ANTIMICROBIAL ACTIVITY OF PITSECELLOBIUM DULCE (ROXB) BENTH. ROOT EXTRACT

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Abstract- The present study was to evaluate the antimicrobial activity of Pithecellobium dulce root against five microorganisms by using Agar well diffusion and broth microdilution methods. The roots of P. dulce were continuously macerated with ethanol and water respectively. The results showed that the ethanolic extract active against Staphylococcus aureus and Bacillus subtilis that showed the largest inhibition zone of 11.67±0.58 mm against Bacillus subtilis, the lowest MIC of 250 µg/ml against Staphylococcus aureus and the lowest MBC of 500 µg/ml against Bacillus subtilis. The water extract did not showed the activity against Escherichia coli and Saccharomyces cerevisiae but showed the largest inhibition zone of 8.00±0.00 mm and the lowest MIC of 62.5 µg/ml against Staphylococcus aureus, the lowest MBC and MFC of >2000 µg/ml against Staphylococcus aureus, Bacillus subtilis and Candida albicans. The results of the present study refer that P. dulce extracts possess bioactive compounds having important antimicrobial properties. Therefore, the plant extracts can be subjected to further isolation of therapeutic antimicrobial agents for pharmacological and toxicological evaluation.

Keywords- Pitsecellobium dulce, Antibacterial activity, Antifungal activity

I. INTRODUCTION

Pithecellobium dulce (Roxb) Benth. is an evergreen tree belong to the family of Leguminosae (subfamily Mimosoideae). It is a native to tropical Asia, America and cultivated throughout India [1]. P. dulce has been used as a folk remedy to treat leprosy, peptic ulcer, dysentery, and toothache [2-4]. The leaves of P. dulce contain steroids, saponins, lipids, phospholipids, glycosides, glycolipids, quericitin, kaempferol, dulcitol and afezilin. Catechol was found in the bark whereas polysaccharide founded in the seeds [5, 6]. The leaves of P. dulce revealed the antifungal, antibacterial, anti-inflammatory, antimicrobial and free radical scavenging activity [7-10]. The seeds extract showed fungistatic and fungicidal effects against plant pathogens [7]. The flower extract exhibited the anti-inflammation properties [11]. Moreover, the root extract have been reported to possess estrogenic activity [12]. Therefore, this study was aimed to evaluate the antimicrobial activities of the different solvent extracts from root of this plant.

II. DETAILS EXPERIMENTAL

Plant material

The roots of P. dulce were collected from Suan Sunandha Rajabhat University, Samut Songkhram Education Center. The plant samples were identified by specialist and compared with the herbarium specimen at Department of Applied Thai Traditional Medicine, College of Allied Health Sciences, Suan Sunandha Rajabhat University, Thailand.

Plant extraction

The roots were shade-dried and ground to coarse powder. The powdered roots were continuously macerated with ethanol and water respectively until exhaustion. The ethanol extract was filtered and evaporated under vacuum, whereas the water extract was lyophilized to dryness. The extract yield was weighed, recorded and dissolved in Dimethyl sulfoxide (DMSO) to obtain a concentration of 200 mg/ml. The crude extracts were then stored at -20 C and further antimicrobial testing.

Microorganisms

Bacillus subtilis ATCC6633, Staphylococcus aureus ATCC6538P, Escherichia coli ATCC25922, Candida albicans ATCC10230 and Saccharomyces cerevisiae ATCC9763 were obtained from Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Preparation of culture media

The bacterial strains were prepared in Mueller Hinton agar (MHA) and Mueller Hinton broth (MHB) for antibacterial testing whereas the fungal strains were prepared in Sabouraud Dextrose agar (SDA) and Sabouraud Dextrose broth (SDB) for antifungal testing.

Preparation of inoculum suspensions

Both bacterial and fungal strains were inoculated on MHA and SDA respectively. They were inoculated at 37° C, for 18-24 hrs for bacteria and 24-48 hrs for fungi. Four to five

of isolated colonies from the overnight culture were suspended in 0.85% of normal saline. The turbidity of the suspension was measured by using the spectrophotometer at 625 nm to obtain the absorbance of 0.08-0.10 which comparable to 0.5 Mc Farland standards (approximately 1 x 10^8 CFU/ml) [13-15].

Determination of zone of inhibition

Agar well diffusion method was used to test antibacterial and antifungal activities. Microorganism standard strains were grown and the inoculums were adjusted the turbidity to 0.5 McFarland standards. Added 100 µl of the suspension was seeded on MHA plates for bacteria and SDA for fungal. Agar wells were cut from seeded agar plates by a cork borer (6 mm.) [16-23]. Twenty microliters of plant extracts of 200 mg/ml and 2 mg/ml for positive controls were transferred into each well with diameter of 6 mm. The plates were incubated at 37° C for 18 to 24 hrs and 24 to 48 hrs for bacterial and fungal strains respectively. The antimicrobial activity was evaluated by measuring the diameters of zone inhibition surrounding the crude extracts. The zones of inhibition were measured in millimeter and the experiment was carried out in triplicates.

Determination of MIC, MBC and MFC

Minimum inhibitory concentration was determined by microdilution method in 96 well microtiter plate. A microbial suspension in broth was prepared by adding 10 µl of normal saline microbial suspensions to 1 ml of MHB or SDB. Into a sterile 96-well microplate, 50 µl of microbial suspended in broth was added to the wells containing 50 µl of plant extract (final concentrations: 3.9-2000 µg /ml with two-fold dilution), positive controls: ampicillin, amikacin and clotrimazole (final concentrations: 0.039-20 µg/ml with two-fold dilution) and negative control (DMSO). All of chemicals was prepared by diluting with broth to obtain final volume of 1 ml and incubated at 37° C, for 18 to 24 hrs for bacteria and 24 to 48 hrs for fungi. The lowest concentration of plant extract inhibiting the growth of the tested microorganisms detected by the lack of visual turbidity compared to the negative control was defined as the MIC for the extract. The samples of the known MIC wells were streaked onto agar plates and incubated at 37 C, for 18-24 hrs for bacteria and 24-48 hrs for fungi. The least concentration with no microbial growth observed on the plate was considered as Minimum bactericidal concentration (MBC) and minimum fungicidal concentrations (MFC) value [24].

III. RESULTS AND DISCUSSION

The antimicrobial activity were investigated by the agar well diffusion method against five microbial strains. The results showed that the ethanolic extract of P. dulce showed the largest inhibition zone of 11.67±0.58 mm against Bacillus, the lowest MIC of 250 µg/ml against Staphylococcus aureus and the lowest MBC of 500 µg/ml against Bacillus subtilis. Whereas, the water extract showed the largest inhibition zone of 8.00±0.00 mm and the lowest MIC of 62.5 µg/ml against Staphylococcus aureus and showed the MBC and MFC of >2000 µg/ml against Staphylococcus aureus, Bacillus subtilis and Candida albicans. The results were shown in Table 1 and Figure 1. The results of this study could be conclude that the water extract showed the potential activities more than the ethanolic extract. This may be due to the better solubility of the active components in organic solvents [25]. However, both extracts did not show the activities against gram-negative bacteria tested. There may be several determinants that will predispose bacteria to agents such as previous encounters with the agents or the nature of medium used, which may affect the diffusability of the agent. Nevertheless, the activities of the extracts were not comparative to those of antibiotics.

IV. CONCLUSIONS

Although this study investigating the antimicrobial activity, the results showed that the extracts from root of P. dulce possessed good antimicrobial activity, approve the great potential of bioactive compounds and are useful for rationalizing the use of this plant in primary health care. The results of this study may be helpful in pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antimicrobial from this plant are the future test.

Table 1 Zone of inhibition (mm), MIC and MBC or MFC (µg/ml) of root extracts from P. dulce, positive and negative controls

Tested microorganisms	Sample	Zone of Inhibition	MIC	MBC/MFC
Staphylococcus aureus	Ethanolic extract	9.67±0.58	250	>2000
	Water extract	8.00±0.00	62.5	>2000
	Ampicillin	46.00±1.01	0.039	1.25
	Amikacin	11.33±0.59	2.5	10
	Clotrimazole	20.67±0.59	2.5	>20
	DMSO	NA	NA	NA
Bacillus subtilis	Ethanolic extract	11.67±0.58	500	500
	Water extract	7.00±0.00	>2000	>2000
	Ampicillin	16.33±0.58	>20	>20
	Amikacin	14.33±0.58	1.25	2.5
	Clotrimazole	22.67±0.58	2.5	10
	DMSO	NA	NA	NA

Escherichia coli	Ethanolic extract	NA	NA	NA
	Water extract	NA	NA	NA
	Ampicillin	23.67±0.58	2.5	10
	Amikacin	15.00±0.00	2.5	5
	Clotrimazole	NA	NA	NA
	DMSO	NA	NA	NA
Candida albicans	Ethanolic extract	NA	NA	NA
	Water extract	7.00±0.00	>2000	>2000
	Ampicillin	NA	NA	NA
	Amikacin	NA	NA	NA
	Clotrimazole	32.00±1.00	0.039	0.312
	DMSO	NA	NA	NA
Saccharomyces cerevisiae	Ethanolic extract	NA	NA	NA
	Water extract	NA	NA	NA
	Ampicillin	NA	NA	NA
	Amikacin	NA	NA	NA
	Clotrimazole	34.67±1.15	0.625	1.25
	DMSO	NA	NA	NA

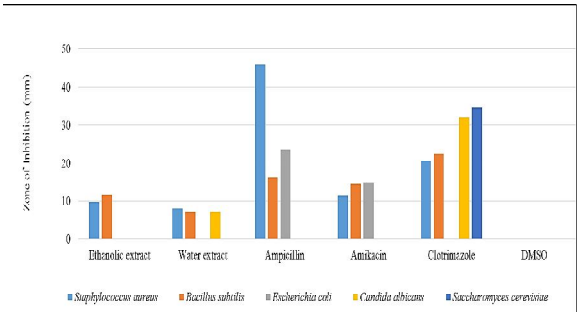

Figure 1 Spectrum of antimicrobial activity of extract, positive and negative controls

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