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Acute Toxicity Assessment of *Jatropha tanjorensis* Chloroform and Aqueous Extract in Wistar Rats

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ABSTRACT

Background: *Over the past couple of decades, public health has grappled with antimicrobial resistance (AMR). This can be attributed to various factors, including antibiotic abuse and the emergence of resistant bacterial species. Hence, plants and other natural products have been sought for medicinal benefits.*
Methods*: Our study assesses the acute toxicity of aqueous and chloroform extracts of Jatropha tanjorensis. Treatment groups of three rats per group (n=21) were given 1200 mg/kg, 2900 mg/kg, and 500 mg/kg of the two extracts, and Histopathological and Haematological results were reported after 24 hours. Phytochemical analysis of Jatropha tanjorensis extracts was also performed prior to the rat experimental analysis.*
Results: *Phytochemical analysis of the extracts revealed the presence of saponins, tannins, and alkaloids. Acute toxicity assessment revealed no toxicity of the extracts, and no rats died after 24 hours. Histopathological analysis revealed no significant differences in kidney, small intestine, liver, or heart lesions between the treatment groups. Haematological analysis showed that the extracts did not clinically significantly affect red blood cell or white blood cell counts.*
Conclusion: *The absence of acute toxicity for Jatropha tanjorensis chloroform aqueous at doses exceeding typical dietary exposure is reassuring, though concentrated extracts require cautious use.*

Keywords: *Jatropha tanjorensis, Sub-acute toxicity, Hepatotoxicity, Chloroform extract.*

Introduction

Man has long relied on plants for food and medicine. Despite the development of several synthetic medications and supplements, plants remain important for maintaining individuals' health and well-being.[1]. Because many medicinal plants are inexpensive, the unequal distribution of wealth in resource-poor parts of the world has led many individuals to choose plant-based therapeutic solutions. [2]. Also, a growing number of individuals—and even in the advanced economies—are turning to plant-based diets and treatment options due to the increased incidence of chronic metabolic conditions worldwide, linked to unhealthy habits and urbanisation.[3].The main reason for this is that several of the purported benefits of plants and plant-based diets have been documented and are perceived as safer than artificial supplements and drugs[4]. Over the years, it has been established that many herbs and plants have potential therapeutic value for infectious diseases. *Jatropha tanjorensis* is one of those plants reported.

A member of the Euphorbiaceae family, *Jatropha tanjorensis* is commonly referred to as a "*Catholic* vegetable" or "*hospital-too-far*" in southern Nigeria. Originally from Central America, *Jatropha tanjorensis* has spread to many tropical and subtropical regions, including Africa, India, and North America, where it is now domiciled. The plant's leaves are a source of edible greens. They are sometimes used for medicinal purposes as a restorative force (embellishment of blood vitality commonly referred to as “blood tonic”) [5]. Historically, it has been utilised as an inebriant derived from the leaves and as a hematinic agent in the treatment of anaemia. Moreover, it has been beneficial for diabetes, skin ailments, malaria, cardiovascular conditions, and bacterial infections. *Jatropha tanjorensis* has attracted significant attention due to its perceived health benefits, availability, and affordability. The study of phytochemicals in these leaves, as reported by [1] and further confirmed by other literature, revealed the presence of tannins, flavonoids, terpenoids, alkaloids, and carbohydrates.
The phytochemical constituents of *Jatropha tanjorensis* possess hypolipidemic and antioxidant characteristics, demonstrating beneficial impacts on the serum lipid profile in albino rats. [6].

Additionally, *Jatropha tanjorensis* has been examined and found to be rich in antioxidant nutrients, including vitamin C, zinc, selenium, and phosphorus.[7]. *Jatropha tanjorensis* latex has also been used to treat skin conditions due to its alkaloid content.[8].

Many plants and plant parts are used in African diets, particularly in Nigeria, or are created and marketed by herbal practitioners as herbal remedies with a variety of health advantages. However, concerns have been expressed about the potential toxic risk associated with eating certain vegetables and herbal preparations. Although it is common to assume that plant-derived natural remedies are non-toxic, scientists believe that toxicological studies should be conducted to ensure their safety.[9].

Methodology

Identification and Collection of Plants

The leaves of *Jatropha tanjorensis* were obtained from March 2024 to May 2024 within the farmlands of Alex Ekwueme Federal University, Ndufu-Alike, Ebonyi State. A botanist at the Department of Biology, AE-FUNAI, authenticated these leaves as *Jatropha tanjorensis*. The leaves collected were labelled and kept to dry at room temperature in the Microbiology laboratory building, labels 0012 and 0013.

Preparation of Plant Samples

The leaves were collected, washed to remove any debris, and dried at room temperature for approximately 21 days. The environment used for drying the leaves was devoid of sunlight and properly aerated to prevent the growth of fungus or other microorganisms. After drying, the leaves were pulverised into fine powder using a mechanical grinder. The powdered plant leaves were then stored in airtight glass jars.

Aqueous and Chloroform Extraction

The procedures employed in this study were based on the methodologies outlined by Udoh *et al.* (2024)[10], with minor modifications. The powdered leaf of *Jatropha tanjorensis* was extracted with water at room temperature and with chloroform, respectively. The powdered leaf, weighing 100g, was soaked in 0.5 L of 95% chloroform for 72 hours. The mixture was periodically stirred until all the desired compounds were fully extracted at room temperature. The chloroform extracts were filtered, and the resulting filtrates were concentrated using a rotator evaporator at decreasing pressure until they completely evaporated. The desiccated raw extracts were stored in hermetically sealed containers. For aqueous extraction, 100g of each powdered plant part was immersed in 0.5 litres of distilled water and kept at room temperature (25 °C – 28 °C) for 24 hours with intermittent stirring. The solution was subsequently filtered to remove impurities, and the resulting liquid was then evaporated in a continuous stream of air.

Sterility Check for Extracts

The [11] The technique was updated and applied to this process. DMSO (10% v/v) was used to dissolve the extracts rather than pure ethanol. To test for contaminants and microbiological growth, each extract was ten-fold diluted in sterile deionised distilled water and filtered through a 0.45 µm Millipore membrane filter (Millipore, India). The extract was then redissolved in 1 ml of sterile deionised distilled water (9 ml) and serially diluted to 10^-6 concentrations.

Using a sterile glass spreader, an aliquot (100 µL) of each extract's 10^{-3} to 10^{-6} dilutions was aseptically added to Petri dishes pre-prepared with nutritional agar and evenly distributed throughout the plate. The plates were incubated for 24 hours at 37°C.

Phytochemical Analysis for Active Compounds

Gas Chromatography-Mass Spectroscopy (GC-MS) was carried out using a Perkin-Elmer Gas Chromatograph, Clarus 500 system, and GC interfaced to a mass spectrometer fitted with an Elite-1, fused silica capillary column (30 X 25mm 1D X µMDF, composed of 100 per cent dimethyl polysiloxane)[12], [13]. Gas chromatography separated compounds in the extracts based on their interactions with the mobile and stationary phases before mass spectrometry, which ionised the compounds (at 70 eV) based on their respective mass-to-charge ratios. The individual components of the sample were determined by comparing the resulting mass spectra to a database of known compounds.

Selection and Acclimatisation of Laboratory Animals

Wistar strain aged 2 – 3 months and weighing between 120 and 140g were used for this study. The Albino rats were purchased from the University of Nigeria, Nsukka animal house before they were transported to the Animal house at Alex Ekwueme Federal University, Ndufu Alike, Ebonyi State, Nigeria, where they were acclimatised for 7 days. Rats that died or became physically sick were excluded from the study. The laboratory animals were fed with an equal proportion of Vital Grower's Feed and water ad libitum for 2 weeks before commencement of the experiment, and also throughout the study period. Throughout this period, the water was supplemented with streptomycin (5 mg mL^{-1}) to reduce the facultative anaerobic bacteria that normally colonise the mouse intestine. All the rats were kept in metabolic cages that were cleaned consistently throughout the experimental period. The animals were managed in accordance with the appropriate ethical guidelines, consistent with the International Standard for the Use of Laboratory Animals.

Acute Toxicity Studies

Acute toxicity studies were carried out according to Lorke's Method. This method permits the estimation of an LD50 in 2 phases, thereby determining the dose range suitable for the experimental animals.[14]. Dosage administrations of 1600mg/kg, 2900mg/kg, and 5000mg/kg were given to 6 groups of 3 experimental animals each for each dose concentration, respectively, for both the aqueous and chloroform extracts of *Jatropha tanjorensis*. Group 1 served as the control for this preliminary study. The extract was administered aseptically by oral gavage to laboratory animals at the animal house at Alex-Ekwueme Federal University, Ndufu-Alike, Ebonyi State. Twenty-four (24) hours after administration, the rats were sacrificed. Standardised methods were used to conduct histopathological analysis on the liver, heart, kidney, and small intestine. Also, haematological parameters were analysed without differentials.

Results

Phytochemical Content of the Extracts

Table 1 below shows the secondary metabolites class identified via their molecular weight for *Jatropha tanjorensis* aqueous and chloroform extracts, respectively. Aqueous and Chloroform extracts of *Jatropha tanjorensis* were found to contain alkaloids, flavonoids, saponins, Phenols, and Terpenoids.

Haematology Analysis During Acute Toxicity Stage

The haematologic results for the acute toxicity test are presented below. Histopathology was performed on the kidneys, small intestine, liver, and heart of experimental animals. After 24 hours, results showed significant differences in the groups' haematological parameters. These results are presented in Table 3 below.

Histopathology Lesion Changes During Acute Toxicity Stage

Histopathological analysis of the Liver, Heart, Kidney, and Small Intestine revealed no significant differences in lesions between the experimental groups and the control. These results are displayed in tables 4.31 – 4.34. Scores were allotted as 0= absent, 1 = affecting less than 30% of lesions, 2= affecting 30 - 70% of lesions and 3 = affecting 71-100% of lesions. On analysis of the changes using the Kruskal-Wallis Test, treatment groups were not significantly different from the control (P<0.05). Results are presented in Tables 2-5

Table 1: Phytochemical Analysis of Jatropha tanjorensis Aqueous and Chloroform Extracts

Extract	Metabolite Class	Retention Time (min)	Major Peaks (m/z)
Aqueous Extract	Alkaloids	10.5 - 12.0	120-150
	Flavonoids	13.0 - 15.0	153, 165, 285
	Tannins	18.5 - 20.0	170-300
	Saponins	23.0 - 25.5	400-600
	Phenols	8.5 - 10.0	94, 107, 121
	Terpenoids	15.0 - 17.0	68, 69, 93
	Glycosides	22.0 - 24.0	300-400
Chloroform Extract	Alkaloids	9.0 - 11.0	120-150
	Flavonoids	12.5 - 14.5	153, 165, 285
	Tannins	18.0 - 19.5	170-180
	Saponins	22.5 - 24.5	400-600
	Phenols	8.0 - 9.5	94, 107, 121
	Terpenoids	14.5 - 16.5	68, 69, 93
	Glycosides	21.5 - 23.5	300-400

Key: *Retention time is measured in minutes; major peaks are shown on the x-axis by their mass-to-charge ratio.*

Table 2: Liver Histomorphological Scores

Sample I. D	Fatty Change	Hematozoin Deposit	Portal Tract Inflammatory Cells	Bile Duct Proliferation	Hepatocellular necrosis with Inflammatory	Haemorrhage	Total (/18)
Group 1/1	1	0	0	0	0	1	2
Group 1/2	0	0	0	0	0	1	1
Group 2/1	0	0	0	0	0	1	1
Group 2/2	0	0	0	0	0	1	1
Group 3/1	0	0	1	0	0	0	1
Group 3/2	0	0	0	0	0	1	1
Group 4/1	0	0	2	0	0	0	2
Group 4/2	0	0	1	0	0	0	1
Group 5/1	0	1	0	0	0	0	1
Group 5/2	1	0	0	0	0	0	1
Group 6/1	0	0	0	0	2	0	2
Group 6/2	0	1	0	0	0	0	1
Group 7/1	0	0	1	0	0	0	1
Group 7/2	1	0	1	0	0	0	2

Key: *Scores are allotted as 0= absent, 1 = affecting less than 30% of lesions, 2 = affecting 30 - 70% of lesions, and 3 = affecting 71-100% of lesions. Group 1= Control, Groups 2-4 = JT aqueous extract, Groups 5-7 = JT chloroform extract. Kruskal-Wallis Test = 0.741 (P<0.005).*

Table 3: Heart Histomorphological Scores

Sample I. D	Myocardial Changes	Fibrosis	Coronary arteriosclerosis	Hyperaemia	Haemorrhage	Inflammation	Total (/18)
Group 1/1	0	0	0	2	0	0	2
Group 1/2	0	0	0	1	0	0	1
Group 2/1	0	0	0	0	1	0	1
Group 2/2	0	0	0	0	2	0	2
Group 3/1	0	0	0	0	2	1	3
Group 3/2	0	0	0	0	2	0	2
Group 4/1	2	0	0	0	2	0	4
Group 4/2	1	0	0	0	2	0	3
Group 5/1	0	0	0	0	2	1	3
Group 5/2	0	0	0	0	1	0	1
Group 6/1	0	0	0	0	1	1	2
Group 6/2	0	0	0	0	1	0	1
Group 7/1	2	0	0	0	2	1	5
Group 7/2	2	0	0	0	1	1	3

Key: *Scores are allotted as 0= absent, 1 = affecting less than 30% of lesions, 2 = affecting 30 - 70% of lesions, and 3 = affecting 71-100% of lesions. Group 1= Control, Groups 2-4 = JT aqueous extract, Groups 5-7 = JT chloroform extract. Kruskal-Wallis Test = 0.741 (P<0.005).*

Table 4: Kidney Histomorphological Scores

Sample I.D	Glomerular hypertrophy or atrophy	Haemorrhage	Hyaline arteriopathy	Inflammatory cell infiltration	Hyperaemia	Tubular dilation	Total (/18)
Group 1/1	0	0	0	0	1	0	1
Group 1/2	0	0	0	0	2	0	2
Group 2/1	0	0	0	0	1	0	1
Group 2/2	0	0	0	0	1	0	1
Group 3/1	0	0	1	0	0	1	2
Group 3/2	0	0	2	0	0	1	3
Group 4/1	0	0	0	2	1	0	3
Group 4/2	0	0	0	1	1	0	2
Group 5/1	2	0	0	0	1	0	3
Group 5/2	1	0	0	0	0	0	1
Group 6/1	0	0	0	0	2	0	2
Group 6/2	0	0	0	0	1	0	1
Group 7/1	0	1	0	0	0	0	1
Group 7/2	0	2	0	0	0	0	2

Key: *Scores are allotted as 0= absent, 1 = affecting less than 30% of lesions, 2 = affecting 30 - 70% of lesions, and 3 = affecting 71-100% of lesions. Group 1= Control, Groups 2-4 = JT aqueous extract, Groups 5-7 = JT chloroform extract. Kruskal-Wallis Test = 0.741 (P<0.005).*

Table 5: Small Intestine Histomorphological Scores

Sample I. D	Erosion or ulceration of the mucosa	Glandular distortion	Epithelial dysplasia	Ectopic Tissues	Inflammation	Congestion of blood vessels	Total (/18)
Group 1/1	0	0	0	1	0	0	1
Group 1/2	0	0	0	0	0	0	1
Group 2/1	0	2	0	0	0	0	2
Group 2/2	0	0	0	0	0	0	0
Group 3/1	0	0	0	0	1	1	2
Group 3/2	0	0	0	0	2	0	2
Group 4/1	0	0	0	0	2	0	2
Group 4/2	0	0	0	0	2	0	2
Group 5/1	0	2	1	0	0	0	3
Group 5/2	0	1	1	0	0	0	2
Group 6/1	0	1	0	0	0	0	2
Group 6/2	0	2	0	0	0	0	1
Group 7/1	0	1	0	0	1	0	2
Group 7/2	0	0	0	0	2	0	2

Table 6: Haematological Analysis of Jatropha tanjorensis aqueous and chloroform extracts

Sample I. D	PCV (%)	HB (g/dl)	RBC (10^3/µl)	WBC (10^3/µl)	MCH (µg)	MCHC (g/dl)	MCV (fl)	PLT (10^3/µl)
Group 1 (Control)	54.00 ± 2.08^{c}	15.30 ± 0.62^{ab}	7.73 ± 0.15^{a}	19.50 ± 0.81^{a}	19.93 ± 1.10^{a}	28.33 ± 2.03^{ab}	71.67 ± 4.41^{b}	663.33 ± 8.82^{e}
Group 2 (A 1600mg/kg)	48.00 ± 1.73^{a}	14.50 ± 0.58^{ab}	7.60 ± 0.35^{a}	21.33 ± 0.88^{a}	20.00 ± 1.15^{a}	27.80 ± 1.56^{ab}	68.40 ± 2.09^{ab}	613.00 ± 8.50^{d}
Group 3 (A 2900mg/kg)	52.00 ± 1.16^{bc}	14.00 ± 0.43^{a}	7.43 ± 0.26^{a}	18.90 ± 0.76^{a}	19.30 ± 0.75^{a}	31.30 ± 0.75^{b}	65.00 ± 1.15^{ab}	558.00 ± 4.62^{b}
Group 4 (A 5000mg/kg)	55.00 ± 0.58^{c}	14.70 ± 0.40^{ab}	7.67 ± 0.38^{a}	20.20 ± 0.52^{a}	19.10 ± 0.64^{a}	28.50 ± 0.87^{ab}	67.10 ± 1.21^{ab}	592.00 ± 6.93^{c}
Group 5 (C 1600mg/kg)	55.00 ± 0.58^{c}	16.10 ± 0.63^{b}	7.93 ± 0.26^{a}	20.80 ± 0.69^{a}	21.60 ± 0.92^{a}	26.80 ± 1.04^{a}	71.30 ± 1.33^{b}	622.00 ± 6.93^{d}
Group 6 (C 2900mg/kg)	46.00 ± 0.58^{a}	13.80 ± 0.46^{a}	7.23 ± 0.15^{a}	19.40 ± 0.75^{a}	19.10 ± 0.58^{a}	30.10 ± 1.21^{ab}	63.40 ± 1.39^{a}	535.00 ± 2.89^{a}
Group 7 (C 5000mg/kg)	49.00 ± 0.58^{ab}	14.10 ± 0.35^{a}	7.40 ± 0.23^{a}	19.80 ± 0.75^{a}	19.60 ± 0.64^{ab}	29.70 ± 0.98^{ab}	65.3 ± 1.33^{ab}	633.00 ± 7.51^{d}

Key: *Group 1= Control, Groups 2-4 – JT aqueous extract, Groups 5-7 – JT chloroform extract. PCV – Pack Cell Volume, HB – Haemoglobin Count RBC – Red Blood Cells, WBC – White Blood Cells, MCH – Mean Corpuscular Hemoglobin, MCHC – Mean Corpuscular Haemoglobin Concentration, MCV – Mean Corpuscular Volume, PLT – Platelet Count, Group parameters with different letters superscript are significantly different (p<0.05). JT = Jatropha tanjorensis.*

Discussion

This study on the acute toxicity of *Jatropha tanjorensis* chloroform and aqueous leaf extracts in Wistar rats revealed no mortality, stable clinical parameters, and minimal histopathological changes, suggesting a low acute toxicity profile (even at doses up to 5,000 mg/kg). These findings generally align with the report's consistent findings. [1], [15], and are in alignment with OECD Guideline 423's low-toxicity category [16].

Certain components of therapeutic plants, such as proteins (toxalbumins), glycosides (cyanophoric, cardiac, steroidal, and lactone glycosides), and alkaloids (tropane, quinoline, and isoquinoline, as well as lupin, senecio, and pyridine-piperidine alkaloids), are associated with toxic effects.[1].

Phytochemical screening (Table 1) identified an array of bioactive compounds, including flavonoids, phenolics, tannins, alkaloids, saponins, terpenoids, and glycosides, in both extracts. It can be inferred that the polyphenols (flavonoids, m/z 153–285; phenolics, m/z 94–121) exerted their antioxidative, anti-inflammatory, and protective abilities on the hepatic and renal structures. This is seen in the incidence of minimal lesions (portal inflammation) and hyperammonaemia in Groups 3–7 (Tables 2- 5, Figures 11, 17). This is consistent with studies of [17] and [18] on *J. tanjorensis,* as well as protection against toxin-induced liver damage reported by [19] and [20]. On the other hand, saponins (m/z 400–600) and alkaloids (m/z 120–150) may explain modest platelet declines at 5,000 mg/kg in Group 7, potentially via membrane perturbation. However, it is noteworthy that these effects were not clinically significant, probably due to the dosage used in this study [21]. The phytochemistry of *J. tanjorensis* is mixed, combining antioxidative flavonoids, tannins, phenols, and membrane-active saponins, glycosides, and terpenoids. This explains the extracts' tolerance to single high doses; however, chronic or excessive use may unleash toxicity from accumulated alkaloids/tannins [22].

The outcome of our haematological analysis complements our phytochemical findings.

This is because there were no significant changes in the haematological parameters under study. Heamatological analysis revealed that most parameters, such as the Red Blood Cell (RBC) counts (7.23–7.93 × 10^3/µL), haemoglobin (Hb) (13.80–16.10 g/dL), and white blood cell counts (18.90–21.33 × 10^3/µL), remained stable across all groups, with no statistically significant differences compared to the control (Table 2, $p > 0.05$). This stability suggests that erythropoiesis and leukocyte dynamics were largely unaffected by the extracts after a single exposure. However, platelet counts fell significantly in Group 6 (chloroform extract, 2900 mg/kg) to 535.00 ± 2.89 × 10^3/µL, compared with the control (663.33 ± 8.82 × 10^3/µL; $p < 0.05$). Likewise, though less pronounced, the platelet count in Group 3 (aqueous extract, 2900 mg/kg) decreased to 558.00 ± 4.62 × 10^3/µL. These reductions could be linked to the presence of saponins, which are known to induce membrane agitations [16] The platelet counts bounced back to 5000 mg/kg (633.00 ± 7.51 × 10^3/µL), indicating a lack of dose dependence and a clinically negligible effect in the context of our study (acute exposure model). This is in contrast with the findings of [1] and [7] who reported an increase in major haematological parameters such as RBC and Hb with chronic dosage, which is consistent with the plant's indigenous use as 'blood tonic" [23], [24]. The exposure model in this study did not allow for such haematological manifestations.

Although there were slight changes to PCV, it is more closely related to physiologic function or dilution than to true erythrocyte loss, and the thrombocytopenia, which is often more consistent with the effects of saponin on platelet function. This is made evident by the lack of bleeding or overt coagulopathy in this acute model. Although they may be needed for repeated-dose studies and a dedicated coagulation/platelet-function assay, the haematological profiles are indicative of the plant's safe use.[1], [7].

Histological assessment of the liver, heart, kidney, and intestine revealed only mild, non-specific changes that were not dose-associated. The hepatic tissues were largely preserved, with only sporadic portal inflammation or fatty change (GROUP 1-2), consistent with adaptive responses and the reported hepatoprotective effects of *J. tanjorensis* flavonoids.[1], [19]. Cardiac tissue remained intact, with only occasional hyperemia or haemorrhage also seen in controls, supporting the absence of acute cardiotoxicity and contrasting with the phorbol ester toxicity reported in other *Jatropha* species.[25]. The renal histology in this study shows normal glomeruli and tubules, with only rare vascular congestion. Such congestion is often regarded as a benign, incidental finding consistent with preserved renal function and has been frequently reported in otherwise normal kidneys.[26]. This confirms that *J. tanjorensis* extracts are not acutely nephrotoxic. Previous chronic studies agree, as [1] reported no renal lesions with repeated dosing, and [22] demonstrated protective effects of *J. tanjorensis* leaves against nephrotoxin-induced injury. Furthermore, intestinal mucosa displayed only minor, reversible alterations, such as glandular distortion or focal inflammation, which may reflect tannin or saponin irritation at high local concentrations.[16]. Collectively, these findings confirm that both aqueous and chloroform extracts of *J. tanjorensis* exhibit low acute organ toxicity, corroborating the aforementioned haematological and clinical profiles observed.

Our acute toxicity findings closely corroborate the extant studies reporting high LD_{50} thresholds for *J. tanjorensis*. For instance, [15] observed no mortality in mice up to 6000 mg/kg, while [1] reported full tolerance in rats at 5000 mg/kg, mirroring our zero deaths at the same dose. Similarly, sub-chronic studies, such as those by [1] and [7], reported minor haematological shifts or mild hepatic changes at high doses. Expanding upon these, our work adds a novel solvent comparison (aqueous vs chloroform) and multi-organ histopathology, reinforcing that both extract types are well tolerated in acute settings.

Conclusion

This study demonstrated that the experimental animals' red blood cell and white blood cell counts were not clinically significantly impacted by extracts from different solvents (water and chloroform). This supports the culinary and medicinal use of *J. tanjorensis* leaves at customary doses. After extended exposure, a decrease in platelet count was associated with potential membrane agitations in the presence of saponin. Hepatic, renal, and cardiac tissues were mostly unaltered, and minor alterations were typically linked to the organs' adaptation to the extract's entry into the animal's system. The absence of acute harm at doses exceeding typical dietary exposure is reassuring, though concentrated extracts require caution. Therefore, formal or informal public health campaigns and regulatory guidance should also promote moderation and standardised preparations to uphold safety while leveraging the plant's nutritional and therapeutic benefits. Future research must address chronic toxicity, the isolation of active compounds, and the pathways underlying the plant's bioactivity.

Abbreviations

JT = *Jatropha tanjorensis,* PCV = Pack Cell Volume, HB = Haemoglobin Count, RBC = Red Blood Cells, WBC = White Blood Cells, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, MCV = Mean Corpuscular Volume, PLT = Platelet Count.

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Biology, Alex Ekwueme Federal University, Ndufu-Alike, Ebonyi State, Nigeria. The leaf samples were deposited in their microbiology laboratory with voucher numbers 0012 and 0013.

Authors' Contributions

UCS, COC, and AMN designed the study. UCS and AKI carried out the experimental protocol, including phytochemistry and animal handling. UCS conducted the statistical analysis, while ACM and OI interpreted the data. All authors contributed to the preparation of the manuscript. All the authors have read and approved the final manuscript for publication.

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Availability of Data and Materials

All data and materials are available on request.

Declarations, Ethics Approval, and Consent to Participate

The Ethics Committee approved this research at Alex Ekwueme Federal University, Ndufu-Alike, Ebonyi State, Nigeria, under reference number REF: FUNAI/SEN/EBC/17/VOL.1/34, and the animals were handled accordingly. In compliance with the International Standard for the use of laboratory animals.

APPENDIX

1: Ethical Clearance

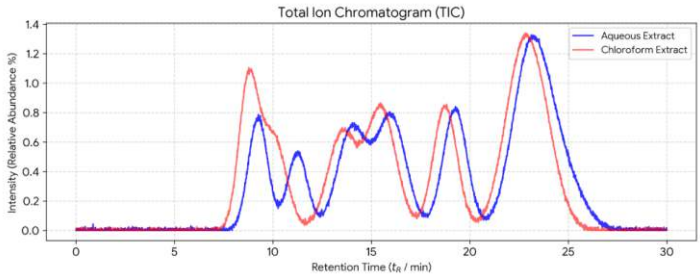
ALEX EKWUEME FEDERAL UNIVERSITY, NDUFU-ALIKE, IKWO
P.M.B. 1010, ABAKALIKI, EBONYI STATE, NIGERIA
REF: FUNAI/SEN/EBC/17/VOL.1/34 20th February, 2024

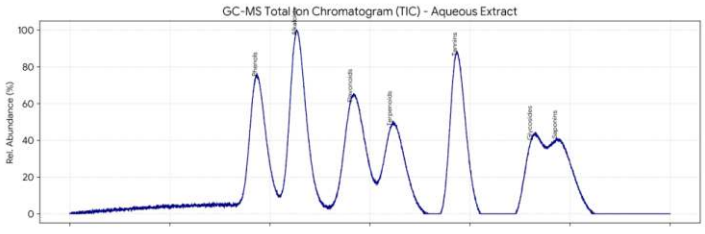
Ugwuocha, Chibuike Sunday,
2020/MS/14848
Department of Microbiology,
Alex Ekwueme Federal University, Ndufu Alike, Ikwo.
ACADEMIC RESEARCH ETHICS ACCEPTANCE LETTER

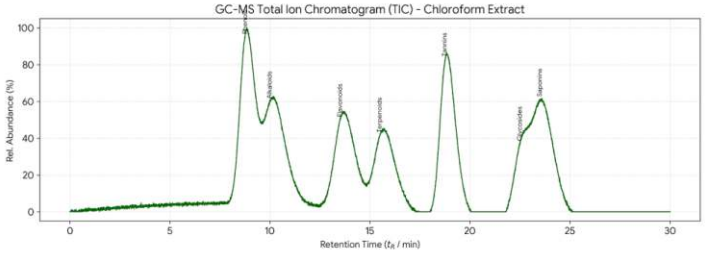
The University Ethics Committee at its 34th Regular Meeting on 20th February, 2024, approved your application and request for investigation in animals with the research topic **"Antimicrobial activity of *Jatropha tanjorensis* against *Escherichia coli* and Salmonella Infection in Wistar Rats"** The duration for this approval is three months from the approval date, 1st March to 1st June, 2024. At this juncture, the Committee's review will either extend or revoke the approval. This is subject to the committee's findings following its review of the research.
By this, you are expected to abide by the terms and conditions in the ethical application forms you signed, as well as the investigators' **declaration page**. Failure to comply with these conditions will automatically result in the withdrawal of acceptance.
Congratulations on your acceptance and best wishes on your research.
Yours sincerely,

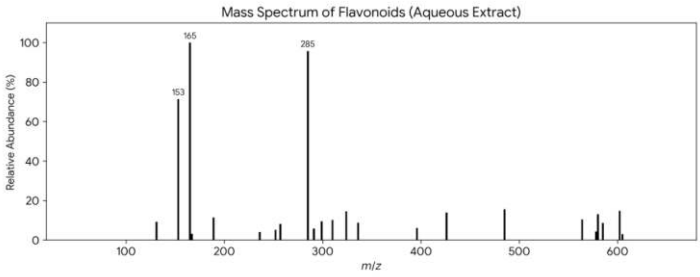
Dr Chukwuemeka O. Nwankiti
Secretary

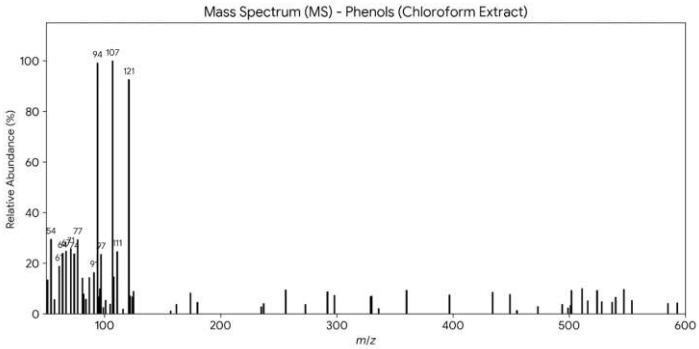
2: PHYTOCHEMICAL ANALYSIS











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