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The antibacterial activities of *Terminalia ivorensis A. Chev* (Combretaceae) leaves against multidrug-resistant bacteria and its toxicity profile

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ABSTRACT

Background: Multidrug resistance (MDR) poses a significant global threat to healthcare. *Terminalia ivorensis*, a plant traditionally used in Africa, has been reported to possess antibacterial and protective properties. However, there is limited scientific evidence regarding its effectiveness against multidrug-resistant strains and its safety profile. This study was therefore conducted to address these knowledge gaps and to support the WHO Traditional Medicine Centre's mission of developing safe, effective, and evidence-based plant-derived therapies. **Objective:** The objective of this study was to investigate the potential of *T. ivorensis* leaf extract as a safe and effective antibacterial agent against multidrug-resistant bacteria. **Materials and Methods:** *T. ivorensis* leaves were ethanol-extracted, phytochemically screened, and tested for antibacterial activity and toxicity using standard microbial assays and Organization for Economic Cooperation and Development (OECD) guidelines for animal studies. Multi-drug-resistant strain obtained from the Department of Pharmaceutical Microbiology, University of Lagos, previously profiled and validated for identity and antimicrobial susceptibility, was utilized in this study. Gram-negative isolates: Seven Extended β-lactamase-producing *Klebsiella pneumoniae* strains [Oga, N3, N24, OG 1(4), N19, Med 1(2), N14], one AmpC β-lactamase strain (Med 5(1)), one efflux pump active *K. pneumoniae* (OG 1(3)), and one *Pseudomonas aeruginosa* (PA 24) clinical isolate were used. *Escherichia coli* ATCC 25922 served as the drug-susceptible control. Gram-positive isolates: Included three oxacillin-resistant, carbapenemase-producing *Staphylococcus aureus* strains [ES 21, ES 44, MED4(2)], one carbapenemase-producing strain (ML 10), one oxacillin-resistant *Staphylococcus lentus* (ES 14), one *Micrococcus* spp. with efflux pump activity (ES 7), one *Staphylococcus xylosus* (ES 16), and one *Enterococcus faecalis* (EF 24). *S. aureus* ATCC 25923 was used as the control. **Results:** The 50% ethanol extract of *T. ivorensis* demonstrated concentration-dependent antibacterial activity against multidrug-resistant microorganisms, with inhibition zones ranging from 13.00 to 31.33 mm at concentrations of 100-400 mg/mL. The standard drug Levofloxacin exhibited bacterial inhibition zones ranging from 15.33 to 45.00 mm at concentrations of 6.25 to 50 µg/mL. While Levofloxacin showed strong activity against several strains, it was ineffective against certain *K. pneumoniae EPA, K. pneumoniae* ESBL producers, and *S. xylosus* ORSA. The extract exhibited minimum inhibitory concentrations (MIC) between 0.4-3.2 mg/mL, while levofloxacin showed ranges from 0.0156 to 8 µg/mL, and minimum bacterial concentrations (MBC), for the extract ranged from 0.8-25.6 mg/mL, compared to levofloxacin's 0.0156 to 8 µg/mL. Phytochemical analysis revealed the presence of alkaloids, tannins, phlobatannins, saponins, terpenoids, cardiac glycosides, reducing sugar, steroids, flavonoids, and phenols. The oral administration of the extracts at doses up to 2000 mg/kg showed no acute toxicity or adverse behavioural effects in mice over 14 days. Sub-acute treatment in rats for 28 days revealed no significant changes in body weight, haematological parameters, or liver function markers, except for a slight but significant increase in creatinine levels. The sub-chronic (60 days) test showed significant elevations in white blood cells, mid-sized Cells absolute, granulocytes absolute, lymphocytes percentage, granulocyte percentage, and platelets. Post-treatment cessation analysis showed notable differences in urea and low-density lipoprotein levels. **Conclusion:** These findings suggest that *T. ivorensis* possesses potent antibacterial properties against multidrug-resistant bacteria tested. Overall, the extract appears relatively safe at tested doses with mild immune activation.

Keywords: Antibacterial activity, *Terminalia ivorensis*, Multidrug resistant, Phytochemistry, Toxicity.

INTRODUCTION

Multidrug resistance (MDR) poses a significant threat to modern medicine, complicating the treatment of infections and increasing risks associated with surgeries and chemotherapy. In 2019, antimicrobial resistance was responsible for 1.27 million deaths and contributed to 4.95 million deaths worldwide [1]. Key resistant bacteria include Methicillin-resistant *Staphylococcus aureus* (MRSA) and Enterobacteriaceae species, such as *Escherichia coli* and *Klebsiella pneumoniae*, which produce enzymes that lead to antibiotic resistance [2]. *Terminalia spp*. is significant in African ethnomedicine for its astringent, haemostatic, and healing properties, particularly in treatment of hemorrhoids [3]. In Nigeria, *Terminalia ivorensis* A. Chev is used for various ailments, including syphilis and burns [4], while in Ghana, it treats fever, stomach aches, and wounds [5]. The plant's bark has shown renal protection and antifungal effects [6,7]. *T. ivorensis* is rich in phytochemicals [8,9], displaying antibacterial, antifungal, antioxidant, and anti-plasmodial effects *in vitro* [9,10], and demonstrating anti-inflammatory, anti-nociceptive, hepatoprotective, and nephroprotective properties *in vivo* studies [7,8,11].

The acetone extract of *T. ivorensis* fresh leaves exhibits antibacterial properties, effective against *E. coli* and *S. aureus*, with *B. subtilis* showing the least sensitivity. Among different solvent fractions, the ethyl acetate fraction had the highest antibacterial activity [12]. Additionally, a study found that the 70% ethanolic extract of *T. ivorensis* trunk barks was significantly more effective against methicillin-resistant Staphylococcus strains compared to the aqueous residue, indicating that the active compounds are more soluble in 70% ethanol. This supports the traditional use of *T. ivorensis* for treating skin diseases and inflammation [13].

In 2017, a study evaluated the intraperitoneal toxicity of *T. ivorensis* bark using adult Wistar rats, finding it moderately toxic with an LD_{50} of 200 mg/kg. No deaths occurred at doses of 2.5, 5.0, and 10.0 mg/kg for the control group, and no significant changes were noted in biochemical or hematological markers compared to controls [14]. Another study in the Ivory Coast assessed the acute and sub-acute toxicity of fractioned extracts. Adult Wistar rats received a single oral dose of 5000 mg/kg of the X_{42} fraction after fasting. Over 14 days, no mortality or significant abnormalities in haemato-biochemical parameters were observed. For sub-acute toxicity, doses of 250, 500, and 1000 mg/kg were administered daily for 28 days, confirming the safety of the X_{42} fraction at these levels [6].

Numerous studies have investigated the pharmacological effects and significance of *T. ivorensis*; however, the potential antibacterial activity against certain multidrug-resistant pathogens, along with the toxicological profile (acute, sub-acute, sub-chronic, and post-administration cessation) of the plant's dried leaves, remains largely unexplored. Therefore, the objective of this study is to address existing knowledge gaps regarding the plant leaves' antibacterial, phytochemical, and toxicological profiles. This aligns with the mission of the WHO Traditional Medicine Centre to provide evidence-based data that will inform policies, standards, and regulatory frameworks for the development of safe and effective formulations derived from *T. ivorensis* leaves.

MATERIAL AND METHODS

Collection and Preparation of Plant Materials

Fresh leaves of *T. ivorensis* were collected from the Botanical Garden of the University of Lagos and authenticated by Mr. Eze Tochukwu at the University Herbarium, where a voucher specimen (LUH 99) was deposited. The leaves were washed to remove surface debris, sorted, and separated from stems. They were air-dried under shade for five days at room temperature (25 ± 2 °C), pulverized into fine powder using a botanical milling machine, and stored in airtight containers in a dark cupboard until required.

Plant Extraction

A total of 800 g of the powdered leaves was extracted with 4 L of 50% ethanol via cold maceration for 72 h with intermittent shaking. The filtrate was obtained using muslin cloth and Whatman No. 2 filter paper, then concentrated under reduced pressure at ≤ 45 °C with a rotary evaporator. The resulting extract was freeze-dried, weighed, and stored in sterile bottles at 4 °C until use.

Phytochemical Screening

Qualitative phytochemical analysis of the ethanol leaf extract of *T. ivorensis* was performed to detect alkaloids, flavonoids, saponins, steroids, tannins, phlobatannins, reducing sugars, terpenoids, cardiac glycosides, and phenolic compounds following the procedures of Ejikeme *et al*. [15].

Antibacterial Activity

Bacterial Isolates

Previously identified and validated Gram-positive and Gram-negative bacterial isolates were obtained from the Department of Pharmaceutical Microbiology, University of Lagos [16,17]. All cultures were standardized to the 0.5 McFarland standard (1×10^8 CFU/mL) using sterile normal saline (0.05 mL of 1.0% $BaCl_2$ in 9.95 mL of 1.0% H_2SO_4).

Gram-negative isolates

Seven Extended β-lactamase-producing *K. pneumoniae* strains [Oga, N3, N24, OG 1(4), N19, Med 1(2), N14], one AmpC β-lactamase strain (Med 5(1)), one efflux pump active *K. pneumoniae* (OG 1(3)), and one *Pseudomonas aeruginosa* (PA 24) clinical isolate were used. *Escherichia coli* ATCC 25922 served as the drug-susceptible control per Clinical and Laboratory Standards Institute (CLSI) [18].

Gram-positive isolates

Included three oxacillin-resistant, carbapenemase-producing *S. aureus* strains [ES 21, ES 44, MED4(2)], one carbapenemase-producing strain (ML 10), one oxacillin-resistant *S. lentus* (ES 14), one *Micrococcus* spp. with efflux pump activity (ES 7), one *S. xylosus* (ES 16), and one *E. faecalis* (EF 24). *S. aureus* ATCC 25923 was used as the control [18].

PREPARATION OF WORKING SOLUTIONS

Extract solutions

For Sample A, 4 g of *T. ivorensis* extract was dissolved in 10 mL of sterile distilled water (400 mg/mL). Sample B was prepared by mixing 2 mL each of Sample A and sterile diluent (200 mg/mL), and Sample C by combining 2 mL of Sample B with 2 mL diluent (100 mg/mL). All were vortexed before use.

Levofloxacin solutions

A 5000 µg/mL stock (Sample I) was serially diluted: Sample II (500 µg/mL), Sample III (50 µg/mL), Sample IV (25 µg/mL), Sample V (12.5 µg/mL), and Sample VI (6.25 µg/mL).

Media Preparation

Mueller–Hinton Agar (MHA) was prepared according to the manufacturer's instructions (ReadyMed®). 38 g of MHA powder was suspended in 1000 mL distilled water, sterilized at 121 °C for 15 min using a Microfield® MF-280A autoclave, and poured into sterile Petri dishes (150 mm × 15 mm) to solidify to a depth of 5–6 mm before inoculation [19].

Antibiotic Susceptibility Testing

Following Bauer *et al*. [19], standardized bacterial suspensions were evenly inoculated on MHA plates using sterile swabs. Each plate (25 mL agar) was divided into four quadrants for different concentrations of extract and/or levofloxacin. Wells (10 mm) were bored aseptically, and 150 µL of extract (400, 200, 100 mg/mL) or levofloxacin (50, 25, 12.5, 6.25 µg/mL) was dispensed into respective wells; sterile water served as control. Plates were left for 2 h to allow diffusion, then incubated at 37 °C for 24 h. Zones of inhibition were measured in millimeters.

Minimum Inhibitory Concentration (MIC)

MICs of the extract and levofloxacin were determined using the modified agar dilution technique described by Hugo and Russell (2004) [20] and CLSI [18, 21]. For the extract, three stock solutions (1.25, 40.00, 80.00 mg/mL) were serially diluted to thirteen working concentrations (0.0125–51.2000 mg/mL). For levofloxacin, four stock concentrations (25, 0.625, 0.0625, 0.00625 µg/mL) generated thirteen working concentrations (0.001953125–8.00 µg/mL).

Sterilized molten MHA (121 °C, 15 min) was mixed with the test solutions, poured into labeled plates, and allowed to set. Each test organism (adjusted to 0.5 McFarland) was inoculated aseptically onto the plates and incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration showing no visible bacterial growth.

Minimum Bactericidal Concentration (MBC)

To determine the MBC, 20 mL of molten single-strength MHA supplemented with 0.1% Tween 80 was sterilized and poured into sterile Petri dishes. From the MIC plates, sections without visible growth were sub-cultured onto MHA–Tween 80 plates using a sterile loop and incubated at 37 °C for 24 h. The MBC corresponded to the lowest concentration at which no bacterial growth was observed.

TOXICOLOGICAL EVALUATION

Experimental Animals and Design

Albino mice (18-22 g) and Wistar rats (130-170 g) were obtained from the University of Lagos. Animals were maintained in the experimental animal facility under controlled conditions (22 ± 3 °C, 50-60% humidity, 12 h light/dark cycle) with free access to water and commercial feed (New Hope Grower Pellets, Nigeria). They were acclimatized for 14 days before experiments, individually marked, and examined by animal health officers for fitness. Bedding was changed regularly, and humane euthanasia was performed via cervical dislocation after the experiments.

Extract suspensions were administered orally based on body weight, not exceeding 0.04 mL/20 g for mice and 2 mL/100 g for rats. Animals were fasted prior to dosing: rats overnight, mice for 3 - 4 h and remained fasted post-dosing (rats 3 - 4 h, mice 1 - 2 h). Toxicity studies followed OECD guidelines No. 425, 407, 408 [22,24].

Acute Oral Toxicity

Eight nulliparous, non-pregnant female mice (8-12 weeks; averaging 20 g) were randomly divided into four groups. Groups received 100, 500, or 2000 mg/kg of *T. ivorensis* extract orally; controls received 0.1 mL distilled water. Animals were observed for 24 h for mortality and behavioral changes (hyperactivity, leaning, scratching, agitation, etc.). Since no lethality occurred, three additional mice were treated at 2000 mg/kg for confirmation of safety.

Sub-Acute Oral Toxicity (28 Days)

Twenty-four adults female Wistar rats (150 ± 20% g) were divided into four groups (n = 6) and administered *T. ivorensis* extract orally at 40, 200, or 1000 mg/kg daily for 28 days; controls received 2 mL distilled water. Rats Were weighed weekly and observed for behavioral or physical changes. After 28 days, animals were fasted overnight before sample collection for hematological and biochemical analysis.

Sub-Chronic Oral Toxicity (60 Days)

Another 24 female Wistar rats were divided similarly and treated daily for 60 days with the same doses (40, 200, 1000 mg/kg). Weight and behavior were monitored weekly. After 60 days, rats were fasted overnight, and blood was collected for hematological and biochemical assays.

Satellite Group (Post-Treatment Observation)

To assess reversibility of any toxic effects, five additional rats per group (from the sub-chronic study) were maintained for 14 days after treatment cessation. Behavioral observations continued during this period, after which blood was collected, and animals were euthanized by cervical dislocation.

Hematological Analysis

During euthanasia, blood samples were drawn via ocular puncture into EDTA tubes. Analyses were performed using the Mindray BC-3200 Fully Automated Hematology Analyzer®, employing 13 µL of blood, 3.5 mL of diluent, and 0.5 mL of lyse reagent to measure total/differential leukocytes, erythrocyte count, hemoglobin, hematocrit, and platelet parameters.

Clinical Biochemistry analysis

Blood for biochemical evaluation was collected into lithium-heparin tubes and centrifuged at 1600 rcf for 5 min (Eppendorf 5702®). Plasma (50 µL) was analyzed with Erba® Mannheim XL-640 Analyzer®, using 100 µL reagent for each parameter. Assays included total cholesterol, bilirubin, urea, creatinine, total protein, albumin, and liver enzymes-alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP).

Ethical Considerations

All animal procedures adhered to national and institutional guidelines for laboratory animal care and use. Ethical approval was granted by the Health Research Ethics Committee (HREC) of the College of Medicine, University of Lagos (Approval No:UNILAGREC/22/06/005). The study complied with the Guide for the Care and Use of Laboratory Animals.

Data Management and Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Graphs were generated using Microsoft Excel. One-way analysis of variance (ANOVA) determined statistical significance among groups, with Tukey's HSD used for post-hoc comparisons. Results were considered significant at $p \leq 0.05$.

RESULTS

Phytochemistry of *Terminalia ivorensis*

The preliminary phytochemical screening of *T. ivorensis* leaf extract revealed the presence of alkaloids, tannins, phlobatannins, saponins, terpenoids, cardiac glycosides, reducing sugars, steroids, flavonoids, and phenols.

Antibacterial Activity

The ethanol extract of *T. ivorensis* demonstrated strong antibacterial efficacy against all tested multidrug-resistant (MDR) bacterial strains. The mean inhibition zones at different concentrations are shown in Table 1. At 400 mg/mL, inhibition ranged between 21.33 mm and 31.33 mm; at 200 mg/mL, 16.33–29.33 mm; and at 100 mg/mL, 13.00–25.33 mm. In comparison, levofloxacin, the reference antibiotic, exhibited inhibition zones ranging from 15.33 mm to 45.00 mm at concentrations between 6.25 and 50 µg/mL. No inhibition zones were recorded for OG 1(3) *K. pneumoniae* EPA, OG 1(4) *K. pneumoniae* EPA (an ESBL producer), Oga *K. pneumoniae* (ESBL producer), and Es 16 *S. xylosus* ORSA at levofloxacin concentrations between 6.25 and 50 µg/mL, except for *S. xylosus* ORSA, which displayed an inhibition zone of 13.33 mm at 50 µg/mL as show in Table 2. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) data are summarized in Table 3. The MIC values for *T. ivorensis* ethanol extract ranged from 0.4 to 3.2 mg/mL, while levofloxacin showed MIC values between 0.0156 and 8 µg/mL. The MBC of the ethanol extract ranged from 0.8 to 25.6 mg/mL, compared to 0.0156 to 8 µg/mL for levofloxacin.

Assessment of Toxicological Profile

Acute Toxicity

Oral administration of *T. ivorensis* ethanol extract at doses up to 2000 mg/kg produced no visible signs of toxicity in mice throughout the 14-day observation period. No changes were observed in behavior, posture, salivation, or excretion, and no mortality occurred. The oral median lethal dose (LD_{50}) was therefore estimated to be greater than 2000 mg/kg. Body weights of treated mice (19.80 ± 1.30 g) did not differ significantly from those of control animals (20.40 ± 1.67 g).

Sub-acute Toxicity

No significant weight differences were observed between control and extract-treated rats after 28 days, as shown in Figure 1. Haematological parameters including red and white blood cell counts, haemoglobin, lymphocytes, and platelets did not differ significantly between treated and control groups ($p < 0.05$). White blood cell counts were slightly reduced in extract-treated animals, while red blood cell counts were marginally higher than those of the control group, except at the 200 mg/kg dose where the count was slightly lower (7.84 ± 0.64 ×10⁶/µL vs. 7.93 ± 0.42 ×10⁶/µL for the control). Biochemical findings showed that creatinine levels were significantly elevated ($p < 0.05$) in the treated groups (68.88 ± 2.58, 80.66 ± 7.06, and 64.60 ± 5.44 µmol/L) at doses of 40, 200, and 1000 mg/kg, respectively, relative to the control (63.61 ± 4.46 µmol/L). Alanine transaminase (ALT) and aspartate aminotransferase (AST) enzyme levels were lower in the treated groups than in the control group, while alkaline phosphatase, triglycerides, and total protein values remained within normal limits. A summary of haematological and biochemical parameters is presented in Table 4.

Sub-chronic Toxicity

At the end of the sub-chronic study, no significant differences in body weight were observed between treated and control groups, as shown in Figure 2. However, white blood cell counts were significantly increased in the treatment groups ($p < 0.05$), with values of 5.93 ± 1.35, 8.85 ± 1.98, and 5.35 ± 0.93 ×10³/µL at 40, 200, and 1000 mg/kg, respectively, compared with 4.30 ± 1.47 ×10³/µL for the control group. Corresponding increases were also observed in absolute granulocyte counts, lymphocyte percentages, granulocyte percentages, mid-sized cell absolute, and platelet count. Red blood cell counts remained unchanged across all treatment groups (8.61 ± 0.38, 8.85 ± 0.50, and 8.95 ± 0.30 ×10⁶/µL) compared with the control (8.69 ± 0.37 ×10⁶/µL). Biochemical parameters summarized in Table 5 show that creatinine levels did not differ significantly among groups. AST values declined slightly at higher doses, from 50.65 ± 5.47 IU/L in the control to 45.28 ± 5.87 IU/L and 45.03 ± 9.51 IU/L at 200 and 1000 mg/kg, respectively.

Satellite Group Analysis

In the satellite (recovery) group assessed after cessation of treatment, haematological and biochemical parameters are summarized in Table 6. White blood cell counts remained elevated at 200 mg/kg, while red blood cell counts were lower at 1000 mg/kg. Platelet counts were significantly higher at 200 mg/kg but decreased toward normal at higher doses. Creatinine and AST levels were lower in all treatment groups relative to the control, while ALT levels decreased at 200 mg/kg. Urea and low-density lipoprotein (LDL) levels showed statistically significant variations.

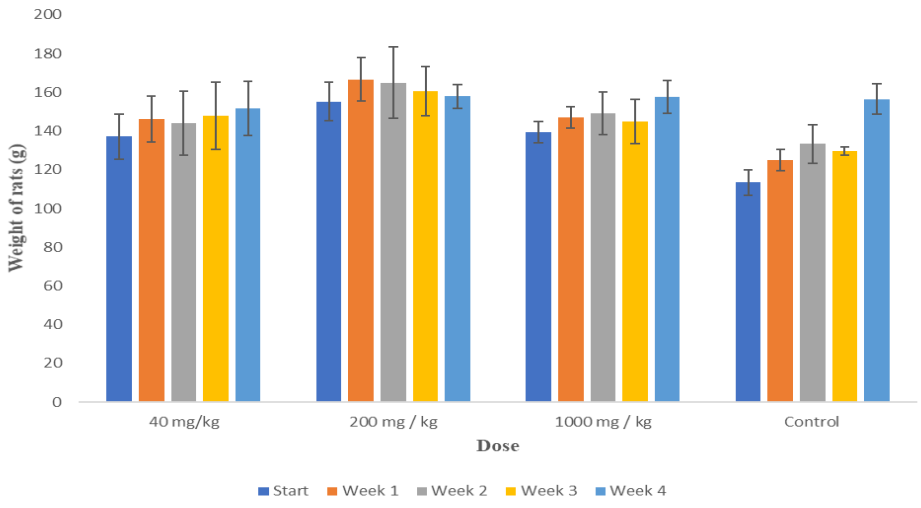

Figure 1: Effect of *T. ivorensis* leave extract on body weight across the sub-acute toxicity

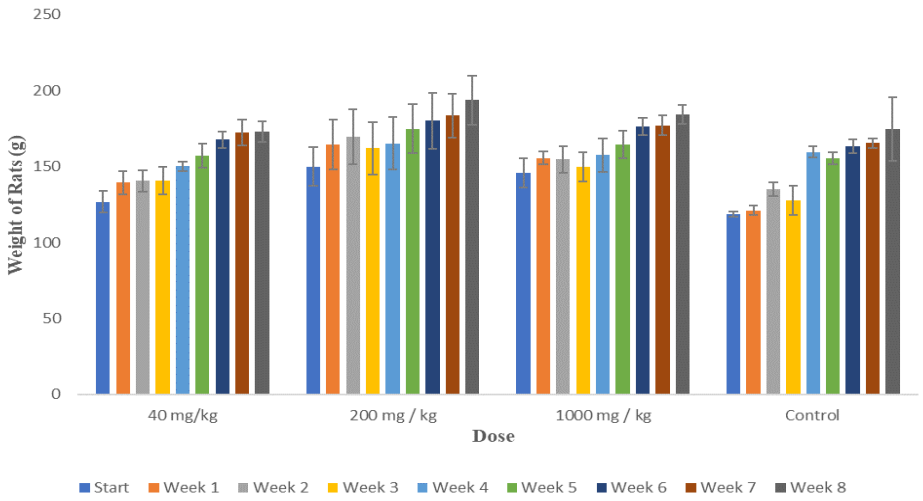

Figure 2: Effect of *T. ivorensis* leave extract on body eight across the sub-chronic toxicity

Table 1: Inhibition zone of *T. ivorensis* against the MDR strains at different concentration

	T. ivorensis				
	Concentration	400 mg/mL	200 mg/mL	100 mg/mL	Solvent
S/N	**ORGANISM CODE** (*Organism, Phenotype)*	**Zone of inhibition (mm)**			
1	**Es 44** *Staphylococcus aureus, EPA, ORSA, Carbapenemase*	28.33 ± 0.58	25.67 ± 1.15	20.67 ± 0.58	0.00 ± 0.00
2	**Med 4 (2)** *Staphylococcus aureus, EPA, ORSA, Carbapenemase*	30.00 ± 1.00	21.67 ± 0.58	19.00 ± 0.00	0.00 ± 0.00
3	**Es 14** *Staphylococcus lentus ORSA*	29.67 ± 0.58	24.33 ± 0.58	18.00 ± 0.00	0.00 ± 0.00
4	**Es 16** *Staphylococcus xylosus ORSA*	30.00 ± 0.00	24.33 ± 0.58	20.67 ± 1.15	0.00 ± 0.00
5	**Es 21** *Staphylococcus aureus, ORSA, Carbapenemase*	22.00 ± 1.00	15.33 ± 0.58	13.33 ± 0.58	0.00 ±0.00
6	**ML 10** *Staphylococcus aureus, Carbapenemase*	31.00 ± 1.00	25.67 ± 0.58	23.33 ± 0.58	0.00 ± 0.00
7	**Es 7** *Micrococcus species EPA, ORSA, Carbapenemase*	25.67 ± 0.58	19.67 ± 0.58	16.33 ± 0.58	0.00 ± 0.00
8	**EF 24** *Enterococcus faecalis*	23.00 ± 0.00	16.33 ± 0.58	13.00 ± 0.00	0.00 ± 0.00
9	**ATCC 25923** *Staphylococcus aureus*	27.33 ± 0.58	25.33 ± 0.58	22.67 ± 0.58	0.00 ± 0.00
10	**N 19** *Klebsiella pneumoniae ESBL producer*	29.33 ± 1.15	26.33 ± 1.53	21.00 ± 1.00	0.00 ± 0.00
11	**N 24** *Klebsiella pneumoniae ESBL producer*	24.67 ± 1.15	18.67 ± 1.15	16.33 ± 0.58	0.00 ± 0.00
12	**Med 1(2)** *Klebsiella pneumoniae EPA; ESBL producer*	31.33 ± 1.53	26.33 ± 5.51	21.67 ± 0.58	0.00 ± 0.00
13	**OG 1(3)** *Klebsiella pneumoniae EPA*	26.33 ± 0.58	22.33 ± 0.58	20.00 ± 1.00	0.00 ± 0.00
14	**OG 1(4)** *Klebsiella pneumoniae EPA; ESBL producer*	30.00 ± 1.00	29.33 ± 0.58	25.33 ± 0.58	0.00 ± 0.00
15	**Oga** *Klebsiella pneumoniae ESBL producer*	26.00 ± 1.00	21.33 ± 0.58	19.00 ± 0.00	0.00 ± 0.00
16	**Med 5(1)** *Klebsiella pneumoniae AmpC β-lactamase producer*	27.33 ± 0.58	25.67 ± 0.58	24.00 ± 0.00	0.00 ± 0.00
17	**N 14** *Enterobacter aerogenes ESBL producer*	29.00 ± 1.73	20.33 ± 1.53	17.67 ± 0.58	0.00 ± 0.00
18	**N 3** *Klebsiella pneumoniae ESBL producer*	27.33 ± 0.58	26.67 ± 1.15	24.67 ± 0.58	0.00 ± 0.00
19	**PA 24** *Pseudomonas aeruginosa*	21.33 ± 0.58	20.33 ± 1.15	17.33 ± 0.58	0.00 ± 0.00
20	**ATCC 25922** *Escherichia coli*	23.33 ± 0.58	19.67 ± 0.58	18.67 ± 1.15	0.00 ± 0.00

Mean ± SD; EPA: Efflux pump activity; ORSA: Oxacillin Resistant *Staphylococcus. Aureus*; ESBL: Extended Spectrum β-Lactamase

Table 2: Inhibition zone of Levofloxacin against the MDR strains at different concentrations

	Levofloxacin				
	Concentration	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL
S/N	**ORGANISM CODE** (*Organism, Phenotype)*	**Zone of Inhibition (mm)**			
1	**Es 44** *Staphylococcus aureus, EPA, ORSA, Carbapenemase*	42.33 ± 0.58	42.67 ± 0.58	35.00± 1.00	33.67 ± 0.58
2	**Med 4 (2)** *Staphylococcus aureus, EPA, ORSA, Carbapenemase*	36.67 ± 0.58	32.33 ± 0.58	29.67 ± 0.58	28.67 ± 0.58
3	**Es 14** *Staphylococcus lentus ORSA*	36.00 ± 0.00	32.33 ± 0.58	26.00 ± 1.00	0.00 ± 0.00
4	**Es 16** *Staphylococcus xylosus ORSA*	13.33 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	**Es 21** *Staphylococcus aureus, ORSA, Carbapenemase*	35.33 ± 0.58	34.33 ± 1.15	28.00 ±0.00	21.33 ± 0.58
6	**ML 10** *Staphylococcus aureus, Carbapenemase*	40.00 ± 0.00	36.00 ± 1.00	23.33 ± 0.58	0.00 ± 0.00
7	**Es 7** *Micrococcus species EPA, ORSA, Carbapenemase*	37.33 ± 0.58	37.00 ± 1.00	34.00 ± 0.00	32.33 ± 0.58
8	**EF 24** *Enterococcus faecalis*	31.67 ± 0.58	29.00 ± 1.00	23.33 ± 0.58	15.33 ± 0.58
9	**ATCC 25923** *Staphylococcus aureus*	31.33 ± 0.58	25.67 ± 0.58	21.00 ± 1.00	0.00 ± 0.00
10	**N 19** *Klebsiella pneumoniae ESBL producer*	28.67 ± 1.15	27.67 ± 0.58	26.33 ± 0.58	15.67 ± 0.58
11	**N 24** *Klebsiella pneumoniae ESBL producer*	34.00 ± 0.00	31.00 ± 1.00	29.00 ± 0.00	23.67 ± 0.58
12	**Med 1(2)** *Klebsiella pneumoniae EPA; ESBL producer*	27.33 ± 0.58	25.67 ± 0.58	22.00 ± 1.00	20.33 ± 0.58
13	**OG 1(3)** *Klebsiella pneumoniae EPA*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
14	**OG 1(4)** *Klebsiella pneumoniae EPA; ESBL producer*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
15	**Oga** *Klebsiella pneumoniae ESBL producer*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
16	**Med 5(1)** *Klebsiella pneumoniae AmpC β-lactamase producer*	30.67 ± 1.15	24.33 ± 0.58	21.67 ± 0.58	21.33 ± 1.15
17	**N 14** *Enterobacter aerogenes ESBL producer*	26.00 ± 1.00	22.33 ± 0.58	21.67 ± 0.58	20.33 ± 0.58
18	**N 3** *Klebsiella pneumoniae ESBL producer*	37.67 ± 0.58	33.33 ± 0.58	28.33 ± 1.53	26.33 ± 1.15
19	**PA 24** *Pseudomonas aeruginosa*	28.33 ± 0.58	22.00 ± 1.00	20.33 ± 0.58	16.33 ± 1.15
20	**ATCC 25922** *Escherichia coli*	34.00 ± 1.00	29.67 ± 0.58	24.00 ± 1.00	21.67 ± 0.58

Mean ± SD; Mean ± SD; EPA: Efflux pump activity; ORSA: Oxacillin Resistant *Staphylococcus. Aureus*; ESBL: Extended Spectrum β-Lactamase

Table 3: Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) for *T. ivorensis* and Levofloxacin

		T. ivorensis		**Levofloxacin**	
S/N	**ORGANISM CODE (***Genus and Species*, *Organism Phenotype***) GRAM POSITVES**	**MIC** (mg/mL)	**MBC** (mg/mL)	**MIC** (µg/mL)	**MBC** (µg/mL)
1	**Es 44** *Staphylococcus aureus, EPA, ORSA, Carbapenemase*	0.8	3.2	8	8
2	**Med 4 (2)** *Staphylococcus aureus, EPA, ORSA, Carbapenemase*	0.8	1.6	0.0625	2
3	**Es 14** *Staphylococcus lentus ORSA*	0.4	1.6	0.25	4
4	**Es 16** *Staphylococcus xylosus ORSA*	0.8	3.2	0.0625	2
5	**Es 21** *Staphylococcus aureus, ORSA, Carbapenemase*	0.4	1.6	1	2
6	**ML 10** *Staphylococcus aureus, Carbapenemase*	1.6	1.6	1	2
7	**Es 7** *Micrococcus species EPA, ORSA, Carbapenemase*	1.6	12.8	0.125	0.125
8	**EF 24** *Enterococcus faecalis*	1.6	12.8	4	4
9	**ATCC 25923** *Staphylococcus aureus*	3.2	3.2	2	2
GRAM NEGATIVE					
10	**N 19** *Klebsiella pneumoniae ESBL producer*	3.2	25.6	0.0156	0.0156
11	**N 24** *Klebsiella pneumoniaeESBL producer*	1.6	12.8	0.5	8
12	**Med 1(2)** *Klebsiella pneumoniae EPA; ESBL producer*	3.2	6.4	0.25	2
13	**OG 1(3)** *Klebsiella pneumoniae EPA*	1.6	1.6	0.25	0.5
14	**OG 1(4)** *Klebsiella pneumoniae EPA; ESBL producer*	1.6	3.2	1	2
15	**Oga** *Klebsiella pneumoniae ESBL producer*	0.8	0.8	1	2
16	**Med 5(1)** *Klebsiella pneumoniae AmpC β-lactamase producer*	3.2	12.8	0.5	0.5
17	**N 14** *Enterobacter aerogenes ESBL producer*	3.2	12.8	0.5	2
18	**N 3** *Klebsiella pneumoniae ESBL producer*	0.8	6.4	0.5	2
19	**PA 24** *Pseudomonas aeruginosa*	0.4	0.8	0.125	0.125
20	**ATCC 25922** *Escherichia coli*	0.4	0.8	0.0313	0.25

Mean ± SD; Mean ± SD; EPA: Efflux pump activity; ORSA: Oxacillin Resistant *Staphylococcus. Aureus*; ESBL: Extended Spectrum β-Lactamase

Table 4: Haematological and Biological effects of 28 days repeated dosing of *T. ivorensis* in the Sub-acute Toxicity Study

Haematological Parameter	Unit	40 mg/kg Treatment Group	200 mg/kg Treatment Group	1000 mg/kg Treatment Group	Control Group
		Mean ± SD			
White Blood Cells	10^3/µL	7.43 ± 0.67	7.78 ± 2.20	7.15 ± 1.73	9.35 ± 1.75
Lymphocytes absolute	10^3/µL	3.20 ± 0.76	2.90 ± 0.86	2.88 ± 0.77	4.28 ± 1.00
Mid-sized Cell absolute	10^9/µL	0.83 ± 0.29	1.08 ± 0.31	0.98 ± 0.43	1.40 ± 0.35
Granulocytes absolute	10^9/µL	3.40 ± 0.32	3.80 ± 1.25	3.30 ± 1.15	3.68 ± 0.53
Lymphocytes percentage	%	42.63 ± 6.44	37.25 ± 4.30	41.40 ± 9.63	45.40 ± 3.41
Mid-sized cell percentage	%	11.55 ± 3.29	14.23 ± 1.56	13.28 ± 3.08	15.08 ± 1.20
Granulocytes percentage	%	45.83 ± 8.14	48.53 ± 5.76	45.33 ± 6.77	39.53 ± 3.21
Haemoglobin	g/dL	14.23 ± 1.01	13.43 ± 0.87	14.40 ± 0.97	13.98 ± 0.75
Red Blood Cell	10^6/µL	8.29 ± 0.41	7.84 ± 0.64	8.22 ± 0.53	7.93 ± 0.42
Haematocrit - Pack Cell Volume	%	41.40 ± 2.96	40.90 ± 2.28	42.80 ± 0.95	42.15 ± 2.55
Mean Cell Volume	fL	49.93 ± 1.35	52.35 ± 2.11	52.23 ± 2.45	53.20 ± 0.41
Mean Cell Haemoglobin	pg.	17.10 ± 0.51	17.10 ± 0.50	17.48 ± 0.38	17.55 ± 0.21
Mean Cell Haemoglobin Concentration	g/dL	34.30 ± 0.33	32.75 ± 0.83	33.58 ± 1.55	33.10 ± 0.45
Red Blood Cell Distribution Width - Coefficient of Variation	%	13.65 ± 0.10	15.15 ± 0.65	14.38 ± 1.42	14.18 ± 0.35
Red Blood Cell Distribution Width - Standard Deviation	fL	25.20 ± 0.80	26.75 ± 1.86	26.78 ± 2.98	26.53 ± 0.45
Platelet	10^9/L	776.25 ± 278.18	640.00 ± 13.06	731.75 ± 61.43	640.00 ± 150.39
Platelet Distribution Width	%	15.75 ± 0.74	15.95 ± 0.37	15.83 ± 0.19	15.93 ± 0.43
Plateletcrit	%	0.35 ± 0.26	0.45 ± 0.03	0.52 ± 0.04	0.46 ± 0.08
Biochemical parameter					
Bilirubin Total	µmol/L	2.40 ± 0.41	2.71 ± 0.81	3.20 ± 1.45	2.39 ± 0.60
High Density Lipoprotein	mmol	1.18 ± 0.11	1.42 ± 0.11	1.45 ± 0.18	1.34 ± 0.28
Creatinine	µmol /L	68.88 ± 2.58	*80.66 ± 7.06	64.60 ± 5.44	63.61 ± 4.46
Alanine Transaminase	IU/L	76.50 ± 16.10	85.63 ± 22.14	75.78 ± 8.88	91.93 ± 29.14
Aspartate aminotransferase	IU/L	164.80 ± 53.15	141.63 ± 40.26	123.78 ± 7.84	171.43 ± 83.18
Alkaline phosphatase	IU/L	259.88 ± 35.91	252.53 ± 184.20	362.65 ± 146.56	445.30 ± 143.57
Urea	mmol/L	5.44 ± 0.89	6.09 ± 0.42	6.04 ± 0.87	5.28 ± 0.22
Triglyceride	mmol/L	0.80 ± 0.17	0.89 ± 0.26	0.76 ± 0.12	0.93 ± 0.28
Cholesterol	mmolL	1.99 ± 0.18	2.10 ± 0.13	1.98 ± 0.24	1.89 ± 0.33
Low Density Lipoprotein	mmolL	0.37 ± 0.15	0.27 ± 0.19	0.20 ± 0.11	0.23 ± 0.13
Protein	g/L	76.43 ± 6.80	76.88 ± 1.47	73.05 ± 4.02	72.48 ± 4.92
Albumin Blood test	g/L	33.28 ± 2.61	32.45 ± 2.90	32.65 ± 0.90	34.48 ± 1.60

Key: *p-value significant at $p<0.05$

Table 5: Haematological and biochemical effects of 60 Days Repeated Dosing of *T. ivorensis* in the Sub-chronic Toxicity Study

Haematological Parameter	Unit	40 mg/kg Treatment Group	200 mg/kg Treatment Group	1000 mg/kg Treatment Group	Control Group
		Mean ± SD			
White Blood Cells	10^3/µL	5.93 ± 1.35	*8.85 ± 1.98	5.35 ± 0.93	4.30 ± 1.47
Lymphocytes absolute	10^3/µL	2.73 ± 0.59	3.20 ± 0.44	2.85 ± 1.35	1.95 ± 0.73
Mid-sized Cell absolute	10^9/µL	0.88 ± 0.34	*1.45 ± 0.26	0.85 ± 0.13	0.78 ± 0.31
Granulocytes absolute	10^9/µL	2.25 ± 0.65	*4.20 ± 1.33	2.40 ± 0.54	1.58 ± 0.50
Lymphocytes percentage	%	46.05 ± 3.53	*36.80 ± 4.77	38.75 ± 1.59	44.73 ± 2.50
Mid-sized cell percentage	%	16.05 ± 0.59	16.73 ± 1.09	17.08 ± 3.08	18.38 ± 3.90
Granulocytes percentage	%	36.90 ± 5.02	*46.48 ± 5.47	44.18 ± 2.91	36.90 ± 3.77
Hemoglobin	g/dL	15.88 ± 1.10	16.18 ± 0.79	15.98 ± 0.66	15.80 ± 0.20
Red Blood Cell	10^6/µL	8.61 ± 0.38	8.85 ± 0.50	8.95 ± 0.30	8.69 ± 0.37
Hematocrit - Pack Cell Volume	%	46.70 ± 3.07	47.85 ± 1.90	47.20 ± 1.83	47.20 ± 0.84
Mean Cell Volume	fl	54.65 ± 1.12	54.15 ± 0.89	52.83 ± 1.15	54.43 ± 1.78
Mean Cell Hemoglobin	pg.	18.40 ± 0.45	18.23 ± 0.15	17.80 ± 0.42	18.15 ± 0.66
Mean Cell Hemoglobin Concentration	g/dL	33.83 ± 0.53	33.75 ± 0.33	33.80 ± 0.20	33.43 ± 0.31
Red Blood Cell Distribution Width - Coefficient of Variation	%	14.10 ± 1.43	13.98 ± 1.47	13.38 ± 0.61	14.53 ± 0.79
Red Blood Cell Distribution Width - Standard Deviation	fL	27.00 ± 1.65	26.75 ± 1.71	25.38 ± 0.97	26.98 ± 1.22
Platelet	10^9/L	805.50 ± 37.28	*988.00 ± 30.36	844.50 ± 102.72	756.25 ± 108.85
Platelet Distribution Width	%	15.70 ± 0.08	15.53 ± 0.10	15.90 ± 0.16	15.75 ± 0.24
Plateletcrit	%	0.58 ± 0.01	0.51 ± 0.34	0.61 ± 0.07	0.53 ± 0.04
Biochemical parameter					
Protein	g/L	81.23 ± 6.55	81.38 ± 5.62	82.45 ± 3.73	79.43 ± 1.42
Cholesterol	mmolL	2.38 ± 0.29	2.32 ± 0.54	2.10 ± 0.37	2.85 ± 0.36
High Density Lipoprotein	mmolL	1.74 ± 0.29	1.79 ± 0.32	1.79 ± 0.30	2.19 ± 0.19
Albumin Blood test	g/L	37.90 ± 3.29	38.45 ± 2.61	39.98 ± 1.21	39.53 ± 0.98
Urea	mmol/L	7.20 ± 1.09	7.76 ± 0.91	9.42 ± 1.75	8.51 ± 1.41
Bilirubin Total	umol/L	16.99 ± 1.35	17.99 ± 11.35	14.81 ± 9.46	22.58 ± 3.14
Alanine transaminase	IU/L	55.58 ± 19.55	45.28 ± 5.87	45.03 ± 9.51	50.65 ± 5.47
Aspartate transaminase	IU/L	116.83 ± 41.32	105.18 ± 9.95	118.08 ± 22.51	113.03 ± 8.19
Alkaline phosphatase	IU/L	174.88 ± 63.22	133.00 ± 46.99	89.15 ± 37.80	152.03 ± 26.04
Creatinine	umolL	82.67 ± 5.81	80.30 ± 6.30	80.78 ± 5.54	79.81 ± 5.70
Triglyceride	mmol/L	*0.36 ± 0.06	0.46 ± 0.07	0.57 ± 0.08	0.47 ± 0.04
Low Density Lipoprotein	mmol/L	0.47 ± 0.14	0.41 ± 0.11	0.25 ± 0.18	0.46 ± 0.18

Key: *p-value significant at $p<0.05$

Table 6: Haematological and biochemical assessment of satellite group post-administration cessation of *T. ivorensis* extract

Haematological assessment	Unit	40 mg/kg Treatment Group	200 mg/kg Treatment Group	1000 mg/kg Treatment Group	Control Group
		Mean ± SD			
White Blood Cells	10^3/µL	5.25 ± 1.08	6.63 ± 0.86	6.05 ± 1.95	6.03 ± 1.39
Lymphocytes absolute	10^3/µL	2.08 ± 0.49	2.63 ± 0.39	2.18 ± 0.56	2.15 ± 0.19
Mid-sized Cell absolute	10^9/µL	0.80 ± 0.08	1.23 ± 0.43	0.98 ± 0.21	0.90 ± 0.08
Granulocytes absolute	10^9/µL	2.38 ± 0.67	2.78 ± 0.73	2.90 ± 1.22	2.98 ± 1.20
Lymphocytes percentage	%	39.93 ± 1.99	40.30 ± 7.65	36.40 ± 3.38	36.23 ± 4.28
Mid-sized cell percentage	%	15.30 ± 3.32	18.35 ± 4.48	16.55 ± 2.14	16.15 ± 3.96
Granulocytes percentage	%	44.78 ± 3.14	41.35 ± 7.65	47.05 ± 4.72	47.63 ± 7.91
Haemoglobin	g/dL	16.85 ± 0.85	16.90 ± 0.83	16.10 ± 0.54	16.10 ± 1.07
Red Blood Cell	10^6/µL	9.41 ± 0.35	9.50 ± 0.43	8.97 ± 0.51	9.16 ± 0.58
Haematocrit - Pack Cell Volume	%	49.95 ± 2.41	50.10 ± 2.99	47.40 ± 1.56	47.80 ± 2.83
Mean Cell Volume	fl	53.18 ± 2.41	52.83 ± 1.80	53.03 ± 3.45	52.28 ± 1.35
Mean Cell Haemoglobin	pg.	17.88 ± 0.90	17.78 ± 0.56	17.93 ± 0.83	17.53 ± 0.34
Mean Cell Haemoglobin Concentration	g/dL	33.70 ± 0.63	33.70 ± 0.42	33.80 ± 0.81	33.63 ± 0.56
Red Blood Cell Distribution Width - Coefficient of Variation	%	13.80 ± 1.64	13.65 ± 1.58	14.05 ± 2.20	13.20 ± 1.60
Red Blood Cell Distribution Width - Standard Deviation	fL	25.98 ± 3.34	25.15 ± 2.51	26.20 ± 3.83	24.75 ± 2.88
Platelet	10^9/L	795.50 ± 151.85	949.25 ± 181.65	724.25 ± 65.76	772.00 ± 126.39
Platelet Distribution Width	%	24.70 ± 18.13	15.68 ± 0.28	16.05 ± 0.34	15.80 ± 0.22
Plateletcrit	%	0.56 ± 0.11	0.29 ± 0.33	0.53 ± 0.06	0.54 ± 0.08
Biochemical parameter					
Protein	g/L	76.98 ± 2.79	68.18 ± 23.13	74.43 ± 3.81	73.90 ± 5.79
Cholesterol	mmolL	2.00 ± 0.27	1.80 ± 0.67	1.92 ± 0.36	1.87 ± 0.29
High Density Lipoprotein	mmolL	1.45 ± 0.27	1.33 ± 0.50	1.50 ± 0.27	1.43 ± 0.25
Alanine transaminase	IU/L	52.10 ± 9.05	45.52 ± 15.36	64.93 ± 19.74	58.60 ± 16.69
Aspartate transaminase	IU/L	116.95 ± 8.67	116.82 ± 48.12	135.53 ± 56.05	142.68 ± 38.86
Alkaline phosphatase	IU/L	217.55 ± 25.91	192.87 ± 65.61	204.63 ± 42.07	228.18 ± 56.97
Albumin Blood test	g/L	29.98 ± 2.70	27.73 ± 9.10	32.50 ± 1.95	31.75 ± 1.46
Urea	mmol/L	*8.46 ± 1.34	*7.20 ± 2.35	8.93 ± 1.24	10.80 ± 0.40
Bilirubin Total	umol/L	9.41 ± 4.11	10.66 ± 8.02	11.29 ± 5.16	8.07 ± 2.07
Creatinine	umolL	86.80 ± 5.63	75.51 ± 25.52	85.36 ± 2.27	82.23 ± 9.57
Triglyceride	mmol/L	0.68 ± 0.05	0.62 ± 0.21	0.75 ± 0.03	0.63 ± 0.11
Low Density Lipoprotein	mmol/L	0.24 ± 0.06	*0.29 ± 0.15	0.21 ± 0.15	0.15 ± 0.09

Key: *p-value significant at $p<0.05$

DISCUSSION

The phytochemicals present in the leaves of *T. ivorensis* is consistent with previous research findings by Kipré *et al*. (2023) [9], who reported a significant presence of polyphenolic compounds in the bark of *T. ivorensis*. Similarly, Coulibaly *et al*. (2014) [13] identified polyphenols, saponins, tannins, and terpenes in aqueous and ethanol stem-bark extracts. The rich phytochemical content of the leaves, particularly polyphenols and flavonoids, supports their traditional medicinal use. These compounds are potent antioxidants known to mitigate oxidative stress, which plays a central role in diseases such as cancer, ulcers, and inflammatory conditions [12].

The observations from the antibacterial activity result with no inhibition zones recorded for OG 1(3) *Klebsiella pneumoniae* EPA, OG 1(4) *K. pneumoniae* EPA (an ESBL producer), Oga *K. pneumoniae* (ESBL producer), and Es 16 *Staphylococcus xylosus* ORSA at levofloxacin concentrations between 6.25 and 50 µg/mL, except for *S. xylosus* ORSA, which displayed an inhibition zone of 13.33 mm at 50 µg/mL correspond with Yang *et al*. [25]. Who reported that isolates with MIC values ≥256 µg/mL exhibited inhibition zones of only 6.5 mm in disk diffusion assays, nonetheless, broth microdilution confirmed inhibition of growth at those elevated concentrations. Fluoroquinolones, including levofloxacin, are important broad-spectrum antibiotics, yet resistance among *K. pneumoniae* strains has increased significantly [26]. *Klebsiella pneumoniae* is a clinically significant pathogen responsible for various hospital-acquired infections and is currently the most prevalent fluoroquinolone-resistant member of the *Enterobacteriaceae* family [27]. Mutations in the quinolone resistance-determining region (QRDR) of *gyrA*, along with porin deficiency, efflux mechanisms, and plasmid-borne *qnrA1*, have been shown to markedly reduce levofloxacin's efficacy both *in vitro* and *in vivo* [28]. The ethanol extract of *T. ivorensis* displayed pronounced inhibition against MDR (Es 44, ES 14, Es 16, Es 21, ML 10 and Med 4(2) *Staphylococcus aureus*, consistent with the findings of Samuel *et al*. [12], who demonstrated that acetone extracts of *T. ivorensis* leaves inhibited both *E. coli* and *S. aureus*. Among the solvent fractions tested in their study, the ethyl acetate fraction exhibited the highest antibacterial activity. The present results also reveal a concentration-dependent increase in inhibition zones, indicating enhanced antibacterial action with higher extract concentrations. The extract's strong antibacterial activity can be attributed to the presence of saponins, terpenes, tannins, and polyphenols compounds known to disrupt microbial membranes, denature proteins, and inhibit essential enzymatic pathways [29]. These findings are in agreement with previous studies demonstrating that the trunk bark of *T. ivorensis*, which contains similar phytochemicals, also exhibits marked bactericidal effects [13].

The absence of mortality or visible toxic symptoms following oral administration of *T. ivorensis* ethanol extract suggests that the extract has a relatively low acute toxicity profile, with an LD_{50} greater than 2000 mg/kg. This finding indicates that the extract may be considered relatively safe under the tested conditions. In contrast, Zaza *et al*. (2018) [14] reported a much lower LD_{50} of 200 mg/kg for an intraperitoneally administered bark fraction (X42), suggesting that the route of administration and type of extract greatly influence toxicity. However, Zaza *et al*. (2016) [6] found that oral administration of 5000 mg/kg of the X42 fraction produced no mortality in rats, supporting the present finding that oral exposure is relatively safe.

Changes in body weight serve as reliable indicators of potential adverse drug effects [30]. The absence of significant differences in body weight between control and treated animals during the sub-acute study indicates that the extract did not adversely affect normal growth or metabolic activity. Haematological parameters, including red and white blood cell counts, haemoglobin, lymphocytes, and platelets, are critical indicators of the pathological and physiological states in both humans and animals [31]. The lack of significant alterations in these parameters suggests that the extract did not induce hematotoxic effects. The elevated creatinine levels observed in the treated groups during the sub-acute study could indicate mild renal stress or extra-renal factors associated with extract metabolism. However, the absence of consistent changes in other renal markers suggests that these effects may be limited. The lower levels of ALT and AST observed in treated animals indicate that the extract did not adversely affect liver function. Because transaminases (AST and ALT) are key indicators of hepatic injury, their stability suggests normal liver function [32]. These findings agree with earlier studies on *T. ivorensis* bark administered orally and intraperitoneally, which also reported no significant changes in serum biochemistry, haematology, body weight, or organ indices compared with controls [6,14].

During the sub-chronic study, the observed increase in white blood cell counts and associated immune-related parameters may reflect an immune or inflammatory response to prolonged exposure to the extract. Such responses may indicate immune activation and possible platelet aggregation, which can influence cardiovascular, metabolic, hematologic, and tissue-level functions [33]. Nevertheless, the stability of red blood cell counts and the maintenance of biochemical parameters within normal physiological ranges suggest that these effects were not severe.

The satellite group analysis showed that most haematological and biochemical parameters returned toward control values after cessation of treatment, indicating that the observed effects were largely reversible. Although urea and LDL levels showed statistically significant variations, the normalization of most parameters during the recovery period suggests that the extract-induced changes were mild and transient rather than indicative of persistent toxicity.

CONCLUSION

The ethanol extract of *T. ivorensis* leaves showed significant antibacterial activity against multidrug-resistant bacterial strains, with inhibition zones increasing with concentration, highlighting its potential for treating resistant infections. In mice, the extract was safe at doses up to 2000 mg/kg, showing no acute toxicity. Sub-acute and sub-chronic studies in rats showed no major changes in body weight or liver function, but a slight increase in creatinine indicated mild renal stress. Elevated white blood cell counts during sub-chronic exposure suggest a prolonged immune response. While the extract appears relatively safe, further research is needed on its immunomodulatory and renal effects.

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Conflict of interest

The authors declared no conflict of interest.

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