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In-vitro Antibacterial Study of Crude extract of Barringtonia asiatica (L.) Kurtz Against Salmonella typhi, Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia

[1, 2]Isaac John Umaru, [1]Fasihuddin Badruddin Ahmed, & [3]Kerenhappuch Isaac Umaru
[1]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak. Malaysia
[2]Department of Biochemistry, Federal University Wukari, Taraba State. Nigeria
[3]Department of Biochemistry, University of Maiduguri Borno State, **Nigeria**
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Abstract

Introduction: The antibiotic-resistant pathogenic bacteria have been recognized as a serious problem for humans and animals. The aims of this study were to evaluate the in-vitro antibacterial study of crude extract of *Barringtonia asiatica* (L.) Kurtz against *Salmonella typhi, Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia.* **Materials and Methods:** Extractions was carried out through conventional method, by soaking the ground, the plant material in non-polar, medium polar and polar solvents in the order of increasing polarity and the crude extracts were tested by the disc diffusion method on nutrient agar medium. **Result:** The growth inhibition was considered and compared based on the inhibition rate as reported and < 9 mm inhibition is considered inactive in this regards the methanol leaves extract was observed to exhibited higher activity at 500 µg/mL on *Klebsiella pneumonia* with growth inhibition zone of 16.23 ± 0.06 mm. And lower inhibition was observed for other organism tested at similar concentration**. Conclusion**: The focus of this study is to provide the prospects of *Barringtonia asiatica* different solvent from non-polar and polar crude extract as a good agent for combating antibacterial menace and disease resistance from modern medicine.

Keywords: Antibacterial, Crude, extract, Barringtonia asiatica, Salmonella typhi, Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia.

1. Introduction

Human since the beginning of civilization, plants, and plant products are used as medicines. The interest in the study of medicinal plants as natural products is increasing day by day in diverse parts of the world. These plants contain active chemical compounds with high antioxidant properties, which help in prevention of various diseases. Generally, bacteria that are used as therapeutic or remedial agents have the genetic ability to transmit and develop resistance to drugs, therefore it has now become imperative to find alternative treatments for bacterial infections (Khatri et al., 2016).

The antibiotic-resistant pathogenic bacteria have been recognized as a serious problem to humans and animals as reported by the World Organization for Animal Health (OIE), the

World Health Organization (WHO) and the Food and Agriculture Organization (FAO). Due to the high frequency of new resistant phenotypes, the only solution is the development of bacterial antibiotic resistance (Polka et al., 2016)

High incidence of resistant microorganisms in the clinical world are in the increases and the only option is to resort to plant and plant products. The plant-based medicines are more effective and cheaper alternative as compared to synthesized compounds in the treatment of diseases (Umaru et al., 2020).

Barringtonia asiatica (L.) Kurtz belongs to a Family of Lecythidaceae is a species native to mangrove habitats in the tropical. It is a common plant in the Malaysian Mangroves, and easily available in Kuching Wetlands Sarawak and Bako National Park. It is also found in tropical Africa especially in Nigeria and Madagascar. Its large pinkish-white, pompon flowers give off a sickly-sweet smell to attract bats and moths which pollinate the flowers at night. It is grown along streets for decorative and shade purposes in some parts of Sarawakian houses and it's also known as sea poison tree (Alfrits & Suriani, 2016) or box fruit due to the distinct box-shaped of the fruit. It is a medium-sized tree growing to 7–25 m tall.

The leaves are narrow obovate, 20–40 cm in length, and 10–20 cm in width matured foliage colour is green, smooth glossy shiny leathery thick simple and evergreen. It is used as sausage food among the native of Sarawak in the Kampong as well as medicinal plants. Inhabitants of several West African countries Nigeria and the Polynesian Islands use the liquid from the crushed bark of *Barringtonia asiatica* to treat chest pains and heart problems (Umaru *et al.,* 2018a). The same plant is used in Papua New Guinea to treat stomach-aches, where the leaves are squeezed into the water and the liquid is taken orally. It is also used for anti-rheumatic medication (Tanor *et al*., 2014)

The aim of this study was to evaluate the in-vitro antifungal study of crude extract of *Barringtonia asiatica (L.) Kurtz a*gainst fungal pathogenic strains

Figure 1: shows *Barringtonia asiatica* leaf and fruit

2. Materials and Methods

Sample Preparation

The fresh samples were washed with distilled water and air-dried. The dried plant material (leaves,) were ground into a fine powder using laboratory pestle and mortar and electric grinder and packed into a clean sample container, labeled and kept for further use.

Extraction

Extractions were carried out through a conventional method as described by Fasihuddin et al. (2010). A total of 2 kg of the powdered sample was extracted using the cold-soaking method. This was achieved by soaking the ground, plant material in non-polar, medium polar and polar solvents in the order of increasing polarity. The dried and ground leaves, of *Barringtonia asiatica* was extracted.

The samples were soaked in hexane with the ratio of 1:3 in 5 liter Erlenmeyer flasks at room temperature for 72 hours. The resulting hexane solution was then filtered using filter paper and the residue was re-extracted with fresh hexane for another 72 hours and filtered. All the extracts were combined and concentrated using the rotary evaporator (model Heidolph Laborota 4000 efficient) under reduced pressure to obtain hexane crude extract.

The residues were then re-extracted using similar procedure with dichloromethane, then chloroform, ethyl acetate, and methanol to obtain dichloromethane, ethyl acetate, chloroform and methanol crude extracts, respectively. At the end of the extraction process the dry weight and yield of each crude extract were determined.

Preparation of Test Sample

Five crude extracts of *Barringtonia asiatica* were used in antifungal assay namely the hexane, dichloromethane, ethyl acetate, chloroform, and methanol crude extracts. The crude extracts were tested by disc diffusion method on nutrient agar medium as described by Mayuri *et al*. (2015). Exactly 5 mg of each crude sample was dissolved homogeneity in 5 mL of methanol giving a stock solution of 1000 µg/mL. Lower concentration of 25, 50, 100, 250, and 500 µg/mL was prepared from the stock solution by proper dilution.

Preparation of Agar Plates

Preparation of agar plates was performed based on the method described by Umaru et al. (2018b) and the nutrient agar according to manufacturer's instruction with 14 g of dried agar dissolved in 500 mL distilled water. The agar solution was heated until boiling followed by sterilization in an autoclave at 121 °C. The agar solution was then poured into a sterile Petri plate and allowed to cool down and forming a gel. The plate was divided into eight sections by making a line marking on the outside surface of the plate. Each section was for each test samples namely the 25 µg, 50 µg, 100 µg, 250 µg and 500 µg samples, tetracycline 30 µg (positive control) and methanol (negative control). The plate was sealed using parafilm and kept at 4 °C upon bacteria inoculation.

Preparation of Bacteria Broth

Several selected bacteria were used to evaluate the antibacterial activities of the crude extracts of *Leptadenia hastata, Barringtonia asiatica* and *Barringtonia racemosa*. *Escherichia coli* (ATCC©25922), *Salmonella typhi* (ATCC©14028), *Staphylococcus aureus* (ATCC©25923) and *Staphylococcus flavin* (ATCC©19155) used in this study were obtained from the stock culture provided by Virology Laboratory, Universiti Malaysia Sarawak. The nutrient broth was prepared according to manufacturer's instruction, with 2.6 g of the dried broth dissolved in 200 mL distilled water followed by sterilization in an autoclave at 121 °C. The bacterial were sub-cultured in 10 mL of broth, each in universal glass vial bottle for 16 hrs inside an incubator equipped with a shaker at 37 °C (Umaru et al., 2018c).

After 16 hours' incubation, turbidity (optical density/OD) of the bacterial broth was measured by using a UV mini spectrophotometer (model 1240 of Shimadzu brand). The measurement of the optical density was performed at wavelength 575 nm and the bacterial broth was ready to be used when its turbidity was between OD 0.6 to 0.9. The nutrient broth was used to adjust the turbidity until the desired value was obtained (Umaru et al., 2018c).

Plate Inoculation

Inoculation of the bacteria was carried out in a biohazard cabinet and the procedure was based on the method described by Umaru et al, (2020). Approximately 1 mL of the ready bacterial broth was transferred into mini centrifuge tubes. A sterile cotton swab was dipped into the mini centrifuge tube containing bacteria broth and streaked over entire of the agar plate surface, performed in 4 different directions. The agar plate was then left for 5-10 min before applying the test samples. The disc used was 6 mm in diameter. A volume of 10 µL of the test samples of concentration 10, 25, 50, 100, 250, and 500 µg/mL was each pipetted into the discs and placed onto the agar plate by using sterile forceps and gently pressed to ensure contact. Next, to be placed on the agar plate was the disc pupated with methanol as a negative control, followed by 30 µg of tetracycline as a standard antibacterial agent (positive control). The plates were left at room temperature for 10 min to allow the diffusion of the test samples and the standards into the agar. Each crude extract was tested in triplicate for each bacterium used. The plate samples were then incubated at 37 °C for 24 hrs before the inhibition zone around every sample disc being examined. The inhibition zone was measured in diameter (mm) to indicate the presence of antibacterial activity for each sample, as compared to the positive control (Umaru et-al., 2020).

3. Result and Discussion

The sensitivity of each crude extract on the microorganism was determined using the disc diffusion technique. The standard used was tetracycline. The growth inhibition was considered and compared based on the inhibition rate as reported and < 9 mm inhibition is considered inactive (Jan Hudzicki, 2009)

The antibacterial activity by Gram-positive or Gram-negative bacteria, it would generally be expected that a much greater number would be active against Gram positive than Gram-negative bacteria (McCutcheon et al. 1992). Whereas, in our study both Gram-negative and Gram-positive bacteria are found to be active against the different solvent crude extract.

The differences in the antibacterial effects of plant extracts may be due to the differences in their phytochemical compositions (Nair et al. 2005

The table shows *Barringtonia asiatica* methanol leaves extract exhibited higher activity at 500 µg/mL on *Klebsiella pneumonia* with a growth inhibition zone of 16.23 ± 0.06 mm. This agrees with the report of Hussain and Kumaresan, (2014) that methanol extract exhibited significant activity against bacteria. In separate studies, it was also reported of the potential and higher activity of methanol crude extract against microorganisms by Habibi *et al*. (2015). Lower inhibition was observed for other organisms tested at similar concentrations. Other extracts at lower concentrations exhibited a lower inhibition zone.

Table : Effect of *Barringtonia asiatica* leaf extract (µg/mL) on Gram positive and Gram-negative bacteria in millimetre (mm)

Conc. (µg/mL)	**Organism**	**Tetracycline (30 µg/mL)**	**Hexane**	**DCM**	**Ethyl acetate**	**Chloroform**	**Methanol**
25	*Salmonella typhi*	20.77 ±0.03	10.43 ± 0.06	8.50 ± 0.10	10.15 ± 0.07	9.15 ± 0.07	9.55 ± 0.07
	Escherichia coli	19.79 ±0.06	10.60±0.10	12.07±0.06	10.10 ± 0.06	8.47 ± 0.31	10.45 ± 0.07
	Staphylococcus aureus	21.16 ±0.11	10.35 ± 0.07	**8.25 ± 0.07**	9.65 ± 0.07b	9.00 ± 0.10	9.80 ± 0.00
	Klebsiella pneumonia	20.76 ±0.18	10.70 ± 0.14	10.00 ± 0.10	9.27 ± 0.12	10.20 ± 0.00	10.40 ± 0.00
50	*Salmonella typhi*	20.77 ±0.03	10.85 ± 0.07	10.90 ± 0.14	12.75 ± 0.07	12.85 ± 0.07	12.90 ± 0.14
	Escherichia coli	19.79 ±0.06	12.00± 0.10	12.25 ± 0.07	12.10 ± 0.10	11.10 ± 0.10	11.40 ± 0.10
	Staphylococcus aureus	21.16 ±0.11	10.57 ± 0.06	12.45 ± 0.07	12.05 ± 0.07	12.57 ± 0.06	12.60 ± 0.10
	Klebsiella pneumonia	20.76 ±0.18	15.03 ± 0.06	15.10 ± 0.10	13.67 ± 0.06	13.63 ± 0.06	**15.30 ± 0.10***
100	*Salmonella typhi*	20.77 ±0.03	13.07 ± 0.06	10.95 ± 0.07	14.25 ± 0.07	10.33 ± 0.06	14.60 ± 0.00
	Escherichia coli	19.79 ±0.06	12.33 ± 0.06	12.33 ± 0.06	12.35 ± 0.07	12.35 ± 0.07	14.07 ± 0.06

	Staphylococcus aureus	21.16 ±0.11	1210 ± 0.10	14.25 ± 0.07	12.65 ± 0.07	14.15 ± 0.07	14.20 ± 0.10
	Klebsiella pneumonia	20.76 ±0.18	14.37 ± 0.06	13.00 ± 0.10	13.13 ± 0.06	14.85 ± 0.07	15.05 ± 0.07*
250	*Salmonella typhi*	20.77 ±0.03	12.35 ± 0.07	12.50 ± 0.10	13.57 ± 0.06	14.35 ± 0.07	14.50 ± 0.00
	Escherichia coli	19.79 ±0.06	12.73 ± 0.12	12.80 ± 0.20	12.20 ± 0.17	12.50 ± 0.10	13.40 ± 0.10
	Staphylococcus aureus	21.16 ±0.11	12.33 ± 0.06	14.60 ± 0.10	14.85 ± 0.07	14.40 ± 0.10	14.53 ± 0.25
	Klebsiella pneumonia	20.76 ±0.18	13.65 ± 0.07	15.27±0.06*	15.55 ± 0.07*	15.00 ± 0.20	**15.70 ± 0.10***
500	*Salmonella typhi*	20.77 ±0.03	14.00 ± 0.10	12.67 ± 0.06	14.45 ± 0.07	13.80 ± 0.00	14.70 ± 0.10
	Escherichia coli	19.79 ±0.06	13.30 ± 0.10	11.90 ± 0.10	13.57 ± 0.06	12.73 ± 0.12	12.70 ± 0.10
	Staphylococcus aureus	21.16 ±0.11	12.70 ± 0.10	14.75 ± 0.07	15.27 ± 0.25	14.80 ± 0.10	15.85 ± 0.07*
	Klebsiella pneumonia	20.76 ±0.18	16.07±0.12*	15.70 ± 0.10	16.20 ± 0.20*	16.05 ± 0.07	**16.23 ± 0.06***

Result is Mean ± SD. N = 3

*= significant activity was observed when compared to the control ($p<0.05$)

Concentration of standard is 30 µg/mL of tetracycline, Conc= Concentration, DCM = Dichloromethane

Conclusion

This study has demonstrated that the administration of Barringtonia asiatica leaves extract on the selected pathogen have shown efficiency in inhibiting the growth rate of the bacteria's. From the result it was observed these extracts of different polarity from *Barringtonia asiatica* could be suggested for pharmaceutical and clinical bodies as an agent to halt the menace of Bacterial.

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Conflict of interest

The authors declare that they have no conflict of interest**.**

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