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Evaluation of the Antibacterial Activity and Toxicity Properties of *Funtumia elastica* (Preuss) Stapf. (TSN 30176), Used in Traditional Medicine in Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. The study's design was conceptualized by author BAA, while authors BCI and WEI performed the experiments. The statistical analysis and result interpretation were handled by authors BAA, BCI, GBM, WEI and COI. Additionally, authors BAA, BCI, GBM and COI did the manuscript draft and contributed to the comprehensive literature review. Proofreading and final approval of the manuscript was carried out by authors BAA, GBM, BCI, COI and WEI.

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ABSTRACT

Background: The urgent need for new and novel antibacterial chemotherapy to combat the worrisome emergence of antimicrobial resistance necessitated the exploration of medicinal plants such as *Funtumia elastica*. This study was designed to evaluate the antibacterial activity, acute and sub-acute toxicity studies of *Funtumia elastica* which is used in the ethnomedical treatment of infections.

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Methods: The leaves and stem bark of *F. elastica* were collected, pulverized, extracted using soxhlet extractor and evaluated phytochemically. Antibacterial activities and Minimum Inhibitory Concentrations (MIC) of the crude extracts were investigated on clinical isolates using agar well diffusion. Bioactive fractions of the dichloromethane extract were obtained through column chromatography and thin layer chromatography. Acute and subacute toxicity studies were evaluated on the dichloromethane extract (DCM) using female Swiss albino mice.
Results: The leaves contain tannins, flavonoids, alkaloids, steroids, saponins, and glycosides while the stem contains tannins and steroids. The hexane extracts lack activities while DCM and ethylacetate extracts of *F. elastica* were found to have some degree of activities on tested clinical isolates. Bioactivity guided fractionation of the DCM extract yielded only fraction 2 been active on the clinical isolates at 3.5625mg/mL. There were no signs of acute toxicity at the maximum dose of 5000mg/kg body weight. Biochemical parameters showed no significant changes in the liver enzymes. This infers that the extract might not have any negative impact on the liver with regards to metabolism.
Conclusion: The moderate antibacterial activities of *Funtumia elastica* extracts justified its ethnomedicinal uses and potential to furnish new antimicrobials.

Keywords: Funtumia elastica; Ethnomedicine; phytochemistry; bioactivity; toxicity.

1. INTRODUCTION

The use of medicinal plant in treatment of sickness and disease is dated back to ancient times, and it is still being used in the 21st century [1,2]. Medicinal plants have been tagged a reservoir for phytochemicals in the production of drugs. Although they are mostly consumed wholly, research has proven that this act could have some deleterious effects on humans [3]. There has been a rapid emergence of resistant bacteria occurring worldwide thus, antimicrobial resistance and its wider implications present the world with a growing healthcare crisis [4]. Hence there is urgent need to look into medicinal plants for new and effective antimicrobials [5].

Funtumia elastica (Preuss) commonly called "Ire'' in Yoruba land Southwest Nigeria [6], belongs to the family Apocynaceae. Members of this family are native to the Europe, Asia, Africa, Australia and America. Many species of the Apocynaceae family are found in the tropical forest, but some grow on temperate regions as perennial herbs. Preliminary phytochemical screening has revealed that *F. elastica* bark contains hydrolysable tannins, sapogenetic glycosides, steroids, and saponins [7], while the leaves contain hydrolysable tannins, flavonoids, starch, and alkaloids with the tannin content of the leaves and stem bark being 2.4% and 1.3% w/w (relative to the dried material), respectively [8].

Decoction of the leaf of *Funtumia elastica* has been found to specifically inhibit the growth of many moulds, including *Aspergillus* sp, *Penicillium* sp, *Candida* sp, as well as the fungi that cause ring worm [9]. Its Antiplasmodial and antileishmanial inhibitory activity has as well been reported [7]. It is also reportedly used in the treatment of wounds [10]. Burkill, [11] has documented that ethanolic extract of *F. elastica* (Preuss) is traditionally used to treat whooping cough, inflammatory diseases such as asthma, blennorhea and painful menstruation. The scientific scrutiny of *Funtumia elastica* is as a result of the need to look for other sources of novel antimicrobial agents particularly from medicinal plants based on ethno-pharmacological information [5]. The present study, was, therefore, designed to evaluate antibacterial activity of fractions from the crude extract of *Funtumia elastica* as well as to assess its acute and subacute toxicity.

2. MATERIALS AND METHODS

2.1 Collection, Identification and Extraction of Plant Materials

Funtumia elastica was collected from University of Ibadan Botanical garden. The plant was earlier identified and authenticated at the herbarium of Federal Research Institute of Nigeria (FRIN), Ibadan with voucher number FHI 112304. The plant parts (leaves and stem bark) were air-dried under shade at ambient temperature, pulverized into fine powder. The pulverized plant materials were weighed and subjected to exhaustive Soxhlet extraction with distilled ethanol. Extracts were collected, concentrated and dried under reduced pressure using rotary evaporator (Xian Ltd, China) at 45ºC, weighed and stored at room temperature before use. A 200g of each part of

Fig. 1. *Funtumia elastica* trees (Source: Author`s work)

the crude extract was dissolved in a mixture 450ml of ethanol and 150ml of distilled water and was partitioned with Hexane, Dichloromethane and Ethyl acetate according to the polarity.

2.2 Animal Stock

Female Swiss albino mice aged 6-7 weeks, weighing 25-30 g were used. The animals were fed on grower feed with free access to water under standard conditions of light (12 h light, 12 h dark), humidity and temperature.

2.3 Phytochemical Screening

Phytochemical screening was carried out to detect the presence of secondary metabolites such as anthraquinones, tanins, saponins, alkaloid, phlobatanins, and steroids, cardiac glycosides, reducing sugar, phenol, glycosides and resins using methods described by Harborne [12].

2.4 Test Microorganisms

Strains of clinical isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumanii* and *Klebsiella spp* were gotten from Clinical Microbiology Laboratory University College Ibadan.

2.5 Antimicrobial Susceptibility Test

The agar cup diffusion technique was adopted using the method of [13]. Each organism was sub-cultured in Tryptic Soy Broth (TSB) overnight, to have them in their log phase of growth. A volume of 0.1ml of each organism from the overnight culture was added to 9.9ml of sterile distilled water to give a 1 in 100 dilution. This was properly mixed to measure homogeneity.

Using a sterile 1ml pipette, 0.2ml of this dilution was withdrawn, introduced into 20ml molten Mueller Hinton agar at 45ºC and was poured into sterile Petri dish (8.5cm diameter). The plate was allowed to set and surface aseptically dried of all moisture. A sterile cork-borer of 6 mm diameter was used to bore equidistant wells on the agar and a 0.2ml volume of the extracts of various concentrations introduced into their respective wells. The positive (Gentamicin 10µg) and negative controls (20% DMSO) were also introduced in their respective wells. The plates were then allowed to stand on the bench for an hour to allow for pre-diffusion of extracts and controls. The plates were incubated at 37ºC for 24 hours, after which zones of inhibitions were measured. All tests were performed in duplicates.

2.6 Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations (MICs) were performed with modifications of agar dilution method as previously explained [14]. Two milliliter (2ml) of each of the reconstituted extracts fractions, prepared to give concentrations of 200 mg/mL, 100 mg/mL, 50 mg/ml and 25 mg/mL, were added to 18ml of molten Mueller-Hinton agar and shaken by rolling it between palms. The properly mixed agar and extract was poured into sterile Petri dishes, allowed to solidify and the surface dried in sterile oven to remove moisture. A loopful of overnight culture of each test bacterial isolate was streaked on the surface of the agar. The plates were appropriately incubated for 24hours at 37ºC and examined for the presence or absence of growth. The lowest concentration that prevented growth of a bacterial isolate was taken as the minimum inhibitory concentration (MIC). All tests were performed in duplicates.

2.7 Determination of Minimum Bactericidal Concentration (MBC) of Crude Extract and Fractions of *Funtumia elastica*

The method of Adeniyi *et al.* [15] was used with some modification. The graded concentrations employed in the MIC with no observable growth on plate, a 0.5ml of test bacterial was added in a test tube of nutrient broth and incubated at 37ºC for 18- 24hrs. To a freshly prepared Muller Hilton agar, samples were taken and streaked out to define the lowest concentration of the extract essential towards the eradication of the bacteria. The failure of the organisms to grow on transference to the media is an indication of kill. The lowest of all the tested concentration that inhibited bacterial growth subsequently after incubation was documented as the minimum bactericidal concentration (MBC). Assay was done in duplicates.

2.8 Acute Toxicity Studies

The method of Lorke [16], was adopted in determining the acute toxicity of the most active fraction of the extracts *Funtumia elastica*, via oral administration. The study was carried out in three phases. In the first phase, three mice were administered 10, 100 and 1000mg/kg body weight of the extract orally (via a cannula), respectively. The mice were observed for signs of adverse effects and death for 24 hours. In the second phase of the study, the procedure was repeated using another set of three mice given 1500, 2000 and 2500mg/kg body weight of the extract respectively. The mice were also observed for signs of toxicity and mortality for 14 days. Based on the results gotten from the second phase, 3000mg/kg body weight and 5000mg/kg body weight of the extract were administered to another set of two mice in the third phase. The mice were observed for signs of toxicity and mortality for the first 4 hours and thereafter daily for 14 days.

2.9 Sub-acute Toxicity Studies of Most Active Fraction of the Extract of *Funtumia elastica*

The method of Aniagu et al., [17], was employed in the sub-acute toxicity study of crude Dichloromethane leaf extract of *Funtumia elastica.* Thirty (30) mice were selected by stratified randomization and divided into six groups of five mice each. Group one, two, three, four and five were administered 25, 50, 100, 200 and 400mg/kg body weight of the crude Dichloromethane leaf extract of *Funtumia elastica* respectively for 28 days. The sixth group was administered 5% DMSO in water used for dissolving the extract as control group. The first day of dosing was taken as D0 whereas the day of sacrifice was designated as D28. The feed intake of the mice in each group was measured once daily over the 28 days period. After sacrifice, heparinized blood sample were taken for hematological examination. The serum from non-heparinized blood was carefully collected for blood chemistry and analysis. All organs were preserved in 10% formal saline solution for histological examination. The effects of the sub-chronic administration of the crude extract for 28 days on the mice were investigated.

2.10 Chromatographic Methods

2.10.1 Column chromatography of most active crude extract

The most active extract was further purified by subjecting it to column chromatography of height, length and internal diameter 95cm by 54cm by 2cm respectively. The one hundred and fifty grams (150g) silica gel was packed wet with *n*-Hexane on the column clamped in a vertical position. As solid phase settles down, the column is gently tapped with a spatula until required height was attained. The sample (10g) was dissolved in a volatile *n*-Hexane and mixed with a little silica gel to form slurry. Gradient elution was carried out starting from least polar solvent (that is 100% *n*- Hexane, *n*-Hexane/Ethylacetate,

100% Ethylacetate, Ethylacetate/ Dichloro-methane, 100% Dichloromethane, Dichloromethane/methanol and 100% Methanol). Sub-fractions were obtained and pooled based on their thin layer chromatography (TLC) profiling.

2.11 Thin Layer Chromatography

The dissolved fractions of the column chromatography were spotted on TLC pre-coated silica gel adsorbent plate activated in an oven at a temperature of 110ºC for about an hour, with the aid of capillary tubes at a distance of 1cm from the base. The spots were equidistant from each other and were allowed to dry on the plates and were subsequently placed in a tank saturated with different solvent system such as *n*-Hexane: Ethylacetate, *n*-Hexane: Dichloromethane etc, at different ratios. The plates were developed using ascending techniques and the developed plates were brought out of the tanks, solvent front marked, before drying the plates. Then using ultraviolet lamp (λmax of 254 and 365 nm), the compound was clearly observed and marked. Their retardation factors (R_f) were obtained using the formula:

$$R_f = \frac{\text{Distance moved by the sample}}{\text{Distance of the solvent front}}$$

2.12 Contact Bioautography

The fractions gotten from column chromatography were spotted on TLC plate and using appropriate solvent system were developed to separate the phyto-chemicals. The developed TLC plate were placed in a Petri dish and cooled molten agar that has been seeded with the organism of interest were poured on them and were allowed to solidify. The plates were incubated at 37ºC for 24hrs. Zones of inhibition were observed, for a clearer observation, the plates were sprayed with tetrazolium salt and re-incubated for 24hour at 37ºC. Clear white zones, against a purple background indicated that the plant extract has antimicrobial activity against the test organism.

2.13 Statistical Analysis

Results were expressed as mean value ± standard deviation (S.E.M). Statistical analysis was evaluated using one way analysis of valiance, followed by a post hoc Newman-Keuls multiple comparison test. All statistical analysis was done using Prism software versions (Graph pad prism software Inc. San Diego, USA). Statistical difference at level $P<0.05$ were considered significant.

3. RESULTS

Dichloromethane fractions consistently gave the highest yield both in the leaves and stem-bark extracts, while the least percentage yield was recorded in the aqueous fraction for the leaves, and n-Hexane for the stem-bark respectively (Table 1). The preliminary phytochemical screening of *Funtumia elastica* leaf and stem bark revealed that the leaves contain tannins, flavonoids, alkaloids, steroids, saponins, and glycosides while the stem contains tannins and steroids (Table 2). The tests organisms were susceptible to Ofloxacin, Gentamicin, ciprofloxacin and Nitrofurantoin but are resistant to Augmentin, Cefotaxime and Ceftazidine (Table 3). The dichloromethane and ethylacetate fractions of ethanolic crude extract of *Funtumia elastica* leaves and bark exhibited different degrees of activities while the aqueous and *n*-hexane fractions showed no activity on the tested pathogenic organisms (Table 4). The minimum inhibitory concentrations (MIC) of the four (4) partitioned extracts (dichloromethane leaves, dichloromethane bark, ethylacetate leaves and ethylacetate bark) on the test organisms ranged between <3.125 mg/mL to >25 mg/mL (Table 5). The minimum bactericidal concentrations of the various fractions of the leaves and stem-bark extracts of *Funtumia elastica,* shows that values ranged between the MIC and 2 X MIC values for each organism (Table 6). Bioactivity guided fractionation showed that *Staphylococcus aureus* ATCC 29213 was most susceptible to the chromatopgraphic fraction 2 out of nine from Dichloromethane fraction of the leaves extract of *F. elastica* (Table 7).

A significant increase was seen in the packed cell volume (PCV) and hemoglobin concentrations as well as red blood cell and white blood cell count (Table 8). Table 9 shows that there were no significant alterations in the serum ALT, AST, and ALP and other biochemical parameters analyzed at various doses of the extract after 28 days administration on the animal models. The histological evaluation of effects of Dichloromethane extract of *Funtumia elastica* on liver tissues of the experimental animals using hematoxylin and eosin, reveals mild diffuse vacuolar degeneration of hepatocytes (Fig. 2). However, the histological evaluation of the effects of Dichloromethane extract of *Funtumia elastica* on kidney tissues using hematoxylin and eosin staining, shows no visible lesions (Fig. 3).

Table 1. Yield and characteristics of crude extract of *Funtumia elastica*

	Sample	Weight of extract (g)	%Yield	Characteristic features
Leaves				
	n-Hexane	29.588	17.93	Coffee brown oily extract
	Dichloromethane	88.263	53.49	Dark brown powdery extract
	Ethylacetate	25.699	15.57	Deep coffee gummy extract
	Aqueous	21.357	12.94	Dark greenish extract
Bark				
	n-Hexane	26.759	12.68	Greasy brown extract
	Dichloromethane	44.257	20.97	Dark brown gummy extract
	Ethylacetate	61.2	29	Dark brown extract
	Aqueous	78.78	37.33	Light brown crispy extract

Table 2. Preliminary phytochemical screening of *Funtumia elastica* leaf and stem bark

Secondary Metabolites	Funtumia elastica (Leaves)	Funtumia elastica (Stem-bark)
Tannins	+	+
Flavonoid	+	-
Alkaloids	+	-
Steroids	+	+
Saponins	+	-
Carbohydrate	+	-
Glycoside	+	-

Key: + = Positive, - = Negative

Table 3. Anti-biogram of tested pathogenic organisms

	Diameter zones of inhibition (in mm)											
DRUGS	**SA 29213**	**SA 6571**	**EC 35218**	**PA 27853**	**9A**	**4B**	**12B**	**P2**	**P4**	**EC1**	**EC23922**	**ACENI 1**
AUG	16	-	-	-	-	-	-	-	-	-	-	-
OFL	11	-	24	-	18	20	24	24	25	-	18	22
CXM	17	11	11	-	-	-	-	-	-	-	-	-
GEN	18	13	17	15	17	15	-	24	12	-	-	-
CTX	-	-	-	-	-	-	-	9	-	-	-	-
CAZ	-	-	-		-	-	-	-	-	-	-	-
CPR	21	27	27	31	-	16	-	26	35	-	19	24
NIT	20	25	26	-	25	30	20	26	-	-	28	21

Key:

SA 29213- Staphylococcus aureus
SA6571- Staphylococcus aureus
EC 3528 - Escherichia coli ATCC35218
PA 27853 – Pseudomonas aeruginosa
9A- Escherichia coli
4B - Escherichia coli
12B – Klebsiella pneumoniae
P2 – Pseudomonas aeruginosa
P4 – Pseudomonas aeruginosa
EC1- Escherichia coli
EC 23922- Escherichia coli
ACENI 1- Acinetobacter baumannii

OFL= Ofloxacin
AUG= Augumentin
CXM= Cefuroxim
GEN= Gentamicin
CTX= Cefotaxime
CAZ= Ceftazidime
CPR= Ciprofloxacin
NIT= Nitrofuratoin

Table 4. Antibacterial activities of different fractions of *F. elastica* leaves and bark extracts

	Diameter Zones of Inhibition (in mm)																	
	Dcm leaves				Dcm bark				Ehtylacetate bark				Ehtyacetate leaves					
	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.125 mg/mL	25 mg/mL	12.5. mg/mL	6.25 mg/mL	3.125 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.125 mg/mL	25 mg/mL	12.5. mg/mL	6.25 mg/mL	3.125 mg/mL	Gent 10µg/mL	DMSO 20%
PA27853	10	8	7	-	8	7	-	-	13	11	9	-	15	12	7	-	-	-
SA29213	23	21	19	17	20	17	10	8	15	13	8	7	15	13	7	-	18	-
SA6571	25	22	18	14	22	20	16	9	14	12	7	-	16	11	-	-	19	-
EC35218	15	8	-	-	13	7	-	-	12	-	-	-	12	-	-	-	18	-
EC23922	15	11	7	7	15	11	7	-	11	-	-	-	14	12	-	-	18	-
P4	-	-	-	-	7	-	-	-	10	-	-	-	-	-	-	-	15	-
ACINE1	16	9	7	-	8	7	-	-	10	-	-	-	14	12	-	-	-	-
4B	16	9	7	-	11	9	7	7	10	-	-	-	11	9	-	-	17	-
12B	13	11	10	8	9	8	-	-	10	8	-	-	-	-	-	-	17	-
9A	-	-	-	-	-	-	-	-	15	12	7	-	10	8	8	-	21	-
P2	12	10	9	8	10	8	-	-	-	-	-	-	-	-	-	-	16	-
EC1	16	13	8	7	14	11	8	-	-	-	-	-	-	-	-	-	17	-

Key:

SA 6571- Staphylococcus aureus
SA 29213- Staphylococcus aureus
4B – Escherichia coli
9A– Escherichia coli
EC 35218 – Escherichia coli
EC 23922 – Escherichia coli
EC1 – Escherichia coli
PA 27853- Pseudomonas aeruginosa
P2 – Pseudomonas aeruginosa
P4 - Pseudomonas aeruginosa
12B – Klebsiella pneumoniae
ACINE1 – Acinetobacter baumannii
DCM- Dichloromethane
DMSO- Dimethylsulfoxide

Table 5. Minimum inhibitory concentration of the fractions of the plant extracts against the test bacterial isolates

Organisms	Dichloromethane leaf (mg/mL)	Ethyl acetate leaf (mg/mL)	Dichloromethane bark (mg/mL)	Ethyl acetate bark (mg/mL)	Gentamicin (µg/mL)
Staphylococcus aureus ATCC 29213	3.125	6.25	3.125	6.25	5
Staphylococcus aureus ATCC 6571	3.125	12.5	3.125	6.25	5
Escherichia coli ATCC 35218	6.25	25	12.5	25	5
Escherichia coli ATCC 23922	3.125	12.5	6.25	25	5
Escherichia coli	>25	6.25	>25	12.5	<1.25
Escherichia coli	6.25	12.5	3.125	25	5
Escherichia coli	3.125	>25	6.25	>25	<1.25
Klebsiella pneumonia	3.125	>25	12.5	12.5	5
Pseudomonas aeruginosa ATCC 27853	6.25	6.25	12.5	6.25	>10
Pseudomonas aeruginosa	>25	>25	25	25	5
Pseudomonas aeruginosa	12.5	>25	12.5	>25	<1.25
Acinetobacter baumanni	>25	12.5	12.5	25	>10

Table 6. Minimum bactericidal concentration (MBC) (mg/mL) of *Funtumia elastica* fractions

Organisms Samples	PA 27853	SA 29213	SA 6571	EC 35218	EC 23922	P4	ACINE 1	4B	12B	9A	P2	EC1
DCM LEAVES	12.5	3.125	3.125	25	6.25	50	12.5	12.5	3.125	50	25	3.125
DCM BARK	25	3.125	3.125	25	12.5	50	25	3.125	25	50	25	12.5
E.A BARK	12.5	3.125	12.5	50	50	50	50	50	25	25	50	50
E.A LEAVES	12.5	12.5	25	50	25	50	25	25	50	12.5	50	50

Key:

PA 27853- Pseudomonas aeruginosa
SA 29213- Staphylococcus aureus
SA 6571- Staphylococcus aureus
EC 35218 – Escherichia coli
EC 23922 – Escherichia coli
P4- Pseudomonas aeruginosa
ACINE 1- Acinetobacter baumannii
4B – Escherichia coli
12B – Klebsiella pneumoniae
9a – Escherichia coli
P2 – Pseudomonas aeruginosa
EC1 – Escherichia coli

Table 7. Antibacterial activities of Fraction 2 of Dichloromethane fraction leaves extract of *F. elastica* against some bacterial isolates

	Diameter zone of inhibition (mm)							
Organisms	**6.25 mg/mL**	**3.125 mg/mL**	**1.625 mg/mL**	**0.781 mg/mL**	**0.39 mg/mL**	**0.195 mg/mL**	**Gent 10µg/mL**	**DMSO 20%**
Staphylococcus aureus ATCC 29213	20	15	10	-	-	-	12	-
Escherichia coli ATCC 23922	14	12	-	-	-	-	10	-
Pseudomonas aeruginosa ATCC 27853	14	12	-	-	-	-	15	-

Key: DMSO- Dimethylsulfoxide. Gent.- Gentamycin.

Table 8. Effect of sub-acute toxicity of Dichloromethane leaves extract of *F. elastica*, on haematological indices in mice

Parameters	Control	25mg/kg	50mg/kg	100mg/kg	200mg/kg	400mg/kg
PCV (%)	45.67 ± 0.33	51.33 ± 0.67*	55.33 ± 2.67*	47.33±0.67	49±1	53±1*
Hemoglobin (g/dl)	15.07 ± 0.13	17.40 ± 0.2l*	18.40 ± 0.3*	15.83±0.03*	16.63±0.07*	16.87±0.07*
RBC(mil/mm$^{3)}$	7.36 ± 0.30	8.57 ±0.01*	8.42 ± 0.07*	8.07 ± 0.1*	8.39±0.02*	8.46±0.05*
WBC(mm^3)	4697±46.7	4250±50	5027 ±367	3507±256.7*	4400 ± 100	2683±183.3*
Platelet(mm^3)	132350±1650	123268±1268	195680±6053*	95000±5000*	115000±5000	103030±8970*
Lymphocytes (%)	73±0.67	78.0 ± 1.0	79.0 ±3.53	76.0 ± 1.0	74.67 ± 0.33	79.67 ± 1.67
Neutrophils (%)	23.67 ± 0.67	21.67 ± 0.33	17.67 ± 1.33	24.67 ± 1.67*	19.67 ± 1.33	20.33 ± 0.33
Monocytes (%)	2.17 ± 0.17	1.17 ± 0.17*	0.93 ± 0.07*	1.13 ± 0.13*	1.83 ± 0.17	2.27 ± 0.27
Eosinophil (%)	1.17 ± 0.17	0.83 ± 0.33	2.83 ± 0.17*	0.83 ±0.17*	1.83 ±0.17	2.13±0.13*

*Data are expressed as Mean ± SEM (n=3). Comparisons were made using one-way ANOVA followed by post-hoc Newman-Keuls test. *P< 0.05 when compared with control.*
Key: PCV= Packed Cell Volume; WBC= White blood cell; RBC= Red blood cell

Table 9. Effect of sub-acute toxicity of dichloromethane leaves extract of *F. elastica* on serum biochemical parameters

Parameters	Control	25mg/kg	50mg/kg	100mg/kg	200mg/kg	400mg/kg
Total Protein(g/dl)	6.4± 0.21	5.93 ± 0.56	6.33 ± 0.17	5.6 ± 0.70	5.6± 0.70	6.1±0.10
Albumin(g/dl)	2.5 ± 0.1	2.47 ± 0.13	2.37 ± 0.13	2.43 ± 0.13	2.37±0.13	2.33±0.17
Globulin(g/dl)	4.0 ± 0.1	3.7 ± 1.2	3.7 ± 0.3	3.47 ± 0.07	3.9±0.2	3.6±0.1
A-G Ratio	0.53±0.03	0.67± 0.03	0.57 ± 0.03	0.47 ± 0.03	0.53± 0.07	0.3±0.1
AST(µl)	41.67±1.67	37.33±0.67	34.67±2.33	35.33±0.67	35.0±2.0	33.67±2.33
ALT(µl)	30.33±1.33	27.67±0.67	26.33±0.67	26.0 ±2.0	26.33±2.67	24.0±1.0
ALP(µl)	89.0 ± 1.0	94.3 ± 3.67	99.0 ± 3.0	81.67±0.67	91.0±3.0	91.33±4.67
BUN (mg/dl)	16.07±0.27	15.23±0.37	15.03±0.47	15.03±0.57	15.93±0.07	16.27±0.03
Creatinine(mg/dl)	0.53 ± 0.07	0.43 ± 0.07	0.47 ± 0.03	0.4 ± 0.1	0.5±0.1	0.47±0.03

*Data are expressed as Mean ± SEM (n=3). Comparisons were made using one-way ANOVA followed by post-hoc Newman-Keuls test. *P< 0.05 when compared with control.*

Key:

A-G Ratio = Albumin-Globulin Ratio

BUN = Bilirubin

AST = Aspartate transaminase

ALT = Alanine transferase, ALP = Alkaline phosphatase

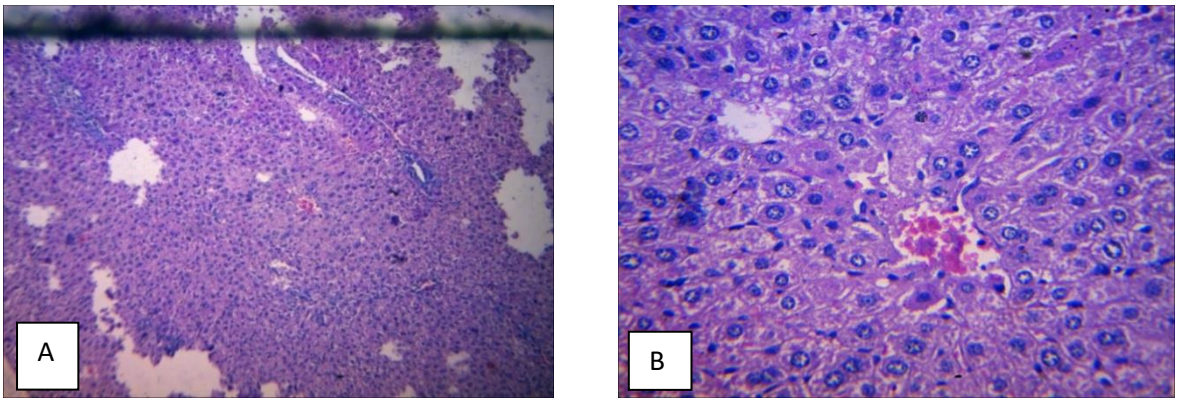

Fig. 2. Histological evaluation of effects of Dichloromethane extract of *Funtumia elastica* using hematoxylin and eosin staining on liver tissues of control in the acute toxicity study in mice. A = X100 magnification; B = X400 magnification

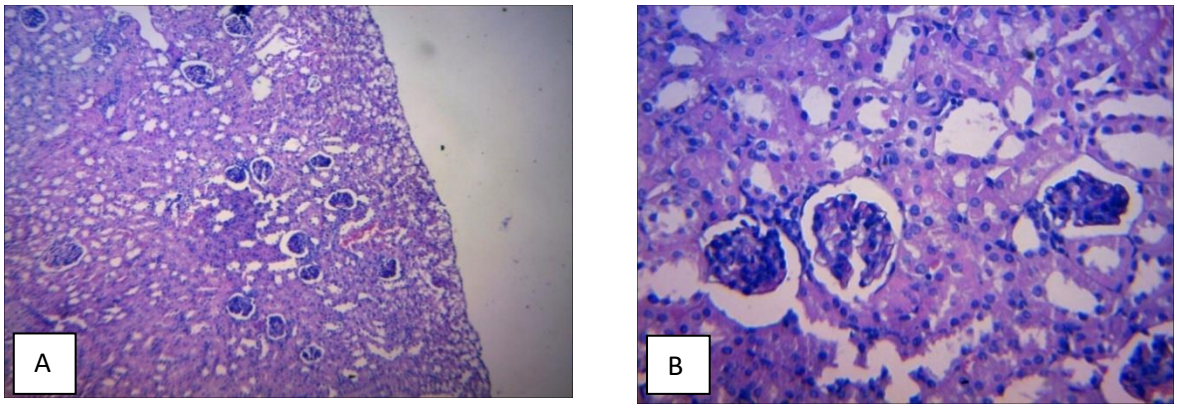

Fig. 3. Histological evaluation of effects of Dichloromethane extract of *Funtumia elastica* using hematoxylin and eosin staining on kidney tissues of control in the acute toxicity study in mice. A = X100 magnification; B = X400 magnification

4. DISCUSSION

The tests organisms were susceptible to Ofloxacin, Gentamicin, ciprofloxacin and Nitrofurantoin but were resistant to Augmentin, Cefotaxime and Ceftazidine. If these organisms are resistant to second and third generation Cephalosporin, there is a need for alternative antibiotics for treatment [18]. The dichloromethane and ethylacetate fractions of ethanolic crude extract of *Funtumia elastica* exhibited different degrees of activities while the aqueous and *n*-hexane fractions showed no activity on the pathogenic organisms. This may be due to the fact that these mid-polar solvents have greater capacities to pull the bioactive compounds from the crude extract during partitioning than the extremely polar and non-polar solvents [19]. This finding is in agreement with that reported by Adekunle and Ikumapayi [10], who found the extremely polar aqueous fractions of the same plant non-active against some fungal species. The minimum inhibitory concentrations (MIC) of the four (4) partitioned crude extracts (dichloromethane leaves, dichloromethane bark, ethylacetate leaves and ethylacetate bark) against the test organisms ranges between <3.125 mg/mL to 25 mg/mL. Though Osei-Djarbeng *et al*., [20] reported MIC ranges of 0.5 mg/mL to 2 mg/mL for the Ethyl-acetate fraction of extract of the same plant against related bacterial strains, the small differences observed could be attributed to strain susceptibility variation amongst the pathogens. The extracts were found to be most effective against *S. aureus* ATCC 29218, *S. aureus* ATCC 6571 and *P. aeruginosa* ATCC 27853 with the MIC range of <3.125 to 6.25 mg/mL but were found to be least effective against *E. coli* ATCC 35218, *P. aeruginosa* (strain P2) and *P. aeruginosa* (strain P4) with the MIC range of 12.5 mg/ml to 25mg/mL. Frempong *et al*., [21],

reported that the extracts of the same plant used in their study, was most active against *E. coli* and *Candida albicans*, but least active against *P. aeruginosa*. It is interesting to note that *S. aureus* ATCC 29213 was the most susceptible to all the fractions with the MIC value of <3.125 mg/mL except for ethylacetate leave extract where it had MIC value of 6.25 mg/mL. Again this pattern of susceptibility which was replicated in the sub-fraction of the dichloromethane fraction agrees with Osei-Djarbeng *et al.* [20], who reported that *S. aureus* NCTC 7447, showed the highest susceptibility in all the fractions of the extracts tested in their study. This could be as a result of the mechanism of action of the bioactive compound(s) in this fraction or the ability of the pathogen to take up sufficient bioactive concentration of the compound(s) into its system. *Pseudomonas aeruginosa* (strain P2) and *Escherichia coli* (strain 1) did not show any significant susceptibility to the ethylacetate leave and bark even at the highest concentration tested. This might not be unconnected to the absence of the bioactive compound(s) present in other fractions of the extracts; or the organism ability to resist the mechanisms of action of these extracts. This is however not surprising since *P. aeruginosa* is notorious for resisting antimicrobial agents [22, 23]. The antibacterial susceptibility testing of different fractions of the dichloromethane leaf revealed activity at fraction 2, with *S. aureus* being the most susceptible as pointed out earlier.

A significant increase was seen in the packed cell volume (PCV) and hemoglobulin concentrations as well as red blood cell and white blood cell count. This indicates a potential hematopoietic potential of the dichloromethane extract with varying increases in the doses. This goes along to support a study [24] which found out those medical and / or medicinal compounds and drugs have been shown to alter physiological range of hematological parameters. Platelet count was seen to reduce as the dose of the extract increases, reflecting a possible dose-dependent effect on the blood clotting system [8].

From this study, various biochemical parameters were assessed in validating the toxicity of *Funtumia elastica* in various systems of the body, using animal models: Alkaline phosphate (ALP), Alanine amino transferase (ALT), Aspartate aminotransferase (AAT), Bilirubin, Albumin and Globulin activities were measured in the serum to evaluate potential pathological effects of *Funtumia elastica* on the liver. However, no alterations were seen in the serum ALT, AST, and ALP of various doses of the extract after 28 days administration. These enzymes are used to access possible damage to the liver [25]. Findings from this study indicate that the extract may not have any negative impact on the functionality of the liver with regards to metabolism. Serum albumin, bilirubin and globulin showed no significant changes in all doses. This indicates that the extract may not have any effect on the state of the liver [25,26].

5. CONCLUSION

The results obtained from the antimicrobial activities of *Funtumia elastica* leaves and bark show that the plant extracts have antibacterial properties and that can yield effective compound for the formation of a new and novel antibiotics, since they inhibited the growth of the tested pathogenic organisms. The effect on hematological parameters shows that *Funtumia elastica* could be a good candidate for blood-normalizing and immune-boasting supplements. Further work is ongoing to isolate and characterize the actual bioactive compounds.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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