https://dx.doi.org/10.4314/jpb.v15i2.6
Vol. 15 no. 2, pp. 139-147 (September 2018)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND
BIORESOURCES

Acute toxicity studies and antibacterial evaluation of *Bombax costatum* stem bark extracts

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Received 13th June 2018; Accepted 20th July 2018

Abstract

Bombax costatum belongs to the family Bombacaceae and is commonly called red-flowered silk-cotton tree or red kapok tree, the plant occurs from Senegal. In Nigeria, different parts of *B. costatum* are employed for various purposes. The extract from the bark is drunk or applied on the head for dizziness; and the gum resin from the bark is pulverized, mixed with oil and used to manage skin diseases such as "craw-craw". This study showed that the, median lethal dose (LD_{50}) via oral route of the extracts was found to be greater than 5000 mg/kg when administered orally in albino rats. The extracts of *B. costatum* stem bark was observed to possess antibacterial activity against four out of the seven bacterial isolates that were used for the study. Ciprofloxacin was used as a control, and it has higher antibacterial activity as compared to the plant extracts. Although, the three extracts showed appreciable zones of inhibition (18-28 mm), the ethyl acetate extract exhibited a wider dimension (26-28mm) and prove to be the best as compared to the hexane and methanol extracts which showed (18-20 mm) and (22-25 mm) respectively. The results from the MIC and MBC also revealed that the ethyl acetate extract (6.25 and 12.5 mg/ml) had higher inhibitory strength than the hexane (25 and 50 mg/ml) and methanol (12 and 25 mg/ml) extracts. The results of this study provide scientific justification for the traditional uses of the stem bark in the treatment of infectious diseases and food poisoning.

Keywords: *Bombax costatum*; Stem bark; Antibacterial activity; Toxicity studies

INTRODUCTION

Antimicrobials are substances that kill or inhibit the growth of microorganisms, could be in the form of antibiotics, which are products of microorganisms or synthesized derivatives [1], antimicrobial peptides produced by complex organisms as well as some microbes [2] and medicinal plants, which appear to be the focus of mainstream medicine today [1]. In an attempt to combat the various forms of disease that have continued to plague humans from time immemorial to this day, different types of antimicrobials have been developed to fight the pathogens responsible for these diseases. Bacteria have evolved numerous defenses against antimicrobial agents, and drug resistant pathogens are on the rise. This resistance is conferred by multidrug resistance (MDR) pumps, membrane translocases that extrude structurally unrelated toxins from the cell. These protect microbial cells from both synthetic and natural antimicrobials [3]. Secondary metabolites resemble endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters

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ISSN 0189-8442

and thus have beneficial medicinal effects on humans due to their recognition in potential target sites [4]. The use of plant extracts and phytochemicals can be of great significance in therapeutic treatments and could help curb the problem of these multi-drug resistant organisms.

Bombax costatum belongs to the family Bombacaceae [5] and is commonly called red-flowered silk-cotton tree or red kapok tree [5]. It is a plant, which grows in woody savannas and from Sahelo-Sudanese to Guinean scattered forests, on many types of soil. It is a deciduous tree growing straight up to about 30 m tall and 100 cm in diameter. The plant occurs from Senegal eastward to Cameroon, southern Chad and the Central African Republic [6]. In Nigeria, different parts of *Bombax costatum* are employed for various purposes. The immature fruits are prepared as an emollient; decoction of young leaves is used as a warm bath for febrile children; the ground bark is taken by pregnant women to increase lactation; the extract from the bark is drunk or applied on the head for dizziness; and the gum resin from the bark is pulverized, mixed with oil and used to manage skin diseases such as "craw-craw" [7], the bark is also used as an emollient; it promotes childbirth and is used in the treatment of diarrhea, bruising, gonorrhea. The plant is locally called '*Kurya*, or *Gujjiya* in Hausa, *Kutupkaci* in Nupe, *Tamu* in Pabur.

EXPERIMENTAL

Collection, identification and preparation of the plant material. The fresh stem bark of *B. costatum* was collected from well-grown and mature trees from *Kudingi* forest, Sabon Gari Local Government Area Zaria, Kaduna State in February 2017. The plant was first taxonomically authenticated at the Herbarium Unit of the Department of Botany, Ahmadu Bello University Zaria, Nigeria with a Voucher specimen number 90005. The stem bark was dusted, cleaned, air-died and pulverized to coarse powder using a clean mortar and pestle. The coarse powdered stem bark sample was stored in an airtight container for subsequent use.

Extraction. The pulverized plant material (700 g) was extracted successively in 2 litres of n-hexane, ethyl acetate and methanol using successive cold maceration. The extract obtained was concentrated to dryness on a water bath and stored in desiccator for further experiment.

Acute oral toxicity study of *Bombax costatum* stem bark extracts. Acute toxicity study was conducted using organization for economic cooperation and development (OECD) guideline 425 of 2001 [8]. The limit dose test procedure was adopted to evaluate the acute toxicity of *B. costatum* samples following oral administration in rats. A total of nine (9) rats were randomly selected and divided into three groups each of three rats. Group 1-3: treated with 5000 mg/kg each of the extract. Prior to treatment, rats were fasted for 4h but allowed free access to water. A rat from each group was picked, weighed and dosed orally with a limit dose of 5000 mg/kg body weight of the extract then food was further withheld for 2 h. Another animal from the same group was given the same dose of the extract; the same applies to two other groups. Each animal was observed after every 15 min in the first 4hrs after dosing, every 30 min for 6hrs and daily for 48hrs for the short-term outcome each time for instant death and then watched for the successive 24 h for the short-term toxicity outcome and finally for the next 13 days for any delayed toxic effects. . Animals were observed for signs of toxicity, which include increased motor activities, rolling, writhing, depression, behavior pattern, diarrhea, sleep, coma, etc. the time of onset, disappearance and length of recovery period from toxic symptoms

were systematically recoded. At the end of the test, surviving rat were weighed.

Histopathological assay. At the completion of the acute toxicity studies, animals were anaesthetized with chloroform, thereafter the Liver, kidney and Lungs of each animal were removed and fixed in 10% formalin, the tissues were dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax. Paraffin sections (5-6 μm) thick transverse sections of the liver and the lung were cut using a rotary microtome and mounted on glass slides. The slides were stained with haematoxylin and eosin (H&E) according to Bancroft and Gamble, (2008) [9]. Stained sections of control and treated rats were examined under the light microscope.

Antibacterial assay of *Bombax costatum* stem bark extracts. The antibacterial activity of the stem bark extracts of *B. costatum* were determined using some pathogenic microbes namely (*Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium ulcerans, Bacillus subtilis, Escherichia coli, Proteus mirabilis and Salmonella* Typhi). The microbes were obtained from the Department of Medical microbiology Ahmadu Bello University Teaching Hospital Zaria

Preparation of stock solution. 0.5g of each of the three extracts of *B. costatum* were weighed and dissolved in 10mls of Dimethylsulfoxide (DMSO) so as to obtain a concentration of 50 mg/ml. These were the initial concentration used to check the antibacterial activities of the plant. From the stock Two-fold serial dilution of the three extracts in the sterilized broth was made to obtain the concentration of 50 mg/ml, 25mglml, 12.5 mg/ml, 6.25 mg/ml and 3.25 mg/ml each. Standard antibiotic Ciprofloxacin at a concentration of 5μg/ml was used as the Positive control drug for the antibacterial activity.

McFarland turbidity standard. This was prepared by dissolving 0.05 ml of 1% Barium sulphate in 9.95 ml 1% sulphuric acid using normal saline. This corresponds to scale 0.5 McFarland standard scale, while the standardized bacteria culture is 1.5 x 10^8 CFU/ml.

Preparation of media plates for antibacterial sensitivity testing. Mueller Hinton agar was the medium for the microbes. The medium was prepared according to manufacturer's instruction. Thirty-eight (38g) of medium was mixed with one litre of distilled water in a screw cap container and autoclaved at 121°C for 15 min. The medium was poured into sterile Petri-dishes; the plates were then allowed to solidify. Agar well Diffusion method was the method employed for the screening of the extract. The sterilized medium was then seeded with 0.1 ml of the standardized inocula (1.5 x 10^8 CFU/ml) of the test microbes. The inocula was spread evenly over the surface of the medium with a sterile swab. By the use of a standard cork-borer of 6 mm in diameter, a well was cut at the center of each inoculated medium; 0.1 ml of the solution of the extracts of the concentration of 50 mg/ml was then introduced into each well on the inoculated media. The inoculated plates were incubated at 37°C for 24hr, after which each plate was observed for zone of inhibition of growth. The zone was measured with a transparent ruler and the results recorded in millimeter.

Minimum inhibitory concentration (MIC) assay using tube dilution method. The minimum inhibitory concentration of the three extracts against *B. costatum* were carried using tube dilution method. Mueller Hinton broth was prepared, 10 ml was dispensed into test tubes and was sterilized at 121°C for 15 min, and the broth was then allowed to cool. Equal volume of the concentrations (50, 25, 12.5, 6.25 and 3.125 mg/ml) were incorporated in nutrient broth in 1:1 and

(0.1ml) of the standardized suspension of the test organisms (1.5 x 10^8 CFU/ml) was added in to each of the test-tube. The tubes were then incubated at 37°C for 24 hours. Tubes containing broth and extract without inocula were incubated to serve as positive control while a tube containing broth and inocula serves as negative control for comparison. The presence of growth (turbid solution) or absence of growth (clear solution) at the end of incubation period was recorded. The lowest concentration of the extract, which had no turbidity, was recorded as the minimum inhibitory concentration (MIC) [10,11].

Minimum bactericidal concentration (MBC). Minimum bactericidal concentration was determined by sub-culturing 0.1ml from the above MIC test dilutions that showed visible growth (turbidity) and all others in which there was no detectable growth on a fresh extract free solid medium and incubated at 370c for 24 hours. The least concentration that shows no single bacteria colony was considered as the minimum bactericidal concentration (MBC) [10,11].

RESULTS

Results of acute toxicity test of *Bombax costatum* stem bark extracts. The acute toxicity (LD_{50}) of the extracts (HE, EE and ME) of *B. costatum* stem bark were determined using OECD method via oral route and showed that the LD_{50} were greater than 5000 mg/kg for each of the extracts. There was no change in food and water intake, there was no behavioural and autonomic changes (pupillary size, lacrimation, salivation, defecation and urination) in the animals after the treatment. There was no death or any sign of toxicity observed on the animals.

Histopathology. Results of the acute toxicity test revealed no gross abnormalities in the Livers, Kidneys and Lungs. However, there were slight distortion in the Liver and Kidney tubules of the rats treated with the extract. Histopathological studies of the Liver revealed normal hepatocytes, well applying nuclei structure and central vein in the control group but slight distortion in the extract treated groups indicated by extravasation of red blood cells (Plates I, II, III and IV). Sections of the Kidney revealed the Bowman's capsule, proximal convoluted tubules in the control group, and distortion of tubules in the extract treated groups (Plates V, VI, VII and VIII). Sections of Lungs revealed alveoli, cilia, bronchea and large air spaces in the control group but slight congestion of cells in between the pockets and inflammation of the broncheo in the extract treated groups (Plates IX, X, XI and XII).

Antibacterial evaluation of extracts of *B. costatum* stem bark

Zones of inhibition of hexane, ethyl acetate and methanol extracts from *B. costatum* against some pathogenic bacteria. Hexane, ethyl acetate and methanol extracts of *B. costatum* stem bark at a concentration of 50mg/ml each showed zones of inhibition between 18 and 28 mm for all the four bacterial strains. All the extracts were observed to have higher zones of inhibition against *E. coli* at 20mm for hexane extract, 28 mm for ethyl acetate extract and 25mm for methanol extract. This was followed by *S. aureus* at 20 mm for hexane extract, 28 mm for ethyl acetate extract and 24 mm for methanol extract. Zones of inhibition for *C. ulcerans* was observed at 18mm for hexane extract, 27 mm for ethyl acetate extract and 23 mm for methanol extract while *S.* Typhi was observe to have the lowest zones of inhibition with hexane extract having 18 mm, 26 mm for ethyl acetate and 22 mm for the methanol extract. Ciprofloxacin showed a wider zone of inhibition between 32 and 41 mm for all the four bacterial strains, and also inhibited the growth of *S. pyogenes*, which were more than the hexane, ethyl acetate and methanol extracts as in Table 1.

Minimum inhibitory concentration of hexane, ethyl acetate and methanol extract from *B. costatum* stem bark against some pathogenic bacteria. The Minimum Inhibition Concentration (MIC) for *S. aureus* were observed at 12.5, 6.25 and 12.5 mg/ml for the hexane, ethyl acetate methanol and extracts respectively while that of *C. ulcerans* were observed at 25, 6.25 and 12.5mg/ml for the hexane, ethyl acetate and methanol extracts respectively. The MIC for *E. coli* were observed at 12.5, 6.25 and 12.5mg/ml, while that of *S. typhi* were observed at 25, 12.5 and 12.5mg/ml for the hexane, ethyl acetate and methanol extracts respectively as in Table 2.

Minimum bactericidal concentration of hexane, ethyl acetate and methanol extract from *B. costatum* against some pathogenic bacteria. Results of this study show that the hexane, ethyl acetate and methanol extract had MBC for *S. aureus* 50, 12.5 and 25mg/ml respectively, *C. ulcerans* at 50, 25 and 25mg/ml for hexane, ethyl acetate and methanol extracts respectively. The MBC for *E. coli* was observed at 50, 12.5 and 25mg/ml for hexane, ethyl acetate and methanol extracts respectively, while that of S. typhi were observed at 50, 25 and 50mg/ml for hexane, ethyl acetate and methanol extracts respectively as in Table 3.

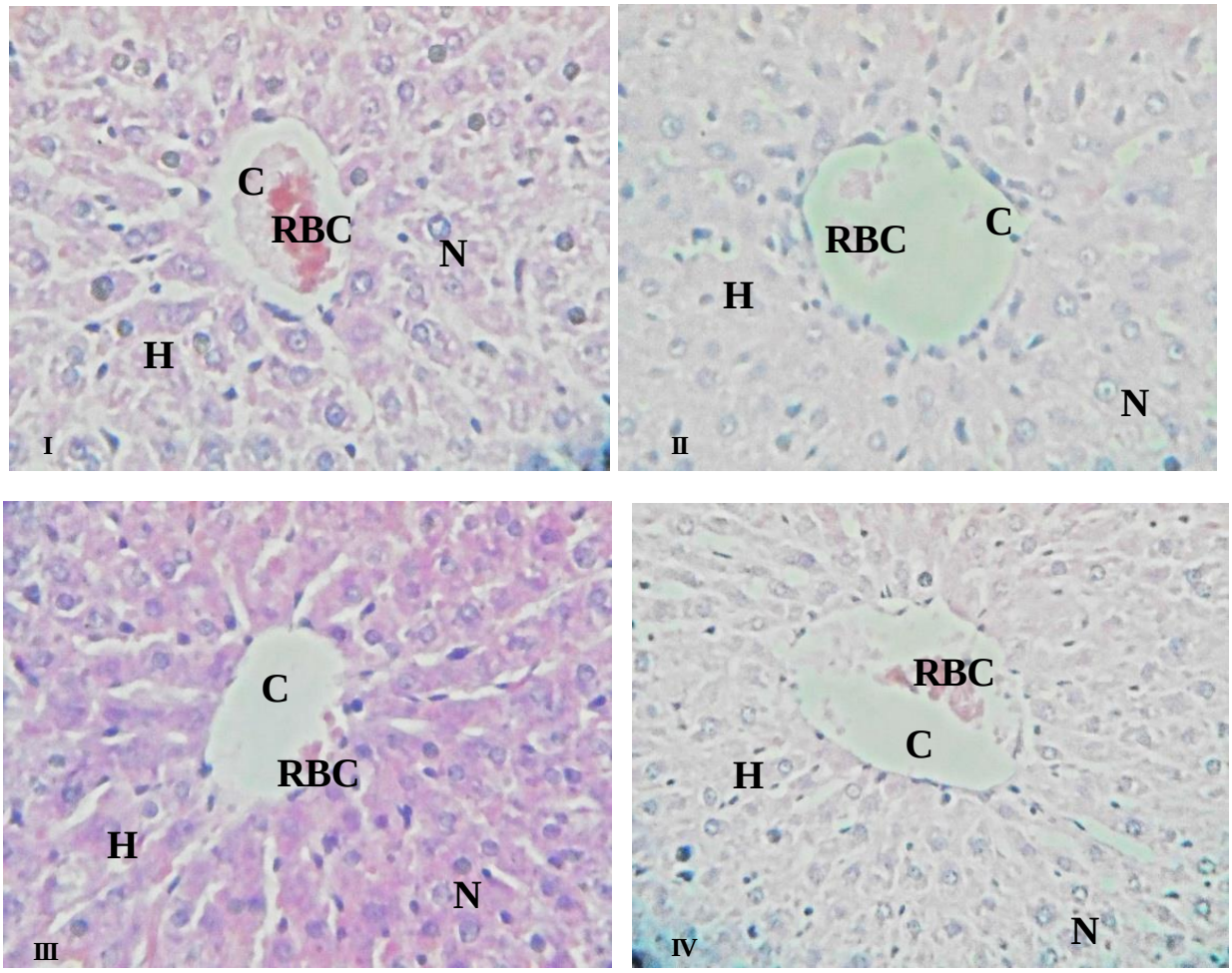

Plate I: Photomicrograph of normal liver of rat showing nuclei (N), hepatocytes (H), central vein (C) and extravasation of red blood cells (RBC). (Transverse section, H&E stain, x400)

Plate II: Photomicrograph of liver of rat treated with hexane extract showing nuclei (N), hepatocytes (H), central vein (C) and extravasation of red blood cells (RBC). (Transverse section, H&E stain, x400)

Plate III: Photomicrograph of liver of rat treated with ethyl acetate extract showing nuclei (N), hepatocytes (H), central vein (C) and extravasation of red blood cells (RBC). (Transverse section, H&E stain, x400)

Plate IV: Photomicrograph of liver of rat treated with methanol extract showing nuclei (N), hepatocytes (H), central vein (C) and extravasation of RBC. (Transverse section, H&E stain, x400)

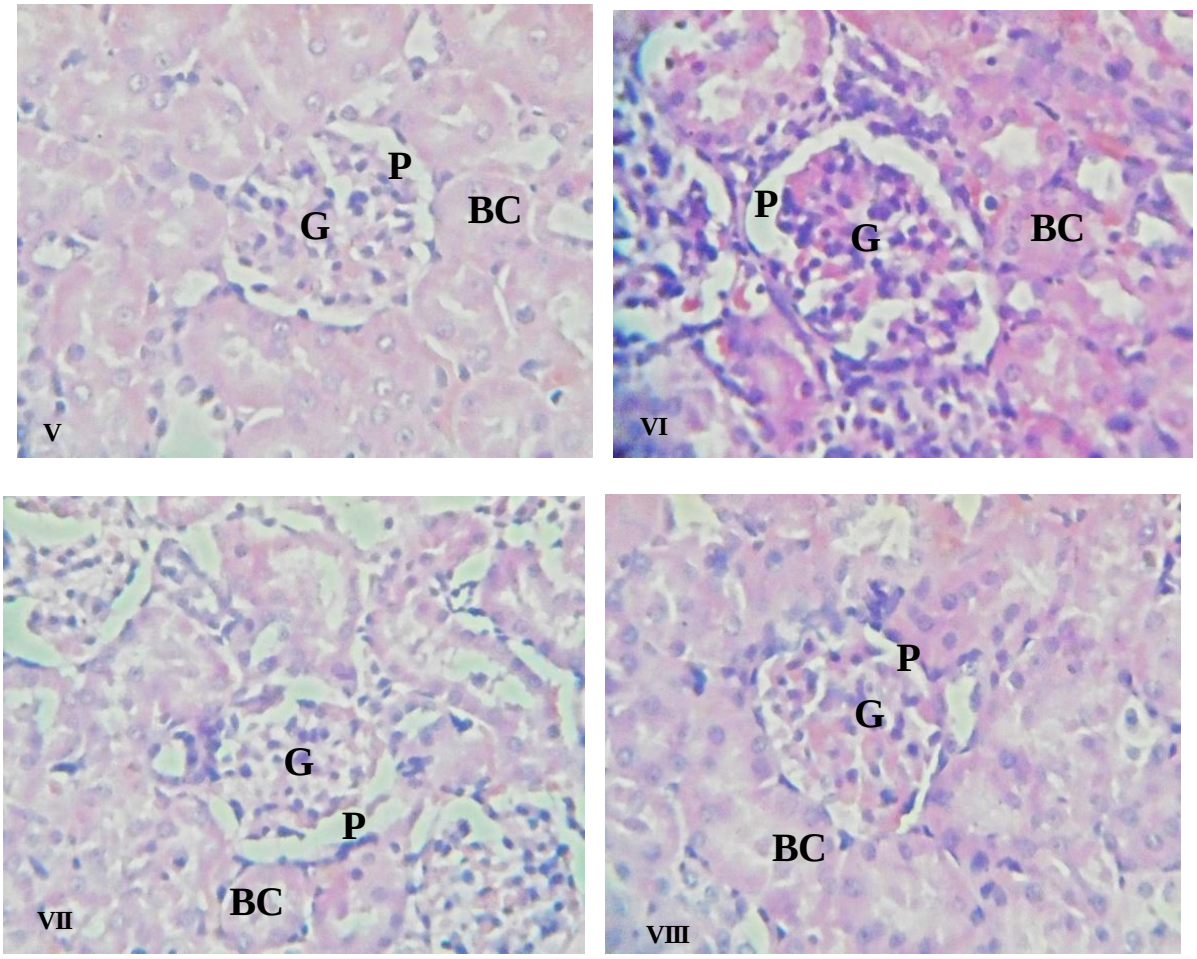

Plate V: Photomicrograph of normal kidney showing Glomerular apparatus (G), Bowman's capsule (BC) and proximal convoluted tubules (P). (Transverse section, H&E stain, x400).

Plate VI: Photomicrograph of kidney of rat treated with hexane extract showing Glomerular apparatus (G), Bowman's capsule (BC) and proximal convoluted tubules (P) (Transverse section, H&E stain, x400).

Plate VII: Photomicrograph of kidney of rat treated with ethyl acetate extract showing Glomerular apparatus (G), Bowman's capsule (BC) and proximal convoluted tubules (P) (Transverse section, H&E stain, x400).

Plate VIII: Photomicrograph of kidney of rat treated with methanol extract showing Glomerular apparatus (G), Bowman's capsule (BC) and proximal convoluted tubules (P) (Transverse section, H&E stain, x400).

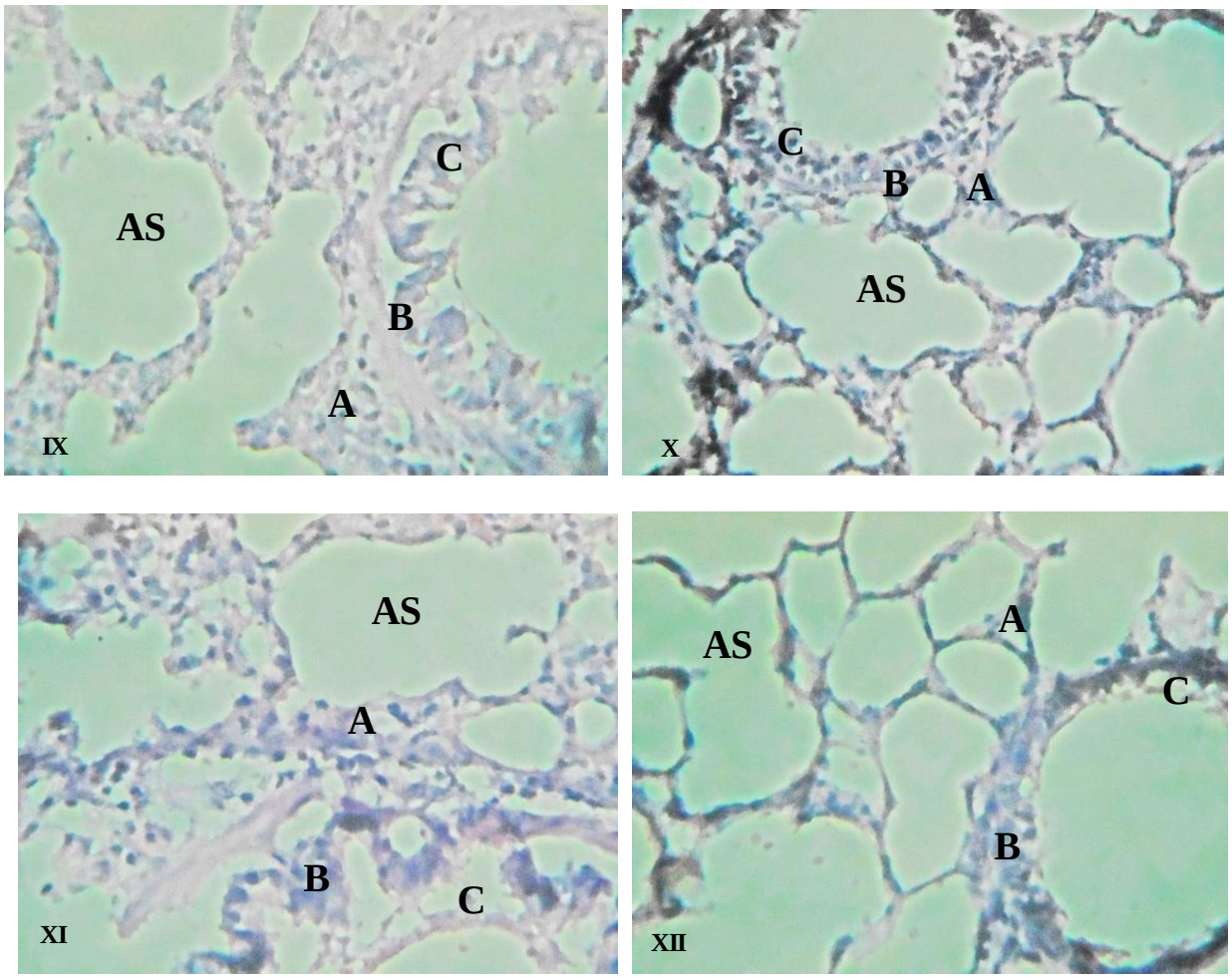

Plate IX: Photomicrograph of normal lung of rat (Control) showing Alveoli (A), air spaces (AS), cilia (C) and bronchea (B) (Transverse section, H&E stain, x400).

Plate X: Photomicrograph of lung of rat treated with hexane extract showing Alveoli (A), air spaces (AS), cilia (C) and bronchea (Transverse section, H&E stain, x400).

Plate XI: Photomicrograph of lung of rat treated with ethyl acetate extract showing Alveoli (A), air spaces (AS), cilia (C) and bronchea (B). (Transverse section, H&E stain, x400).

Plate XII: Photomicrograph of lung of rat treated with methanol extract showing Alveoli (A), air spaces (AS), cilia (C) and bronchea (B). (Transverse section, H&E stain, x400).

Table 1: Antibacterial Sensitivity profile of extracts of B. costatumagainst test microorganisms: Control (Ciprofloxacin) Zones of Inhibition (mm)

Test organism	Hexane 50mg/ml	Ethyl acetate 50mg/ml	Methanol 50mg/ml	Ciprofloxacin
Staphylococcus aureus	20	28	24	35
Streptococcus pyogenes	0	0	0	32
Corynebacterium ulcerans	18	27	23	0
Bacillus subtilis	0	0	0	34
Escherichia coli	20	28	25	38
Proteus mirabilis	0	0	0	0
Salmonella Typhi	18	26	22	41

Table 2: Minimum Inhibition Concentration of hexane, ethyl acetate and methanol extracts of *B. costatum* against test microorganisms

Organism	Hexane					Ethyl acetate					Methanol				
	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml
S. aureus	-	-	o*	+	++	-	-	-	o*	+	-	-	o*	+	++
C. ulcerans	-	o*	+	++	+++	-	-	-	o*	+	-	-	o*	+	++
E. coli	-	-	o*	+	++	-	-	-	o*	+	-	-	o*	+	++
S. typhi	-	o*	+	++	+++	-	-	o*	+	++	-	-	o*	+	++

Key: -= No turbidity (no growth) o*= MIC, += Turbid (high growth), ++= Moderate turbidity, +++= High turbidity.
Minimum bactericidal concentration of hexane, ethyl acetate and methanol extract from *B. costatum* against some pathogenic bacteria

Table 3: Minimum Bactericidal Concentration of hexane, ethyl acetate and methanol extracts of *B. costatum* against test microorganisms

Test organism	Hexane					Ethyl acetate					Methanol				
	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml
S. aureus	o*	+	++	+++	+++	-	-	o*	+	++	-	o*	+	++	+++
C. ulcerans	o*	+	++	+++	+++	-	o*	+	++	+++	-	o*	+	++	+++
E. coli	o*	+	++	+++	+++	-	-	o*	+	++	-	o*	+	++	+++
S. typhi	o*	+	++	+++	+++	-	o*	+	++	+++	o*	+	++	+++	+++

Key: - = No colony growth, o*= MBC += Scanty colony growth, ++= Moderate colony growth turbidity, +++= Heavy colony growth.

DISCUSSION

In order to determine the safety margin of drugs and plant products for human use, toxicological evaluation was carried out in experimental animals using OECD Guideline 2011 (limit test method) to predict toxicity and to provide guidelines for selecting a "safe" dose in animals and also used to estimate the therapeutic index (LD_{50}/ED_{50}) of drugs [12-14]. The median lethal dose (LD_{50}) of the extracts (hexane, ethyl acetate and methanol) of the stem-bark of *B. costatum* was carried out orally in rats. The LD_{50} was found to be greater than 5000 mg/kg when administered orally in rats. These studies showed the extracts of *B. costatum* stem bark are practically non-toxic when administered using the oral route. This is based on the toxicity classification, which states that substances with LD_{50} values of 5000 to 15,000 mg/kg body weight are practically non-toxic [15]. Histo-pathological studies was carried out on the rats, the organs of animals treated with the extracts showed no histological change from the normal group which suggested that there is no tissue damage. Hexane, ethyl acetate and methanol extracts of *B. costatum* stem bark was observed to possess antibacterial activity against four out of the seven bacterial isolates that were used during the present study. Ciprofloxacin was observed to have wider zones of inhibition (32-41 mm), which indicates that it has higher antibacterial activity as compared to the plant extracts. Although, the three extracts showed appreciable zones of inhibition (18-28 mm), the ethyl acetate extract exhibited a wider dimension (26-28mm) as compared to the hexane and methanol extracts which showed

(18-20 mm) and (22-25 mm) respectively. The results from the MIC and MBC also revealed that the ethyl acetate extract (6.25 and 12.5 mg/ml) had higher inhibitory strength than the hexane (25 and 50 mg/ml) and methanol (12 and 25 mg/ml) extracts. Conclusively, from the overall studies, ethyl acetate extract was observed to have higher antibacterial activity at various concentrations than the hexane and methanol extracts, this can be supported by the claim that different solvents have different ability of extracting phytoconstituents depending on their polarity an indication that the plant contains antibiotic substances that have broad spectrum of activity including antibacterial activity [16].

Conclusion. The ethyl acetate extract was observed to have the best antibacterial activity compared to other extracts; the results support the traditional use of the plant in treatment of infectious diseases. The presence of pharmacologically active principles such as flavonoids, tannins and saponins may be responsible for the antibacterial activity. This plant may therefore serve as potential antibacterial agent.

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