THE ANTIBACTERIAL ACTIVITY STUDY OF SENNA ALATA LEAF EXTRACT AND FRACTION TOWARDS MRSA (METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS) AND ITS MODE OF ACTION

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ABSTRACT

Introduction: The increasing prevalence of healthcare-associated MRSA infections need new approach to overcome of growing problems. Medicinal plants are promising as the most valuable resources for antibiotic development. Senna alata had shown antibacterial effect in Str. pyogenes and S. aureus. Objective: to evaluate the antibacterial activity of Senna alata extract and fraction against MRSA. Method: The antibacterial activity calculated based on Minimum Inhibitory Concentration (MIC) using Mueller Hinton broth in microdilution method and the mode of action was conducted using Scanning Electron Microscopy (SEM). Result: Phytochemical screening of dried Senna alata leaf and its extract showed the presence of flavonoid, alcaloid, saponin, quinone, tannin, and sterol. The antibacterial activity showed the MIC value of the extract against MRSA was 512 µg/mL. The ethyl acetate fraction showed the best MIC value at 256 µg/mL. The SEM observation of MRSA treated by ethyl acetate fraction of Senna alata showed membrane shrinkage. Conclusion: Senna alata was promising to be developed as antibacterial agent especially MRSA strain, but the further in vivo research and discover the mode of its action still needed to shed light the effect.

KEYWORDS: Senna alata, antibacterial activity, MRSA, MIC, SEM.
INTRODUCTION

MRSA is methicillin-resistant *Staphylococcus aureus*, a type of staph bacteria that is resistant to commonly used antibiotics such as e.g., macrolides, tetracyclines, aminoglycosides, vancomycin. MRSA showed highly morbidity and mortality in many geographic areas both in the hospital and in the community.[1] Increasing prevalence of healthcare-associated MRSA infections is usually associated with a wide dissemination of particular epidemic clonal lineages of the *S. aureus* population.[2] The WHO has acknowledged the need to identify new antibiotics and/or new approaches to overcome the growing problems associated with such infectious agents.

Due to the emerging of MRSA resistant, there is a pressing need to develop new and innovative antimicrobial agents. Medical plants has potential source for treatment of infection beside has low toxicity and acessable. Ketepeng cina (*Senna alata*) is commonly known as Candlebrush, is a tropical shrub having yellow flowers and large leaves whose juice is used as a cure for ringworm and poisonous bites. *Senna alata* originally from Argentina which grows well in forested areas such as in Indonesia. The pharmacological activity show that *Senna alata* potential to be laxative, antifungal, analgesic activity, antihyperglycemic activity, hepatoprotector activity, anti-inflammatory activity, choleretic effect and antimicrobial activity.[3][4][5][6][7][8] On the previous study, ketepeng cina (*Senna alata*) had shown the activity as antimicrobial towards gram positive bacteria and negative gram bacteria. In our continuous research, we designed to determine the antibacterial activity of *Senna alata* extract and fraction towards MRSA and conducted the mode of its action using Scanning Electron Microscopy (SEM).

MATERIALS AND METHOD

Plants

The fresh *Senna alata* leaves were collected from Manoko field in Bandung at winter. The collected plants were identified and classified according to the herbarium Bandungense at the School of Technology and Life Science research centre. The fresh *Senna alata* then dried under sunlight exposure in two days. The dried leaves were milled into fine powder using a milling machine.

Materials

96 microwell plate, autoclave, shaker, *Laminar Air Flow* (LAF), eppendorf, micropipette, separation funnel, glass set, chromatography set, MRSA collected from patients in hospital,

**Preparations of extract and fraction**

Dried *Senna alata* allowing the extraction process by reflux method using ethanol 96%. The solvent from obtained extract was completely removed by using rotary evaporator to obtain semi-solid mass and the randement will be calculated based on weighen dried *Senna alata*. A portion of resulting crude extract wasfractioned by separation funnel using solvent n hexane, ethyl acetate and water. Eluates were collected in 1-L Erlenmeyer flasks and each fraction was subjected to evaporation under reduced pressure in a rotary evaporator. Fractions were stored at 4°C until assayed.

**Phytochemical screening**

The plant materials were screened for the presence of different classes of secondary metabolites including alkaloids, flavonoids, alcaloid, saponins, tannins, quinones, sterols/triterpenes using previously described method.

**MRSA preparation**

The MRSA were taken from isolated specimen in hospitalized patients. The MRSA were over night cultured (18-24 h) at 37°C on nutrient broth for the preparation of cell suspensions. MRSA cell suspension was homogenized and adjusted to 0.5 McFarland standards (5x10⁵ CFU/mL) using spectrophotometry.

**Antimicrobial Susceptibility Assays**

The minimum inhibitory concentration (MIC) of *Senna alata* was initially determined using *Mueller-Hinton Broth Microdilution*.[⁹] MIC determination was performed by a serial dilution technique using 96-well microtiter plates. The 100 µL extract were put into the column and row number 12 of the well. Then 100 µL MRSA suspension were put into each well/plates. Microplates were incubated for 24 h at 37°C. The lowest concentrations without visible growth were defined as concentrations which completely inhibited bacterial (MICs). DMSO was used as a control, while tetracycline and vancomycine were used as a positive control, MHB as negative control. The assay was repeated twice with three replicate per assay.
Scanning Electron Microscopy

The preparation of Scanning Electron Microscopy was performed to make MRSA inoculum in liquid medium of Mueller Hinton Broth then added with the standard antibiotics (tetracycline hydrochloride and vancomycin hydrochloride) and the ethyl acetat fraction of Senna alata. The inoculum was incubated for 24 h at 37 ° C. MRSA were harvested by centrifugation using glutaraldehyde 2% v / v in buffer Na-cocodilate and Na-cocodilate buffer solution and washed once in phosphate-buffered saline, pH 7.4 and added to the end of the fixation solution (OsO4 solution of 1% v / v in phosphate buffer pH 7.4). The next process is dehydrated by adding ethanol-rise starts from 10 to 100% ethanol (in this process begins with ethanol 50%, 70%, 80%, 95%, up to 100% v / v). Cells that have been dehydrated and then suspended in t-butanol. This suspension was dropped into the small space in the cover slip. The cover slips coated with metals freeze drying and gold (Au) and observed in the SEM. The target of antibacterial fraction action can be determined by observing changes in the bacterial cell morphology compared with normal bacterial cells.

RESULT

The discovery for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones.

Phytochemical Screening

Dried and freshly prepared extracts were subjected to a preliminary phytochemical screening for various constituents. The results (Table 1) revealed the presence of flavonoid, alcaloid, saponin, quinones, tannin, sterol/treterpenoid in both dried and extract. These bioactive compounds have been reported to be used by plants for protection against bacterial, fungal and pesticidal infections and are responsible for antimicrobial activity.\textsuperscript{[10]}

Table 1. Results of Phytochemical Analysis of dried \textit{Senna Alata} and its Extracts.

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>\textit{Senna alata}</th>
<th>Dried</th>
<th>Freshly Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Quinones</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Alcaloid</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Steroid/triterpenoid</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

+ : Present , - : Absent
Antibacterial activity

The in vitro test on antibacterial activity revealed that ethanol extracts of *Senna alata* leaf inhibited the growth of MRSA in a concentration dependent manner. The antibacterial activity was detected at 512 µg/µL leaf extract concentration for the ethanol extraction (Fig. 1). The fraction of n-hexane, ethyl acetate and water showed MIC value >1024, 256, >1024 µg/mL (table 2).

![Fig.1: It shows the ethanolic extract of Senna alata leaf](image1)

Table 2. The antibacterial activity of *Senna alata* against MRSA

<table>
<thead>
<tr>
<th>Fraction</th>
<th>MIC value of Senna alata (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-hexane</td>
<td>&gt;1024</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>256</td>
</tr>
<tr>
<td>water</td>
<td>&gt;1024</td>
</tr>
</tbody>
</table>

![Fig.2: It shows the ethyl acetate fraction of Senna alata leaf](image2)

Mode of action

The observation by SEM (Fig. 3) showed the changes of morphology in MRSA cell which was contacted to ethyl acetate fraction of *Senna alata* compared to the normal cell of MRSA. The MRSA cell, which was in contact with ethyl acetate fraction of *Senna alata* had an abnormal cell wall, which was indicated by the presence of pores and shrinkage in the cell wall. The pore in cell surface and the shrinkage in Cell wall were hypothesized due to the imperfect synthesis of the cell wall. The damage in the cell wall ensure the cause of the cell death.
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Figure 1. Scanning Electron Microscopy of MRSA bacterial cells, (a) normal cells, (b) the cells was contacted with tetracycline HCl, (c) The cells was contacted with vancomycin HCl, (d) the cell was contacted with ethyl acetate fraction of *Senna alata*

DISCUSSION

Search for new antibacterial is very important in recent times, considering the escalating levels of antibiotic resistance among pathogenic bacteria. Medicinal plants are commonly available resources, have less side effects, economic and have antimicrobial properties. Medicinal plants are promising as the most valuable resources for antibiotic development. Pathogenic strains of antibiotic resistant bacteria have emerged due to the misuse of antibiotics. As a result, bacteria become resistant to antibiotics, which are in turn less effective after extended periods of use. Pharmaceutical companies whose efforts are focused on the production and manufacture of antibiotics strive to manufacture new generations of antibiotics capable of treating such antibiotic-resistant bacterial strains.
MRSA is one of the leading causes of skin, soft tissue, bone, joint, abscess, and normal heart valve infections. Herein, we examined the anti-MRSA activity of extracts and fraction from *Senna alata* leaves.

Preliminary phytochemical analyses revealed that *Senna alata* contained flavonoid, alcaloid, saponin, tannin, qionones and sterol/triterpendoid as shown in Table 1. These bioactive compounds have been reported to be used by plants for protection against bacterial and responsible for antimicrobal activity.[12]

Previous study resulted *Senna alata* susceptible to *Str. Pyogenes* and *S. aureus*[12] while in the present study showed the MIC value of *Senna alata* leaf extract (512 µg/mL) and its fraction in ethyl acetate (256 µg/mL). It is believed that the membrane would be the target for antimicrobial peptides.[12] Our results demonstrate that SEM of MRSA treated by ethyl acetate fraction of *Senna alata* shows membrane shrinkage. This result supports the possibility that the antimicrobial peptide might be the response molecule for the ability to inhibit MRSA of ethyl acetate fraction of *Senna alata*.

The results obtained from the present study provide evidence that ethanolic extracts and ethyl acetate fraction of the *Senna alata* exhibit useful as antibacterial against isolated MRSA strain, suggesting that they may be clinically useful. Further search in respect of these findings are needed and promising.

**CONCLUSION**

The ethanolic extract and ethyl acetate fraction of *Senna alata* leaf is potential to be developed as antibacterial agent especially against MRSA strain due to the the result of present study. The further in vivo research and mode of its action is needed to shed light the antibacterial effect.

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**REFERENCES**


