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In vitro study of antibacterial activity of *Entada spiralis Ridl.* crude extract on selected skin infection-causing bacteria

Raihanah Hasaniah Abdullah[a], Risyawati Mohamed Ismail[b]

[a,b]School of Technology Management and Logistics, Universiti Utara Malaysia, 06010 UUM Sintok, Kedah

corresponding author: *risyawati@uum.edu.my*

Abstract

In Malaysia, *Entada spiralis Ridl*. from Leguminoceae family grows wildly and the scientific study of it has not been explored deeply. Entada spiralis Ridl. is a liana or woody climber plant which is locally known as "akarbeluru" or "Sintok". This study is performed to evaluate the antibacterial activity of Sintok extract from the stem bark of Entada spiralis against skin bacteria infections by the disc diffusion method. Two types of skin bacteria known as Staphylococcus aureus and Streptococcus pyogenes were selected and tested against the water extracted Sintok. Sintok concentration of 1600mg/ml gave the highest inhibition zone diameter against Streptococcus pyogene rather than Staphylococcus aureus with a diameter of 25.5 mm and 18. mm, respectively. This study indicates clear evidence supporting the traditional use of Entada spiralis in treating skin infections related to bacteria.

***Keywords*:** Entada spiralis, Sintok, antibacterial, bacteria, skin disease

1.0 Introduction

In prehistoric times, old folk used medicinal plants as a direct therapeutic agent because of the cheap cost and it is easy to obtain it (Bouyahya, Abrini, Et-Touys, Bakri, &Dakka, 2017; Ripen &Noweg, 2016). This includes plants used to treat skin-related afflictions. In 2013, the fourth most leading cause of disability worldwide was skin disease and it remains a major cause of disability worldwide. According to the Institute for Health Metrics and Evaluation, skin diseases in Malaysia were recorded as the top two health problems in 2016. However, the increasing number of antibiotics resistant strains such as Methicillin-resistant Staphylococcus aureus (MRSA) are causing scientists to look for alternative solutions for skin problems (Kuete et al., 2009). One of the plants that fit such needs is namely Entada spiralis, also known as Sintok or Beluru. Sintok is a tree climber and was traditionally used as soaps and shampoo by our ancestors. A previous study showed that Sintok showed antifungal activity but no study was yet carried out to investigate its antibacterial activity (Harun, So'ad, Hassan, & Ramli, 2014).

Apart from that, the previous researcher used different solvents such as petroleum ether, ethyl acetate, methanol, and chloroform when extracting Sintok. However, due to the sensitivity of the skin (of sufferers), we explore a different

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method of Sintok extraction i.e. water which is considered as a green solvent and does not cause environmental pollution because of the sensitive and fragile skin condition suffered by the patients (Castro-puyana, Marina, & Plaza, 2017).

2.0 Materials and Methods

Plant materials and extraction

Source of stem bark of E. spiralis were collected from the forest in Perlis. About 2kg of crushed dry stem bark was cut into pieces for about 1 to 1.5inch long. The crushed stem bark was then soaked in distilled water with a ratio of 1:5. Then, Sintok was extracted using a double boil method. The Sintok extracts were extracted for an hour at a temperature of 200°C. The crude extracts were then filtered and transferred into Schott bottles and kept in a refrigerator until used.

Microorganisms

The microorganisms used in this study were bacteria (Staphylococcus aureus ATCC 33591 and Streptococcus pyogenes ATCC 19615). The stock cultures for bacterial strains were maintained on Tryptic Soy Agar (TSA) medium for 24 hours at room temperature in the dark.

Preparation of discs from crude extracts

Stock solution of 1600 mg/ml of crude extract was prepared by dissolving 1.6g of extracts in 1 ml of water. Then, make stock solution into serial two-fold dilution to get 1600 mg/ml, 800 mg/ml, 400mg/ml, 200 mg/ml, and 100 mg/ml, respectively. 20 µL of each dilution was impregnated onto a sterile paper disk using a micropipette and allowed the solvent to dry at room temperature. All discs were stored at -5°C before use.

Chemicals for antibacterial activity

Amoxycillin, Cephalexin, Clindamycin, Cefoxitin, and Penicillin (Oxoid, England) were used as antibacterial reference against bacteria used. These antibiotic discs were used as positive controls, while empty discs as negative control. Tryptic Soy Agar (TSA) was purchased from Merck, Germany.

Bacterial suspension

The bacteria were subcultured in TSA and incubated in an incubator for 24 hours. The bacterial suspension was standardized to 1.0 x 101 cells/ml. It was used as an inoculum for antibacterial susceptibility testing.

Minimum inhibition concentration (MIC)

The disc diffusion method was followed for the antibacterial susceptibility test to determine the minimum inhibition concentration. Test plates were prepared by pouring 15 ml sterile molten of TSA for bacteria into Petri dishes and allowed to solidify. Plates were dried and 0.1 ml of standardized inoculum suspension was poured and uniformly spread. The excess inoculum was drained and the inoculum was allowed to dry for 5 min.

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The discs were then applied and the plates were incubated at 37°C for 24 h (bacteria). The inhibition zone was measured from the edge of the disc to the inner margin of the surrounding pathogens. Amoxycillin, Cephalexin, Clindamycin, Cefoxitin, and Penicillin were used as standard reference. The empty discs were used as a negative control. Each assay in this experiment was performed in triplicate.

3.0 Results

The result for the antibacterial activity test of different Sintok extract concentration by using disc diffusion agar method are demonstrated in Table 1 and Table.2. The growth of Staphylococcus aureus and Streptococcus pyogenes were inhibited by Sintok extract in a concentration-dependent manner. However, both bacteria were not inhibited at concentrations of 100mg/ml. Statistically, the inhibition zone for Sintok concentration from 400 mg/ml to 1600 mg/ml in Table 2 were bigger compared to Table 1. Sintok extract with a concentration of 1600mg/ml was found to be the most effective concentration.

Nevertheless, it gives the highest inhibition zone diameter against Streptococcus pyogene rather than Staphylococcus aureus with a diameter of 25.5 mm and 18. mm, respectively. Sintok concentration at 200mg.ml displayed the lowest activity against Staphylococcus aureus and Streptococcus pyogene. Therefore, this condition is called as the minimum inhibition concentration (MIC).

The negative controls did not affect the growth of the bacteria. Four types of antibiotics were used in this study as positive controls or standard reference. They are Amoxycillin, Cephalexin, Cefoxitin, and Penicillin. Each of the antibiotics has a different dose per each disc. The antibiotics with the highest dose are Cephalexin and Cefoxitin which gives the most elevated inhibition zone compared to Amoxycillin and Penicillin. This showed that both bacteria were sensitive and susceptible to all of the positive controls used.

Table 1: *In vitro antibacterial activity of different Sintok concentration extract and antibiotics against Staphylococcus aureus bacteria*

Sintok Concentration (mg/ml)	**Inhibition Zone (mm)**					
	Empty Disc (Control)	**Sintok**	**AML (25µL)**	**CL (30µL)**	**FOX (30µL)**	**P (10µL)**
1600	-	18.0 ± 2.82	9.0 ± 0	23.5 ± 0.71	21.5 ± 0.71	7.5 ± 0.71
800	-	12.0 ± 0	8.5 ± 0.71	19.5 ± 2.12	17.0 ± 5.66	7.0 ± 0
400	-	9.0 ± 0	9.0 ± 1.41	22.5 ± 0.71	16.0 ± 5.66	8.0 ± 0
200	-	7.0 ± 0	8.0 ± 1.41	24.0 ± 0	19.0 ± 0	7.5 ± 0.71
100	-	-	8.0 ± 0	21.5 ± 0.71	20.0 ± 0	7.5 ± 0.71

-, No activity; AML, Amoxycillin; CL, Cephalexin; FOX, Cefoxitin; P, Penicillin, control antibacterial drug

Table 2: *In vitro antibacterial activity of different Sintok concentration extract and antibiotics against Streptococcus pyogenes bacteria*

Sintok Concentration (mg/ml)	**Inhibition Zone (mm)**					
	Empty Disc (Control)	**Sintok**	**AML (25µL)**	**CL (30µL)**	**FOX (30µL)**	**P (10µL)**
1600	-	25.5 ± 0.71	11.5 ± 0.71	24.5 ± 0.71	18.5 ± 0.71	10.5 ± 0.71
800	-	15.5 ± 2.12	15.0 ± 0	29.5 ± 0.71	22.5 ± 2.12	15.5 ± 2.12

400	-	10.5 ± 0.71	17.5 ± 0.71	26.0 ± 2.83	21.5 ± 2.12	15.5 ± 2.12
200	-	6.5 ± 0.71	22.0 ± 0	27.5 ± 0.71	26.0 ± 0	14.0 ± 1.41
100	-	-	21.5 ± 0.71	31.0 ± 1.41	26.5 ± 2.12	20.0 ± 0

-, No activity; AML, Amoxycillin; CL, Cephalexin; FOX, Cefoxitin; P, Penicillin, control antibacterial drug

4.0 Discussion

The Sintok extract showed significant MIC value against both selected skin infection-causing bacteria. In Malaysia, Sintok was traditionally used as a shampoo for scalp treatments, as soap for washing agents and body soap for the post-natal bath. This positive results scientifically proved that traditionally used Sintok extract has an antibacterial property. Furthermore, in a study by Lalitha, Rajeshwaran, Kumar, Deepa and Gowthami (2010), the extract of Acacia mellifera from Leguminosae family showed an antibacterial activity against Staphylococcus aureus. This observation was strongly supported by Chew *et al*. (2011) and Elechi andIgboh (2017) with different plant extract from the same family.

In order to, examine the effectiveness of the extracts to inhibit the growth of the tested bacteria, MIC assay was employed. A higher concentration of Sintok extract gave larger inhibition zones. This could be due to the presence of bioactive phytocomponents found in the Sintok extract. The reason both of the bacteria were sensitive towards the extract was most probably because of the interference of the active compounds of the extracts, which caused the cell membrane to leaked and results in cell lysis. Finally, caused the cell death of the bacteria. As eloquently stated by Gonelimali *et al.* (2018), the effect of plant extracts on cytoplasmic pH and membrane of bacteria cell caused the changes of cell pH and results in the damage to the bacterial cell membrane.

Moreover, the sensitivity of bacteria towards Sintok extract was also due to the synergistic effect. Probably, the main reasons for this are sequential inhibition of a common biochemical pathway and disintegration of the outer membrane. Different groups of bioactive chemical agents contained in the extracts also plays an important role in cell death. Saponin is an active compound in Sintok extract can form complex with extracellular proteins, soluble proteins, and bacterial cell membrane (Mummed, Abraha, Assefa, Feyera&Nigusse, 2018).

This study indicates that extracts from Entada spiralis possess an antibacterial activity against skin disease bacteria. However, further studies with different solvents use to extract Sintok are required in order tocompare higher concentration of active antibacterial compounds presence.

5.0 Conclusion

The water extracted Sintok inhibited the growth of skin disease bacteria which are *Staphylococcus aureus* ATCC 33591 and *Streptococcus pyogenes* ATCC 19615. Therefore, the water extracted Sintok has a potency as an antimicrobial for the skin disease.

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