ANTIMYCOBATERIAL AND ANTIBACTERIAL ACTIVITIES OF EXTRACTS FROM *Caesalpinia bonduc* (L.) Roxb

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Abstract

Aim of study: The study focused on searching bioactives that can be used to combat pathogenic microbes from leaves, stem wood and stem bark of *Caesalpinia bonduc* Roxb.

Background: Many pathogenic microbes have developed resistance to most of the recommended first line and second line drugs. Antimicrobial resistances endanger the control of infectious diseases by increasing morbidity and mortality and impose massive costs on societies in both developed and developing countries. Medicinal plants used for treatment of infectious diseases are potential of bioactives to combat this problem. *Caesalpinia bonduc* (L.) Roxb is ethnomedically used by communities in Africa and India but little is known on the bioactive compounds therefrom. In order to know the bioactive on other parts of this plant it contains, it was necessary to undertake phytochemical investigation and antimicrobial analysis of extracts.

Material and Method: The plant materials of *Caesalpinia bonduc* were collected from Kisakasaka mangrove reserve, Zanzibar. Micro-organisms were collected from Department of Microbiology at MUHAS. Methanol, chloroform and *n*-hexane extracts from stem wood, stem bark and leaves of *Caesalpinia bonduc* were evaluated for antimicrobials. Antibacterial activity was evaluated against *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC29953) and *Pseudomonas aeruginosa* (ATCC25922). Antimycobacterial activity was performed against two non-pathogenic mycobacteria species namely *Mycobacteria indicus pranii* and *Mycobacteria madagascariense*. The two-fold serial dilution method was used to evaluate MIC for antimicrobial activities. Chromatographic techniques employed in sequential extractions.

Results: Extracts with high activity had MIC of 2.5 mg/mL while extracts with low activity had MIC of 5.0 mg/mL.

Conclusion: Antimicrobial activity exhibited by extracts indicated that, *Caesalpinia bonduc* is a potential for bioactive metabolites which can be used as leads for the development of antimicrobial agents.

Key words: *Caesalpinia bonduc*, antibacterial, antimycobacterial

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Introduction

Antibacterial resistance is one of a global health care problem in the 21st century for the fact that many bacteria have developed resistance to most of the recommended first line and second line drugs. For instance, *Mycobacterium tuberculosis* have developed resistance against first and second line anti-TB drugs [1], *Staphylococcus aureus* have developed resistance against most classes of antibiotics [2,3,4], *Escherichia coli* and *Vibrio cholera* have developed resistance to the common used antibiotics [5]. These antimicrobial resistances endanger the control of infectious diseases by increasing morbidity and mortality and impose massive costs on societies in both developed and developing countries [6,7,8]. If the current trend continues, in future there may not be effective antimicrobials to treat patients with serious infections. Consequently, there is an urgent need to search for new drugs with unique mechanism.

Medicinal plants are promising good natural source for searching new antimicrobial agents because more than 25% of the currently in use chemotherapeutic agents have been derived from them [9,10]. For instance, *Caesalpinia bonduc* (L.) Roxb is a coastal, higher plant that is used traditionally for treatment of various diseases worldwide. Among those diseases includes arthritis, fever, cough, worms, malaria, diabetes, dyspepsia, jaundice, dysmenorrhea and amenorrhea [11]. This indicates presence of bioactives in the *Caesalpinia bonduc.* In order to validate its ethnomedical use, it was necessary to undertake antimicrobial analysis of extracts

from *Caesalpinia bonduc*. Thus, this study was focused on the antimicrobial analysis of extracts from *Caesalpinia bonduc.*

Material and Methods

Collection of Plant Materials and Microorganisms

The plant materials were collected from the Kisakasaka Mangrove Reserves. Plant species was identified by in the field by Mwadini Mtumwa and confirmed at the Institute of Marine Sciences, University of Dar es Salaam where a voucher specimen (SH-CS2365) is preserved. The *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC29953), *Pseudomonas aeruginosa* (ATCC25922), *Mycobacterium madagascariense* (*MM*) and *Mycobacterium indicus pranii* (*MIP*) were collected from department of microbiology and immunology at Mhimbili University of Health and Allied Sciences.

Extraction of Plant Materials

The plant materials were air-dried and pulverized into fine powder. The air-dried pulverized plant materials were soaked in dichloromethane-methanol (4:1) followed by Ethyl acetate-methanol (4:1) for 48x2 hours and then filtered. The filtrate of crude extracts were concentrated *in vacuo* using a rotary evaporator and combined. The combined extracts were stored in a refrigerator under 4^{o}C for further total fractionation and bioassay. Total fractionation was performed to obtain fraction of extracts for bioassay. The extracts of stem bark, stem wood and leaves were adsorbed in silica gel, then after were packed in a Column and elution was performed by gradual change of solvents, started with hexane followed by chloroform and finally methanol. Therefore, six extract fractions (20 mg of each extract) of the *Caesalpinia bonduc* were obtained for antimicrobial test.

Antimycobacterial Assay

The antimycobacterial assay to search for anti-TB was conducted for the crude extracts against two selected non-pathogenic mycobacteria species so as to determine their efficacy. The two-

fold serial dilution method was done to examine the Minimal Inhibitory Concentration (MIC). The experiment was done at the Institute of Traditional Medicines, MUHAS. The mycobacteria species were sub-cultured in middle-brook 7H9 broth base that was supplemented with glycerol (AR). The culturing medium of 1.18 g of middlebrook 7H9 broth base suspended in 230 mL of distilled water in scotch bottle of 500 mL followed by addition of glycerol was prepared. The mixture was heated to dissolve the broth completely, thereafter autoclaved at 121^0C for 15 minutes. Then, the mixture was left to cool and followed by inoculation. Finally, the inoculums were incubated at 31^0C and 35^0C for *MM* and *MIP* respectively and were cultured for five days for optimal growth before biological assay.

In order to determine Minimal Inhibitory Concentration (MIC), the two-fold serial dilution method was performed in the sterilized 96 wells of polystyrene microtiter plates as described by Ellof, (1998) [12]. Twenty milligrams of each crude extract fractions were used to prepare stock solutions (20 mg/mL) by dissolving 20mg of crude extracts in 1mL of 10% DMSO and 90% sterile middle brook 7H9 broth in eppendorf tube. The bacterial inoculums obtained from grown cultures into middlebrook 7H9 broth base containing 0.1% tween 80, the turbidity was adjusted to standard solution which is equivalent to the McFarland unit (Approximately $1.2x10^8$CFU/mL). The inoculums were therefore ready for bioassay. Fifty microlitres (50 µL) containing 0.1% tween 80 were added to each microtitre plate well first row followed by 50 µL of the extracts, this halved stock concentration from 20 mg/mL to 10 mg/mL. From the mixture, 50 µL were withdrawn from each well of the first wells to the next wells to half the concentration. A series of concentration (10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625 mg/mL) were prepared through serial dilution. Thenafter, 50 µL of the prepared mycobacteria cultures were added to 50 µL in each well thus completing the two fold dilution by halving concentrations. Some wells having inoculums were left without drug and were used as growth control (negative control), to those wells with inoculums and a drug (ciprofloxacin) were used as positive control. The inoculated microtitre plates were then incubated for 24 hours. After incubation, 30 µL of Iodonitrotetrazolium (INT) chloride salt was added into each well and plates were incubated for one hour ready for the results. The indicator (INT) was used to indicate either pink colour for the presence of microbes or clear for the growth inhibition. MIC values of extracts were read at the lowest concentration where a marked reduction in colour formation was noted and recorded.

Antibacterial Assay

The antibacterial assay was conducted for all crude extracts to determine their efficacy against three bacteria strains; *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC29953), and *Pseudomonas aeruginosa* (ATCC25922). The two-fold serial dilution method was employed to determine the Minimal Inhibitory Concentration. The experiment was done at the ITM (MUHAS). The collected bacteria species were sub-cultured under tryptone agar nutrient which support growth of bacteria at pH 7.56. The tryptone agar nutrient (8.0 g) was suspended in 230 mL of distilled water in 500 mL scotch bottle and the mixture was heated to dissolve the agar completely, then after mixture was autoclaved at 121^0C for 15 minutes. The mixture was left to cool then followed by inoculation, and finally, the inoculums were incubated at 37^0C for 24 hour. MIC determination procedures were similar as in antimycobacterial assay above. The slightly difference for this section is that, Tryptone broth was used over normal bacteria instead of the middle-brook broth base containing 0.1% tween which was used on *Mycobacteria species*. Gentamycin was used as drug for positive control instead of ciprofloxacin which was used to *Mycobacteria species.*

Results and Discussion

The bioassay results of extracts from *C. bonduc* showed that, fractions with high activities had Minimal Inhibitory Concentration (MIC) of 2.5 mg/mL while those with low activities had MIC of 5.0 mg/mL (Table 1). Hexane extract fractions were not active against all assayed microbes up to maximum concentration of 5.0 mg/mL. This embraced that, the species assayed were not susceptible to less polar compounds found in the hexane fractions.

In addition, the chloroform extract from leaves showed the inhibition activity against *E. coli* and *P. aeruginosa* at MIC of 5.0 mg/mL and 2.5 mg/mL respectively. This is an indicative for the assayed bacteria being susceptible to potent bioactive metabolites found in chloroform extract fractions. The methanolic extracts fractions of leaves were the most active against the *S. aureus* and *E. coli* because exhibited the inhibition activity at MIC of 2.5 mg/mL. On the other hand, leaves were evaluated as most active part of the plant for exhibiting the inhibition activity against

all assayed microbes species by methanolic. High susceptibility of assayed bacteria toward methanolic extracts indicated the presence of polar compounds that are bioactive metabolites against bacteria like flavonoids, tannins and saponins [13,14].

The *Staphylococcus aureus*, a gram positive bacterium was only susceptible to methanolic extract fractions of leaves at MIC of 2.5 mg/mL while the gram negative bacteria, *P. aeruginosa* and *E. coli*, were susceptible to four extract fractions. Gram negative bacteria were susceptible to chloroform and methanolic extract fractions from stem wood at MIC of 5.0 mg/mL. The *P. aeruginosa* and *E. coli* were susceptible to leaves chloroform extract fractions at MIC of 2.5 mg/mL and 5.0 mg/mL respectively. The *P. aeruginosa* and *E. coli* were susceptible to leaves methanolic extract fractions at MIC of 5.0 mg/mL and 2.5 mg/mL respectively. In evaluation for antibacterial activities, the study revealed that *E. coli* and *P. aeruginosa* were more susceptible than *Staphylococcus aureus*. This circumstance of gram negative bacteria being more susceptible than gram positive bacteria is an unusual phenomenon since it was expected the greater number of extracts to be active against gram positive than gram negative [15]. In addition, majority of the conventional antibiotics are generally more active against the gram positive bacteria than gram negative bacteria because of capsule resistance that gram negative bacteria they have [16]. Thus, this signifies the presence of bioactive metabolites in extract fractions of *C. bonduc* having unique mechanism against pathogenic gram negative bacteria.

On the other hand, the chloroform extract fractions of stem wood were exhibited inhibition activity at MIC of 2.5 mg/mL against *Mycobacterium indicus pranii* (*MIP).* Methanolic extract fractions of leaves exhibited high inhibition activity against *M. madagascarience* at MIC of 2.5 mg/mL but exhibited least inhibition activity against *MIP* at MIC of 5.0 mg/mL. The susceptibility of the assayed mycobacteria species was an indicative for the presence of potential bioactive metabolites in the chloroform extract fractions of stem wood and leaves. The extract fractions that showed antimycobacterial activities revealed the presence of potential bioactive metabolites such as terpenoids, alkaloids, coumarins and phenolics [17, 18].

Extracts from medicinal plants have been used by humans for a wide range to resolve human health problems including treatment of infectious diseases [19]. Accordingly, this emphasizes extensive biological assay investigations on medicinal plants for searching antibiotics to combat the emerging and overgrowing drug resistant strains.

Table 1: Antimycobacterial (Anti-TB) and antibacterial activities of stem wood and leaves extracts from *Caesalpinia bonduc*

Extract Fractions	Minimum Inhibitory Concentration, MIC (mg/mL) for two Mycobacteria & three Pathogenic Bacteria species				
	Mycobacterium madagascariense	*Mycobacterium indicus pranii*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Stem wood, hexane extract	NA	NA	NA	NA	NA
Stem wood, chloroform extract	NA	2.5	NA	5.0	5.0
Stem wood, methanolic extract	NA	NA	NA	5.0	5.0
Leaves, hexane extract	NA	NA	NA	NA	NA
Leaves, chloroform extract	NA	NA	NA	5.0	2.5
Leaves, methanolic extract	2.5	5.0	2.5	2.5	5.0

Key: NA = No activity (not active)

Conclusion

Bioassay results attained from the plant extracts for the antibacterial and antimycobacterial activities showed strong correlation with medicinal use of the *C. bonduc* for healing bacterial infections diseases traditionally. The antimicrobial activities of the plant extracts fractions embraced the presence of potential bioactive metabolites for the development of antibiotics and anti-TB. Accordingly, the antimicrobial activities exhibited by extracts revealed that, the *Caesalpinia bonduc* is a potentially good source of bioactive metabolites for development of broad spectrum antibiotics. Conversely, the plant inspired for further extensive isolation of bioactives and investigations for antimicrobials, pharmacological and toxicology evaluations.

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Authors' contributions

All authors worked together to achieve this work. All authors have cordially supported the work and preparation of the manuscript. Author SHM designed and supervised the study and prepared the first draft of the manuscript. Authors MC and RP advised and guided the final draft of the manuscript. All authors read and approved the final manuscript.

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