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Antibacterial and Cytotoxicity Studies of Barringtonia Asiatica

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Abstract

Objective: The hexane leaf extract of *Barringtonia asiatica* has biological activity, however, the study was carried out with an objective to ascertain its effects on Escherichia coli (ATCC©25922), Salmonella typhi (ATCC©14028), Staphylococcus aureus (ATCC©25923) and Kliebsielia Pheumonia and to evaluate the cytotoxicity of the leaves extract using brine shrimp lethality assay.

Methods: *Barringtonia asiatica* extract was evaluated for its antibacterial activity. Antibacterial activity assessment was performed by Disc diffusion assay the leaves of the plant were extracted with n-hexane, dichloromethane, ethyl acetate, chloroform and methanol and then vaporized to give respective extracts. Antimicrobial activity against Escherichia coli, salmonella typhi, staphylococcus aureus and Klebsielia pneumonia, was determined. The optical density of the broth using UV mini spectrophotometer and zone of inhibition by the crude extract were determined.

Results: The results showed that of n-hexane extracts of varying concentration the 500ppm and 1000ppm displayed more activity with 4.00 ± 0.10, 4.30 ± 0.10b, 3.70 ± 0.10, 4.07 ± 0.12mm and 4.67 ± 0.12a, 4.35 ± 0.07a, 4.05 ± 0.07a, 4.55 ± 0.07mm respectively on all the pathogen subjected to the studies displayed where aSignificantly ($p<0.05$) higher compared to different extract at the same concentration b Significantly ($p<0.05$) lower compared to the control, than others at 25-1000 ppm per disc of the extracts concentration tested. However, the result of the cytotoxicity showed that *Barringtonia asiatica* Leaf extract were toxic on brine shrimp larvae with LC_{50} value of 208.091 when compared with the control 7.455 thus having toxicity when referred to the fact that LC_{50} value of less than 1000µg/mL is toxic while LC_{50} value of greater than 1000µg/mL is non-toxic.

Conclusion: The present results showed the potential of the medicinal plant used by traditional herbal medical practitioners as natural antimicrobial agents, thus can be further used to determine the bioactive products that may provide as leads in the development of new drugs.

Keywords: Barringtonia asiatica; Extract; Cytotoxicity; Antibacterial

Introduction

Plants are important sources of medicinal products, they are recognized for their ability to produce a rich source of secondary metabolites and humans have long before now used many species to treat various kind of disease and ailment [1]. *Barringtonia asiatica* is a species of Barringtonia native to mangrove habitats on the tropical, it is a common plant in the Malaysian Mangroves and wetlands such as the Kuching wetlands Sarawak and Bako National Park,

It is also found in tropical Africa, 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 Box Fruit due to the distinct box-shaped of the fruit, it is a medium-sized tree growing to 7-25 m tall. [2,3].

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 sarawakian in the kampong as well as a medicinal plant, inhabitants of several West African countries, Nigeria and the Polynesian Islands use liquid from the crushed bark of *Barringtonia asiatica* to treat chest pains and heart troubles. The same plant is used in Papua New Guinea to treat stomach-aches, the top leaves from this tree are squeezed into water and the liquid taken orally [4]. The plant when mature the

bark texture is smooth and woody with the root type fibrous and tap root.

In cytotoxicity study, the brine shrimp cytotoxicity assay is considered as a convenient method for preliminary assessment of toxicity, testing. However, limited studies have reported bioactivities of *Barringtonia asiatica* and the antimicrobial activity

Thus, this *in-vivo* lethality assay is the simplest zoological organism (brine shrimp) which can be used as a convenient monitor for screening and fractionation in the discovery and monitoring of bioactive natural product, it is a general assay and capable of detecting various bioactivity present in crude extracts of medicinal plants and has been used as an indicator for general toxicity and as a guide for the detection of antitumor and pesticidal compounds. Since its introduction by Meyer et al. [5].

The aim of this research is study the hexane leaf extract of *Barringtonia asiatica* has biological activity and to ascertain its effects on *Escherichia coli* (ATCC©25922), *Salmonella typhi* (ATCC©14028), *Staphylococcus aureus* (ATCC©25923) and Klebsiella Pneumonia as well as to evaluate the cytotoxicity of the leaves extract using brine shrimp lethality assay.

Material and Method

All chemicals used in this investigation were of analytical grade and were obtained from SIGMA. Standard antibacterial agent (30µg) tetracycline, antimicrobial susceptibility test discs and Nutrient agar (CM0003) were obtained from Oxoid Ltd, Wade Road, Basingstoke, Hants, RG2 8PW, UK.

Preparation of agar plates

Preparation of agar plates was performed based on method described by Pundir and Jain [6].

Preparation of bacteria broth

All the selected bacteria were used to evaluate the antibacterial activities of the crude extracts of Barringtonia asiatica; *Escherichia coli* (ATCC©25922), *Salmonella typhi*, (ATCC©14028), *Staphylococcus aureus* (ATCC©25923) and *Kliebselia pneumonia*, (ATCC©19155). They were all 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 autoclave at 121°C.

The bacterial was sub-cultured in a 10 mL of broth, each in universal glass bottle for 16 hours inside an incubator equipped with shaker at 37°C [7]. After 16 hours incubation, turbidity (optical density/OD) of the bacterial broth was measured by using UV mini spectrophotometer (model 1240 of Shimadzu brand), comparable to that of nutrient broth standard tube for further use [8]. Measurement 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. Nutrient broth was used to adjust the turbidity until the desired value was obtained.

Plate Inoculation

Inoculation of the bacteria was carried out in a biohazard cabinet and the procedure was based on method described by Pundir & Jain [6]. Approximately 1 mL of the ready bacterial broth were transferred into mini centrifuge tubes. A sterile cotton swap 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 minutes before applying the test samples.

The disc used was 6 mm diameter. A volume of 10 µL of the test samples of concentration 25, 50, 100, 250, 500, 1000 ppm were each pupated onto 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 negative control, followed by 30 µg of tetracycline as standard antibacterial agent (positive control). The plates were left at room temperature for 10 minutes 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 hours before the inhibition zone around every sample disc being examined. The inhibition zone was measured in diameter to indicate the presence of antibacterial activity for each sample, as compared to the positive control.

Brine Shrimp (Artemia salina) Lethality Test

Toxicity test against brine shrimp (Artemia salina) developed by [5] was used in this study. The brine shrimp hatch, 1.5 g of Artemia salina cysts (Sanders Great Salt Lake, Brine Shrimp Company U. S. A.) was aerated in 1 L capacity glass container containing filtered seawater (collected from Damai beach in Kuching-Sarawak).

Air pump was fitted to the water to ensure complete aeration of the cysts after 48 hrs. of incubation at room temperature between 27-29°C under continuous illumination of fluorescence lamp, newly hatched free-swimming nauplii were harvested from the bottom of the glass container. The freshly hatched nauplii were used for the bioassay.

Exactly 5mg of sample was dissolved in 5 mL methanol, and the mixture was sonicated to ensure homogeneity of the extract. six different volumes of 500, 100, 50, 25, 10 and 1µL each from the stock solution were transferred into NUNC multidisc in triplicate. Solvent could evaporate under a running fume hood for overnight and followed by the addition of 0.2 mL DMSO and 4.8 mL seawater to give final concentration of 500, 100, 50, 25, 10 and 1 µg/mL, respectively.

Ten brine shrimp nauplii were transferred into each concentration in NUNC multidisc, and was observed every 6

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hours for 24 hours. The amount of dead nauplii were calculated. Thymol was used as positive control, whereas 0.2 mL DMSO and 4.8 mL seawater was used as negative control. The data was analyzed to determine the concentration of the samples that kill 50% of brine shrimp at 24 hours or known as LC_{50}.

Statistical analysis

The results were expressed as means ± Standard deviation (SD) of three parallel measurements with one-way ANOVA. The LC_{50} values for toxicity assay was calculated and determined by performing Profit analysis in IBM SPSS Statistic software of version 21.

Result and Discussion

Result

In the antibacterial and cytotoxicity studies, the hexane extract of *Barringtonia asiatica* exhibited the presence of antibacterial bioactive component (Table 1). The antibacterial activity of the extract was in concentration dependent manner. Activity was gradually increased with the concentration, from low concentration level to higher concentration level. The hexane extract exhibited dose dependent inhibition of bactericidal in comparison to the control.

Table 1: Antibacterial Activity of *Barringtonia asiatica* Leaf Extract.

	Concentration (ppm)						
Organisms	**Control**	**25**	**50**	**100**	**250**	**500**	**1000**
Salmonella typhi	5.50±0.91	2.43 ± 0.06	2.85 ± 0.07	3.07 ± 0.06	3.35 ± 0.07	4.00 ± 0.10	4.67 ± 0.12[a]
E. coli	5.68±0.59	2.60 ± 0.10	3.00 ± 0.10	3.33 ± 0.06	3.73 ± 0.12b	4.30 ± 0.10b	4.35 ± 0.07[a]
Staphy aureus	5.83±0.29	2.35 ± 0.07	2.57 ± 0.06	3.10 ± 0.10	3.33 ± 0.06	3.70 ± 0.10	4.05 ± 0.07[a]
Staphy aureus	5.83±0.29	2.35 ± 0.07	2.57 ± 0.06	3.10 ± 0.10	3.33 ± 0.06	3.70 ± 0.10	4.05 ± 0.07[a]

Determination Values are Mean ± SD for five

[a]Significantly (p< 0.05) higher compared to different concentration on same organism in each row bSignificantly (p< 0.05) higher compared to at the same organism at different concentration in each column.

The extract showed 4.00 ± 0.10mm, 4.30 ± 0.10mm, 3.70 ± 0.10mm, 4.07 ± 0.12 mm and 4.67 ± 0.12mm, 4.35 ± 0.07mm, 4.05 ± 0.07mm, 4.55 ± 0.07mm inhibition of activity at the doses of 500 and 1000 ppm, respectively while tetracycline showed 5.50±0.91mm, 5.68±0.59mm, 5.83±0.29mm, 6.73±0.77 inhibition of bacteria. While, at various concentration of the extract (1, 10, 25, 50, 100 and 500ppm) the average death of Artemia salina of hexane crude extract of the Leaf caused the death rate to increase with increase in concentration, given rise to LC_{50} 208.091 µg/mL when compared to the test control thymol with LC_{50} 7.455µg/mL. The result is mean+SD. N = 30 (Table 2).

Table 2: Average death of Artemia salina at different concentration of Hexane crude extract of *Barringtonia asiatica* Leaf.

Hexane Crude extract	**Hexane Crude extract**					**LC_{50}(µg/mL)**
Leaves	1	10	25	50	100	500
	2.70±0.58	3.70±0.58	4.70±0.58	5.00±1.00	7.30±0.58	208.091
(-ve control)	0	0	0	0	0	-
(+ve control	5±0.00	7±0.00	10±0.00	10±0.00	10±0.00	7.455

Discussion

The hexane extract of *Barringtonia asiatica* various concentration gave an impressive inhibition against the pathogen (*Salmonella typhi, E. coli, Staphylococcus aureus, Klebsielia Pneumonia*) with a diameter of inhibition within the range of 2.35 ± 0.07mm and 4.67 ± 0.12mm for 25pmm-1000ppm. However, the crude extract showed a greater antibacterial activity against *Salmonella typhi* with inhibition zone of 4.67 ± 0.12mm at concentrations of 1000ppm, when compared to positive standard of tetracycline as well as other concentration with the inhibition zone.

The inhibition of *Escherichia coli* (*E. coli*) by the crude extract at various concentration is within the diameter range of 2.60 ± 0.10 to 4.35 ± 0.07mm for 25-1000ppm. Most of the crude extract inhibition gave an increase in the inhibition with increase in concentration, this was followed by *Staphylococcus aureus* and Klebsielia Pneumonia as shown in the (Table 1). *Barringtonia asiatica* at 1000ppm is significant and active on all the pathogen, with aSignificantly (p< 0.05) higher compared to different concentration in each rows and bSignificantly (p< 0.05) higher compared to different extract at the same concentration in each column.

However, the result of the cytotoxicity showed that *Barringtonia asiatica* Leaf extract were toxic on brine shrimp larvae with LC_{50} value of 208.091 when compared with the control thymol at LC_{50} 7.455 thus having toxicity when referred to the fact that LC_{50} value of less than 1000µg/mL is toxic while LC_{50} value of greater than 1000µg/mL is non-toxic.

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Conclusion

The hexane extract of the leaves of *Barringtonia asiatica* indicated varied levels of antibacterial activities. The concentration of the plant extracts at various concentration level exhibited a high cytotoxicity activity on Brine shrimps. Thus, the plant is said to have a reasonable potential as antimicrobial compounds against microorganisms especially in the case of *Salmonella typhi, E. coli, Klebsielia Pneumonia* and lastly *Staphylococcus aureus* with increase in concentration. However, the plant extracts can be used as a novel drug against the development of resistance strains and in the treatment of infectious diseases caused by resistance bacteria.

Conflict of Interests

All authors have none to declare.

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