an excessive (LH) response with a delayed return to normal in both dialysis groups
4	Kidney H	Proton (H^+) identified in the kidney tissue	The kidney plays key roles in extracellular fluid pH homeostasis by reclaiming bicarbonate (HCO_3^-) filtered at the glomerulus and generating the consumed HCO_3^- by secreting protons (H^+) into the urine (renal acidification)
5	Liver VCN	Virtual non-contrast	Virtual non-contrast imaging is an image post-processing technique used to create 'non-contrast' images of contrast-enhanced scans via the subtraction of iodine.
6	Kidney G	Glomerulus	The glomerulus, the kidney's filtering unit, is a specialized bundle of capillaries uniquely situated between two resistance vessels.
7	Kidney T	Tissue	Kidney tubules are tiny tubes in the kidneys that return nutrients, fluids, and other substances filtered from the blood but the body needs back to the blood. They are essential to an organism's blood clearance mechanism, recovering essential metabolites from glomerular filtration.

Table 9: Haematological assessment of Mice with local and foreign *Azanza garckeana* extract

Blood cells	Results (local)	Results (foreign)	Units	Normal limits	Flags
WBCs	4.6±0.3	4.3±0.3	× 10-3/mL	4.0-12.0	
LYM	3.7±0.1	3.1±0.2	× 10-3/mL	1.0-5.0	
MON	0.6±0.05	0.7±0.07	× 10-3/mL	0.1-1.0	
GRA	0.3±0.03	0.5±0.04	× 10-3/mL	2.0-8.0	L
LYM%	80.2±10.0	72.9±10.4	%	25.0-50.0	H
MON%	14.1±0.4	15.2±0.5	%	2.0-10.0	H
GRA%	5.7±0.6	11.9±0.6	%	50.0-80.0	L
RBC	8.54±1.0	6.58±0.5	× 10-6/mL	4.00-6.20	D/h
HGB	17.3±0.5	13.0±0.2	g/dl	11.0-17.0	h
HCT	59.7±1.1	43.5±0.8	%	35.0-55.0	h
MCV	69.9±9.4	66.1±8.3	mm^{-3}	80.0-100.0	L
MCH	20.3±2.3	19.8±1.4	pg	26.0-34.0	L
MCHC	29.0±2.6	29.9±2.8		31.0-35.5	h/l
RDWC	23.0±1.8	17.0±1.2	g/dl	10.0-16.0	h
RDWS	57.1±1.5	40.8±1.1	mm^{-3}	37.0-46.0	h
PLT	625±34.2	727±33.9	× 10-3/mL	150-400	H
MPV	8.3±1.0	7.7±0.8	%	7.0-11.0	
PCT	0.519±0.04	0.560±0.05	%	0.200-0.500	H
PDW	14.5±0.5	15.1±0.7	%	10.0-18.0	
PLCR	16.7±0.8	11.5±0.4	%	12.0-42.0	l

Key; RBC = Red blood cell; HGB = haemoglobin concentration; HCT = Haemotocrit; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration; WBC = White blood cell count; LYM% = lymphocytes percentage; MON% = Monocytes percentage, MON = Monocytes count; GRA = Granulocytes count, PLT = platelet count

Gas Chromatography-Mass Spectroscopy



GCMS Chromatogram

DISCUSSION

All the seed extracts from the three different solvents (Aqueous, Methanol, Petroleum ether) of both local and foreign *A. garckeana* were gummy in texture, but some were dark brown and others light brown. The color variations could be caused by differences in the inherent color of the components of the seeds used to make the extracts. Aqueous extracts were generally gummier in texture than petroleum ether and methanol *A. garckeana* extracts, which could be due to the nature of solvents. The variance in the % recovery may result from variations in the seeds' metabolite contents' solubility in a given solvent. These findings align with earlier research findings that secondary metabolites in medicinal plants differ depending on how solubility of solvents employed for extraction (Lawal *et al.*, 2014). These study findings show that Petroleum ether extracts are the ideal extraction solvent for seeds of *A. garckeana*.

The current study confirms earlier research findings by demonstrating the presence of secondary metabolites, including flavonoids, tannins, alkaloids, saponins, and carbohydrates in *A. garckeana*. Numerous writers have demonstrated phenols, flavonoids, alkaloids, and tannins' antibacterial, antioxidant, anti-inflammatory, antimalarial, and analgesic properties (Carini *et al.*, 2001). Earlier studies by Lawal *et al.* (2015) revealed that secondary plant metabolites are found in many parts of plants and have been used to cure, prevent, and manage various medical diseases. They have also been shown to have physiological effects promoting natural healing with minimal negative side effects. The findings demonstrated the presence of alkaloids, carbohydrates, saponins, flavonoids, and tannins in the methanol extract of all plant seeds. These findings also agreed with the previous literature, as a report by Usman *et al.* (2009) stated that the preliminary phytochemical studies of the partitioned portion of *A. garckeana* seeds showed that tannins, saponins, and resins were present. The study findings based on the GCMS analysis show the existence of several hydrocarbon molecules, including Dodecanoic acid, ethyl ester, Octadecenoic acid, Undecanoic acid, and methyl ether. The compounds present in *A. garckeana* have a wide range of biological and therapeutic qualities that have been documented, including the ability to inhibit the human immunodeficiency virus and possess antibacterial, anti-malarial, anti-inflammatory, anthelmintic, antinociceptive, and anti-cancer characteristics (Alakurtti, 2006).

The findings of this investigation show that all the extracts of both the local and foreign seed exhibited an antibacterial action on the test isolates and that the extract of foreign petroleum ether *A. garckeana* exhibited higher antimicrobial activities on the bacterial isolates tested than the extract of local petroleum ether *A. garckeana*. *S. aureus* was more sensitive to the foreign extracts of petroleum ether *A. garckena* compared to local extracts, while *K. pneumoniae* was found to be more sensitive to the methanol extract of both local and foreign seeds of *A. garckeana*.

Escherichia coli was more sensitive to the local petroleum ether A. garckeana extracts than foreign ones. *Pseudomonas aeruginosa* was more sensitive to the foreign *A. garckena* Aqueous extracts. These findings indicated that *A. garckeana* might be a possible medicinal agent against pathogenic bacteria.

The result of LD_{50} local and foreign seed of *A. garckeana* extracts has been determined to be more than 600 mg/kg as at about 1000mg/kg nine (9) mice were dead due to the high concentration administered. This indicates that the dosage level should be considered, as it might cause damage if not regulated, especially bearing the fact that the histopathological and hematological studies show that the heart with normal features, kidney with slight hyperplasia of inflammatory cells, and liver with slight hepatic necrosis at 1000 mg/kg.

The weight loss of the body and its organs observed in the study compared with the control concurs with the opinion of Teo *et al*. (2002) and Michael *et al*. (2015), who observed that such changes are a significant and accurate marker of changes to the body and organs brought about by chemicals following exposure to toxicants. The absence of significant differences in body weight index between the Aqueous, Petroleum ether, and Methanol *A. garckeana* groups suggests that the phytochemicals in the extracts did not alter the mass composition of the mice's body or organs by influencing biochemical processes that determine body and organ weight or by lowering food intake.

CONCLUSION

The study demonstrated that both the local and foreign seeds of *Azanza garckeana* contain important metabolites (Dodecanoic acid, ethyl ester, Octadecenoic acid, Undecanoic acid, methyl ether) and had antimicrobial therapeutic potential, with the foreign extract exhibiting higher activity. Toxicity and histopathological study demonstrated that extracts of local and foreign seeds of *A. garckeana* extracts were slightly toxic at the concentrations used in the study. Thus, it is recommended that using Azanza garckeana as a traditional herb be done cautiously and dose-dependent. Further studies are needed to establish its safety.

REFERENCES

Adoum, O.A. Akinniyi, J.A. and Omar, T. (1997). The Effect of Geographical Location on the Antimicrobial Activities and Trace Element Concentration in the Root of *Calotropis procera* (Ait.) R. Br. *Annals of Borno*, 13 (14): 199-207.

Ahmed, R.H., El Hassan, M.S. and El Hadi, H.M. (2016). Potential Capability of *Azanza garckeana* Fruits Aqueous Extract on Enhancement of Iron Absorption in Wistar Albino Rats. *Int J Adv Res Biol Sci*, 3: 245-50.

Alakurtti, S., Mäkelä, T, Koskimies, S. and Kauhaluoma, S. (2006) Pharmacological Properties of the Ubiquitous Natural Product Botulin. *European Journal of Pharmaceutical Sciences*, 29 (1): 1-13. **[Crossref]**

Ali, M., Yahaya, A., Zage, A. U. and Yusuf, Z. M. (2017). *In-vitro* Antibacterial Activity and Phytochemical Screening of *Psidium guajava* on some Enteric Bacterial Isolates of Public Health Importance. *Journal of Advances in Medical and Pharmaceutical Sciences*, 12 (3): 1-7. **[Crossref]**

Aliyu, M. and Samaila, S. C. (2015). Analgesic and Anti-inflammatory Activities of Ethanolic Extract of Rheumatic Tea Formula (RTF) in Rats and Mice. *International Journal of Herbs and Pharmacological Research*, 4 (2): 17-24.

Hassan, A.I. (2023). *Antimicrobial Activity and Toxicity Study of Local and Foreign Seeds of Azanza Garckeana* (*Goron Tula*) *Extracts*. (Unpublshed Master's

Association of Official Analytical chemist (AOAC), Official Method (2012), in: Official Methods of Analysis of AOAC International, 19th ed. AOAC International, Gaithersburg, MD, USA, 2012.

Carini, M., Adlini, G., Furlanetto, S., Stefani, R., and Facino, R. M. (2001). LC Coupled to ion-Trap MS for the Rapid Screening Detection of Polyphenol Antioxidant from *Helichrysum stoechas*. *Journal Pharmaceutical Biomedical Analysis*, 24: 517-526. **[Crossref]**

Cheesbrough, M. (2006). Laboratory Practice in Tropical Countries Part II, 2 (Ed) Cambridge University Press, New York, pp: 65-67. **[Crossref]**

Ciulei, 1. (1994). Methodology for the Analysis of Vegetable Drugs Chemical Industries Branch. Division of Industrial Operations. UNIDO, Romania: Pp.24-67.

Demetrio, L., Valle, J. R., Jeamie, I. A. and Windell, L. R. (2015). Antibacterial activities of ethanol extracts of Phillipine medicinal plants against MDR bacteria. *Asian Journal of Tropical Bio-Medicine*

Drury, R. A., Wallington, E. A., and Cancerson, R. (1976). Carlton's Histopathological Techniques. Fourth ed. University Press, Oxford. London, New York. 1: 20-30.

Dissertation), Bayero University, Kano, Nigeria.

Henry, R.J. (1974). Clincal Chemistry, Principles and Techniques. 2nd Edition, Harper and Row, Hagerstown, MD.

Jendrasskik, J. and Grof, P. (1938) In-vitro Determination of Total and Direct Bilirubin in Serumor Plasma. *Biochemistry*, 297 (1): 81-89.

Karmen, A. (1955). A Note on the Spectrophotometric Assay of Glutamic-oxalacetic Transaminase in Human Blood Serum. *Journal clinical Investment*, 34: 131-133. **[Crossref]**

Lako, J., Trenerry, V. C., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran., S., and Prer R. (2007). Phytochemical Flavonoids, Carotenoids and the Antioxidant Properties of a Selection of Fijian Fruit, Vegetables and other Readily Available Foods. *Food Chem*. 101: 1727-1741. **[Crossref]**

Lawal, B., Ossai, P.C., Shittu, O.K. and Abubakar, A. N. (2014). Evaluation of Phytochemicals, Proximate, Minerals Anti-nutritional Compositions of Yam Peel, Maize Chaff Bean Coat, *International Journal of Applied Biological Research*, 6: 1-17. **[Crossref]**

Lawal, B., Shittu, O. K., Kabiru, A. Y., Jigam, A. A., Umar, M. B., Berinyuy, E. B., *et al*. (2015) Potential Antimalarials from African Natural Products: A Review. *Journal Intercult Ethnopharmacol*, 4: 318-343.

Lalitha, T. P. and Jayanthi, P. (2012). Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of *Eichhornia crassipes* (Mart.) Solms. *Asian Journal of Plant Science and Research*, **2** (2): 115-122.

Levy, D.R., Hunter, N., Lin, S. and Anyoha, R. (2023). Mouth spontaneous behavior reflects individual variation rather than estrous state. *Current Biol*, 33 (7): 1358-1364. **[Crossref]**

Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing. *Arch Toxicol*, 54: 275- 87. **[Crossref]**

Michael, K.G., L.U. Onyia and S.B. Jidauna, (2015). Evaluation of phytochemicals in *Azanza garckeana* (Goron tula) seed. IOSR J. *Agric. Vet. Sci.*, 8: 71-74.

Momodu, I. B., Okungbowa, E. S., Agoreyo, B. O. and Maliki, M. M. (2022). Gas Chromatography – Mass Spectrometry Identification of Bioactive Compounds in Methanol and Aqueous Seed Extracts of *Azanza garckeana* Fruits, Nigerian Journal of Biotechnology, Spec. Edtn: 25-38. **[Crossref]**

Michel. S., Nayak, B.S., Sandiford, S. and Maxwell, A. (2009). Evaluation of the Wound healing Activity of Extract of *Morindacitri folia* L. Evidence-Based. *Complementary and Alternative Medicine*, 6: 351-356. **[Crossref]**

Mshelia, E. H. et al. (2016). The quantitative phytochemicals content of the root bark of *Grewia mollis*, the antioxidant and cytotoxicity activities of its extracts. *European Journal of Pure and Applied Chemistry*, 3 (2): 1-11.

Mukhtar, M. D. and Tukur, A. (1999). Antibacterial activities of aqueous and ethanolic extracts of *Pistia statiotes*. *Nigerian Society for Experimental Biology*, 1: 51-58

Nester, E.W., Anderson, D.G., Roberts, C.E., Pearnall. N. N. and Nester, M.T. (2004). Microbiology: A human Perspective, 4th ed., McGraw Hill, New York, USA: pp 17.

Organisation for Economic Co-opeartion and Development (OECD) (1998). Report of the First Meeting of the OECD Endocrine Disrupter Testing and Assessment (EDTA) Task Force, 10th-11th March 1998. Available Upon Request at Organisation for Economic Cooperation and Development, Paris.

Parekh, J. and Chanda, S.V. (2007). Invitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turkish Journal of Biology*, 31: 53-58.

Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*; 28(1):56-63. **[Crossref]**

Sofowora, A. (1993). Medicinal Plants and Traditional Medicinal in Africa: 2nd Ed. Sunshine House, Ibadan, Nigeria: Spectrum Books Ltd. Screening Plants for Bioactive Agents: pp. 134-156.

Taura, D.W. and Oyeyi, T.L. (2009). *In-vitro* Antibacterial Activity of the Combinations of Ethanolic Extracts of *Annova comosus*, *Allium sativum* and *Aloe barbadensis* in Comparison with Ciprofloxacin. Biological and Environmental Sciences Journal for the Tropics, 6 (1): 146- 151.

Taylor, F. and Kwerepe, B. (1995). Towards Demonstration of Indigenous Fruit Trees in Botswana. In: J.A. Maghebe, Y. Ntulpanyama, and P. W. Chirwa (eds).

Improvements of indigenous of Miambo wood land of South Africa ICRAF Nairobi Kenya Pages: 400.

Teo, S., Stirling, D., Thomas, S., Hoberman, A., Kiorpes, A. and Khetano,V. (2002). A 90-day oral gavage toxicity study of d-methylphenidate and d, 1-methylphenidate in Sprague-Dawley rats. *Toxicol*, 179 93): 183-196. **[Crossref]**

Usman, H., Abdulrahman, F.1., Kaita, A.H. and Khan, L.Z. (2009). Antibacterial Assays of the Solvents Partitioned Portions of Methanol Stem Bark Extract of *Bauchinia rufescens* Lam (Leguminosae-Ceaesalpinioideae). *The Pacific Journal of Science and Technology*, 10: 2.

Van Wyk, B. E. (2011). The potential of South African Plants in The Development of New. Medicinal Products. *S Afr J Bot*, 77: 812-829. **[Crossref]**

Vennaposa, S. Devareddy, S. K., Sumathi, N., and Senthil, K. (2013). Phytochemical and Antimicrobial Evaluations of *Dichrostachys cinerea*. *International Research Journal of Pharmacy*, 4 *(1)*.

WHO (1992). Research guidelines for evaluating the safety and efficacy of herbal medicines World Health Organization, 5 (33): 3-33.

World Health Organization (2010). Use of Antibacterials Outside Human Medicine and Result and Antibacterial Resistance in Humans. World Health Organization. Archieved from the Original.

Yusuf , A.A., Lawal, B., Sani, S., Garba, R., Mohammed, B.A., Oshevire, D.B. and Adesina, A.A. (2020). Pharmacological activities of *Azanza garckeana* (Goron Tula) grown in Nigeria. *Clinical Phytoscience* (2020) 6: 27. **[Crossref]**.