Antimicrobial activity of the constituents of Smallanthus sonchifolius leaves against methicillin-resistant Staphylococcus aureus


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Abstract. – Background and Objectives: Methicillin-resistant Staphylococcus aureus (MRSA) has been a serious problem as its infection is associated with higher mortality and increase cost worldwide. In the present study, the antibacterial activity of enhydrin, polymatin B, allo-schkuhriolide from the leaves of Smallanthus sonchifolius was investigated.

Material and Methods: Enhydrin, polymatin B, allo-schkuhriolide from the leaves of Smallanthus sonchifolius were tested for antimicrobial activity using micro dilution broth method against 2 strains of ATCC 33591, ATCC 25923 and 15 strains of clinical isolates MRSA.

Results: The antibacterial activity of Smallanthus sonchifolius can safely be attributed to enhydrin as polymatin B, and allo-schkuhriolide are not showing any activity against Staphylococcus aureus strains. The enhydrin showed good antibacterial activity against all tested strains (MIC = 125-500 µg/ml).

Discussion: These results suggest that only enhydrin can be considered as an antibacterial drug against MRSA.

Key Words: Antibacterial activity, MRSA, Smallanthus sonchifolius.

Introduction

Staphylococcus aureus is a public, fatal pathogen associated with a variety of infections and the principal cause of skin and soft tissue infections, surgical site and catheter infections, pneumonia, osteoarticular and bacteremia infections. Moreover, because Staphylococcus aureus generally is an intracellular pathogenic organ, infections caused by this microbe can be difficult for medical treatment and can survive and relapse. Also, the overuse of drugs causes resistance. Currently, over 50% of the Staphylococcus aureus infections around the world are caused by methicillin-resistant Staphylococcus aureus (MRSA). The rise in antibiotic-resistant pathogens has led to the development of new therapeutic agents that are effective against these bacteria. Recently, there has been considerable interest in the use of plant materials as an alternative method to control pathogenic microorganisms and many compounds of plant products have been shown to be specifically targeted against resistant pathogenic bacteria.

Yacon [Smallanthus sonchifolius H. Robinson; Asteraceae; syn Polymnia sonchifolia] is an Andean crop used for centuries by the native inhabitants of South America as food and in traditional medicine. Smallanthus sonchifolius is reported to have antioxidative, antiinflammatory and antimicrobial activity properties. The constituents of tubers of Smallanthus sonchifolius include fructooligosaccharide and phenolic compounds and the leaves have several kaurene diterpenoids, acetophenone-type phytoalexins, and melampolide-type sesquiterpene lactones. However, to date, no studies regarding the antimicrobial activity of the leaves of Smallanthus sonchifolius against MRSA have been conducted. Therefore, the goal of this study is to evaluate the antimicrobial activity of enhydrin, polymatin B and allo-schkuhriolide from the leaves of Smallanthus sonchifolius.
Materials and Methods

Plant Materials and Sample Preparation
The leaves of yacon (Smallanthus sonchifolius) were collected from Bonghwa, Gyeongbuk, Korea in September 2005. The plant material was identified by Emeritus Professor Kyong Soon Lee at Chungbuk National University. A voucher specimen of this plant was deposited at the Herbarium of College of Pharmacy, Chungbuk National University, South Korea. Isolated compounds used were enhydrin, polymatin B, allo-schkuhriolide from the leaves of Smallanthus sonchifolius.

Bacterial Strains and Growth Conditions
Staphylococcus aureus and MRSA strains (Table I) were selected as test microorganisms as for decades, therapeutics options have been very limited. In the case of MRSA, it is resistant not only to β-lactams but to other types of antibiotics. Microorganisms were suspended in Mueller Hinton broth (Becton Dickinson, Sparks, MD, USA) and then incubated at 37°C for 24 h. Mueller Hinton agar (Becton Dickinson, Sparks, MD, USA) was used for the agar diffusion method.

Determination of the MecA Gene
Detection of the mecA gene in the MRSA strains was performed by PCR (Polymerase Chain Reaction) amplification. Prior to the DNA extraction, bacteria stock cultures were subcultured twice onto Mueller Hinton agar plates (MHA plates). For rapid extraction, one to five bacterial colonies were suspended in 300 µl of cell lysis buffer and heated at 100°C for 20 minutes. After centrifugation at 12,000 rpm for 10 minutes, 2 µl of the supernatant was used for the DNA extraction. PCR reactions were performed using a MRSA Primer Mix Kit (Genotek Co, Daejeon, Yuseong-gu, Republic of Korea). The PCR amplification consisted of 30 cycles (94°C, 60 sec; 55°C, 60 sec; 72°C, 60 sec). The primers used in this study were as follows: mecA – forward primer: 5’-ATGAGATTCGCTCTTTC-3’ reverse primer: 5’-TGGATTGACAGACTGAC3’. The final PCR products were separated on 2% agarose gel.

Minimum Inhibitory Concentration
The minimum inhibitory concentration (MIC) was determined using the broth dilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Briefly, a

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Table I. The Staphylococcus aureus strains used in the experiments.

<table>
<thead>
<tr>
<th>Staphylococcus aureus strain</th>
<th>Class</th>
<th>Mec A gene</th>
<th>Antibiotic resistance pattern</th>
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<tbody>
<tr>
<td>ATCC25923</td>
<td>MSSA</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ATCC33591</td>
<td>MRSA</td>
<td>+</td>
<td>AM, OX</td>
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<tr>
<td><strong>Clinical isolates</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>DPS-1</td>
<td>MRSA</td>
<td>+</td>
<td>AM, OX</td>
</tr>
<tr>
<td>DPS-2</td>
<td>MRSA</td>
<td>+</td>
<td>AM, OX</td>
</tr>
<tr>
<td>DPS-3</td>
<td>MRSA</td>
<td>+</td>
<td>AM, OX</td>
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<tr>
<td>DPS-4</td>
<td>MRSA</td>
<td>+</td>
<td>AM, OX</td>
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<td>DPS-5</td>
<td>MRSA</td>
<td>+</td>
<td>AM, OX</td>
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<tr>
<td>DPS-6</td>
<td>MRSA</td>
<td>+</td>
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<td>DPS-7</td>
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<td>DPS-8</td>
<td>MRSA</td>
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<td>DPS-9</td>
<td>MRSA</td>
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<td>DPS-10</td>
<td>MRSA</td>
<td>+</td>
<td>AM, OX</td>
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<td>DPS-11</td>
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<td>DPS-13</td>
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<td>AM, OX</td>
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<tr>
<td>DPS-14</td>
<td>MRSA</td>
<td>+</td>
<td>AM, OX</td>
</tr>
<tr>
<td>DPS-15</td>
<td>MRSA</td>
<td>+</td>
<td>AM, OX</td>
</tr>
</tbody>
</table>

+ = Positive; – = Negative; AM = Ampicillin; OX = Oxacillin.
preparation of the microorganisms inocula was done on 24 h broth cultures and the suspensions were adjusted to a 0.5 McFarland standard turbidity (approximately 10^8 CFU/ml). Final inoculums were adjusted to the 10^4 CFU/ml. The MHB was supplemented with serial ampicillin and enhydrin, polymatin B, allo-schkuhriolide concentrations. The MIC was defined as the lowest concentration in which there is no visible growth after 24 h of incubation at 37°C.12.

**Statistical Analysis**

All the experiments were performed in triplicates. The MIC data for each microorganism were analyzed using one-way analysis of variance (ANOVA) and the differences among group means were analyzed using the Dunnett's multiple comparisons test. P value < 0.05 was considered as significant.

**Results**

Enhydrin, polymatin B, allo-schkuhriolide (Figure 1) from the leaves of *Smallanthus sonchifolius* were screen tested for antimicrobial activity using micro dilution broth method against 3 strains of ATCC 33591, ATCC 25923 and DPS-1. The results are presented in Table II as MIC. The bioactivity is only seen in enhydrin compared to the polymatin B, and allo-schkuhriolide. Enhydrin appears to be the sole compound showing antibacterial activity with MICs ranging from 125 to 500 µg/ml (Table III).

**Discussion**

Today, the ongoing emergence of multi-drug resistant bacteria and the infectious diseases caused by them are serious global problems1. MRSA is very dangerous, and produces serious medical problems since its infection is often associated with acquired multi-drugs resistance. Today, with this emergence of antibiotic resistant pathogens like MRSA, a new approach using natural products must be taken. These natural products are more and more in demand by their non side effect benefit13, creating the need to develop alternative antimicrobial drugs for the

![Figure 1](image1.png)

**Figure 1.** A, Chemical structure of enhydrin. B, Polymatin B. C, Allo-schkuhriolide.
treatment of infectious diseases\textsuperscript{14,15}. Thus, our ongoing efforts to find bioactive natural products have led us to study the antibacterial activity of \textit{Smallanthus sonchifolius} against \textit{Staphylococcus aureus} strains.

The antibacterial activity of \textit{Smallanthus sonchifolius} can safely be attributed to enhydrin as polymatin B, and \textit{allo}-schkuhriolide are not showing any activity against \textit{Staphylococcus aureus} strains. The enhydrin showed good antibacterial activity against all tested strains (MIC = 125-500 µg/ml). From the structure of melamolides derivatives isolated from \textit{Smallanthus sonchifolius}, enhydrin like the other two are different in their polymatin B, and \textit{allo}-schkuhriolide enhydrin seems, by its different biological properties, to be an object of extensive research projects to better the health of human kind.

In light of the results obtained, the antibacterial activity of \textit{Smallanthus sonchifolius} can safely be attributed to enhydrin as polymatin B, and \textit{allo}-schkuhriolide are not showing any activity against \textit{Staphylococcus aureus} strains. This com-

\begin{table}[h]
\centering
\caption{Antimicrobial activity of enhydrin (ED), polymatin B (PM), \textit{allo}-schkuhriolide (AS) from the leaves of \textit{Smallanthus sonchifolius} against \textit{Staphylococcus aureus} (ATCC 33591, ATCC 25923, DPS-1), strains.}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{Staphylococcus aureus strain} & \textbf{MIC (µg/ml)} & & & & \\
 & \textbf{ED} & \textbf{PM} & \textbf{AS} & \textbf{AM}\textsuperscript{c} & \textbf{OX}\textsuperscript{c} \\
\hline
ATCC25923 & 250 & ND\textsuperscript{d} & ND & 0.06 & 0.97 \\
ATCC33591 & 125 & ND & ND & 500 & 500 \\
DPS-1\textsuperscript{b} & 250 & ND & ND & 250 & 250 \\
\hline
\textsuperscript{a}ND; no detected activity at this concentration; \textsuperscript{b}DPS, clinical isolates from Wonkwang University Hospital; \textsuperscript{c}Positive control; ampicillin (AM) and oxacillin (OX).
\end{table}

\begin{table}[h]
\centering
\caption{Antimicrobial activity of enhydrin(ED) isolated \textit{Smallanthus sonchifolius} and ampicillin against 16 strains of \textit{Staphylococcus aureus}.}
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
\textbf{Staphylococcus aureus strain} & \textbf{Class} & \textbf{Mec A gene} & \textbf{MIC\textsuperscript{a} (µg/ml)} & & & & \\
 & & & \textbf{ED} & \textbf{AM}\textsuperscript{c} & \textbf{OX}\textsuperscript{c} & & \\
\hline
ATCC25923 & MSSA & – & 250 & 0.06 & 0.97 & & \\
ATCC33591 & MRSA & + & 125 & 500 & 500 & & \\
\hline
\textbf{Clinical isolates} & & & & & & & \\
DPS-1\textsuperscript{b} & MRSA & + & 250 & 250 & 250 & & \\
DPS-2 & MRSA & + & 500 & 62.5 & 500 & & \\
DPS-3 & MRSA & + & 500 & 250 & 500 & & \\
DPS-4 & MRSA & + & 500 & 62.5 & 250 & & \\
DPS-5 & MRSA & + & 250 & 31.25 & 250 & & \\
DPS-6 & MRSA & + & 250 & 31.25 & 500 & & \\
DPS-7 & MRSA & + & 250 & 31.25 & 500 & & \\
DPS-8 & MRSA & + & 250 & 31.25 & 500 & & \\
DPS-9 & MRSA & + & 250 & 31.25 & 250 & & \\
DPS-10 & MRSA & + & 500 & 31.25 & 250 & & \\
DPS-11 & MRSA & + & 500 & 31.25 & 500 & & \\
DPS-12 & MRSA & + & 500 & 31.25 & 500 & & \\
DPS-13 & MRSA & + & 250 & 31.25 & 500 & & \\
DPS-14 & MRSA & + & 250 & 31.25 & 500 & & \\
DPS-15 & MRSA & + & 250 & 31.25 & 500 & & \\
\hline
\textsuperscript{a}MIC = Minimum inhibitory concentration; \textsuperscript{b}DPS = Clinical isolates from Wonkwang University Hospital; \textsuperscript{c}Positive control = Ampicillin (AM) and oxacillin (OX).
\end{tabular}
\end{table}
pound isolated from *Smallanthus sonchifolius* possessed antimicrobial activity *in vitro* and can be considered as potential candidate drug in the treatment of infectious diseases caused by MRSA. However, a measure of caution should be taken as herbal medicines can be toxic particularly for the liver. Further investigations are therefore on the way regarding the biological activities of enhydrin and its toxicity.

Acknowledgements

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References


